POST GRADUATE DEGREE PROGRAMME (CBCS)

IN

BOTANY

SEMESTER - II

Course: BOHCT 2.2

(Plant Physiology and Biochemistry)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

KALYANI-741 235

WEST BENGAL

COURSE PREPARATION TEAM

Dr. Bapi Ghosh
Assistant professor
Department of Botany, DODL
Kalyani University

Dr. Pallab Kumar Ghosh
Assistant professor
Department of Botany, DODL
Kalyani University

Dr. Sudipta Roy
HOD & Associate professor
Department of Botany
Kalyani University

Dr. Zahed Hossain
Associate professor
Department of Botany
Kalyani University

Dr. Sudha Gupta
Assistant professor
Department of Botany
Kalyani University

Dr. Malay Kr. Adak
Assistant professor
Department of Botany
Kalyani University

Dr. Neera Sen Sarkar
Assistant professor
Department of Botany
Kalyani University

June, 2019

Directorate of Open and Distance Learning, University of Kalyani

Published by the Directorate of Open and Distance Learning,
University of Kalyani, Kalyani-741235, West Bengal and Printed by
Printtech, 15A, Ambika Mukherjee Road, Kolkata-700056

All right reserved. No part of this work should be reproduced in any form without the permission in writing from the Directorate of Open and Distance Learning, University of Kalyani.

Authors are responsible for the academic contents of the course as far as copyright laws are concerned.

Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2017 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Sankar Kumar Ghosh, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every member of PGBOS (DODL), University of Kalyani. Heartfelt thanks is also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and co-ordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

Self Learning Materials (SLMs) have been published by the Directorate of Open and Distance Learning, University of Kalyani, Kalyani-741235, West Bengal and all the copyright reserved for University of Kalyani. No part of this work should be reproduced in any from without permission in writing from the appropriate authority of the University of Kalyani.

All the Self Learning Materials are-self writing and collected from e-book, journals and websites.

Director
Directorate of Open and Distance Learning
University of Kalyani

SYLLABUS

COURSE–BOHCT 2.2

Plant Physiology and Biochemistry

(Full Marks–75)

Course	Group	Details Contents Structure		Study hour
BOHCT 2.2	Plant Physiology and Biochemistry	**Unit 1. Water and Plant Cells and water balance of Plants**	1. Water and Plant Cells: Water in Plant life, structure and properties of water, water potential chemical properties of water, water potential of Plant Cells, major factors contributing cell water potential, cell wall and membrane properties.	1
			2. Water balance of Plants: Water in the Soil, Water absorption by roots, Water transport through Xylem, Water movement from leaf to atmosphere, Soil-Plant-atmosphere continuum.	
		Unit 2. Cell wall, transport and signal transduction	3. Cell wall structure, biogenesis; membrane transport processes; solute transport and photo assimilate translocation.	1
			4. Signal transduction: Signal transduction in eukaryotes	
		Unit 3. Sensory photobiology	5. Sensory photobiology: Light control of plant development; phytochrome: properties, phytochromeinduced response, phytochrome signaling pathways, blue light responses.	1
		Unit 4. Plant hormones and growth regulators	6. Plant hormones and growth regulators: Chemistry, biosynthesis, physiological effects, and signal transduction pathways of auxins, gibberellins, cytokinin, ethylene, abscisic acid, brassinosteroids, polyamines, jasmonates	1
		Unit 5. Growth, developmental physiology, Senescence and programmed cell death	7. Growth and developmental physiology: Embryogenesis and differentiation of plant organs Seedgermination and seedling growth.	1
			8. Senescence and programmed cell death: Types of senescence, metabolic changes associated with senescence and its regulation, influence of hormones.	
		Unit 6. Control of flowering:	9. Control of flowering: Floral meristem and floral organ development, floral evocation.	1

Course	Group	Details Contents Structure		Study hour
BOHCT 2.2	Plant Physiology and Biochemistry	**Unit 7. Stress physiology**	10. Stress physiology: Response and adaptation to abiotic stress: water stress, temperature stress (heat and cold stress). Gene regulation and proteomics of stress tolerance, Development of transgenic plants to stress tolerance.	1
		Unit 8. Principle of biophysical chemistry and Nitrogen metabolism	11. Principle of biophysical chemistry: pH, buffer, reaction kinetics, thermodynamics, law of mass action, acid base reactions, bond energy, energy rich compounds, redox potential, free energy.	1
			12. Nitrogen metabolism: Biological and non biological nitrogen fixation, nitrate and ammonium assimilation	
		Unit 9. Enzyme	13. Enzyme: Enzyme kinetics, catalytic reactions and regulatory properties, inhibitions, iso-enzymes, allosterism, ribozyme and abzymes, vitamins as coenzymes.	1
		Unit 10. Chemistry of plant products: structure and properties	14. Chemistry of plant products: structure and properties of carbohydrates, lipids, amino acids, proteins, nucleic acids, secondary metabolites.	1
		Unit 11. Photosynthesis	15. Photosynthesis: Light reactions, organization of light absorbing system, mechanism of electron and proton transport, Carbon concentrating mechanisms	1
		Unit 12. Energy yielding metabolisms	16. Energy yielding metabolisms: Paths of energy synthesis through Glycolysis, Citric acid cycle, plant mitochondrial electron transport chain, alternative oxidase, PPP cycle, regulation of respiratory pathways, Lipid metabolism: fatty acid biosynthesis and oxidation.	1

Content

	COURSE – BOHCT 2.2 Plant Physiology and Biochemistry	Page No.
Unit 1.	Water and Plant Cells and water balance of Plants	10-17
Unit 2.	Cell wall, transport and signal transduction	17-38
Unit 3.	Sensory photobiology	38-50
Unit 4.	Plant hormones and growth regulators	50-81
Unit 5.	Growth, developmental physiology, Senescence and programmed cell death	81-95
Unit 6.	Control of flowering:	95-114
Unit 7.	Stress physiology	114-121
Unit 8.	Principle of biophysical chemistry and Nitrogen metabolism	121-127, 233-236
Unit 9.	Enzyme	127-142
Unit 10.	Chemistry of plant products: structure and properties	142-198
Unit 11.	Photosynthesis	198-210
Unit 12.	Energy yielding metabolisms	210-233

COURSE – BOHCT2.2

PLANT PHYSIOLOGY AND BIOCHEMISTRY

Hard Core Theory Paper **Credit: = 3**

Content Structure

1. Introduction
2. Course Objective
3. Water and Plant Cells: Water in Plant life, structure and properties of water, water potential chemical properties of water, water potential of Plant Cells, major factors contributing cell water potential, cell wall and membrane properties.
4. Water balance of Plants: Water in the Soil, Water absorption by roots, Water transport through Xylem, Water movement from leaf to atmosphere, Soil-Plant-atmosphere continuum..
5. Cell wall structure, biogenesis; membrane transport processes; solute transport and photo assimilate translocation.
6. Signal transduction: Signal transduction in eukaryotes.
7. Sensory photobiology: Light control of plant development; phytochrome: properties, phytochromeinduced response, phytochrome signaling pathways, blue light responses.
8. Plant hormones and growth regulators: Chemistry, biosynthesis, physiological effects, and signal transduction pathways of auxins, gibberellins, cytokinin, ethylene, abscisic acid, brassinosteroids, polyamines, jasmonates.
9. Growth and developmental physiology: Embryogenesis and differentiation of plant organs Seedgermination and seedling growth.
10. Senescence and programmed cell death: Types of senescence, metabolic changes associated with senescence and its regulation, influence of hormones.
11. Control of flowering: Floral meristem and floral organ development, floral evocation.
12. Stress physiology: Response and adaptation to abiotic stress: water stress, temperature stress (heat and cold stress). Gene regulation and proteomics of stress tolerance, Development of transgenic plants to stress tolerance.
13. Principle of biophysical chemistry: pH, buffer, reaction kinetics, thermodynamics, law of mass action, acid base reactions, bond energy, energy rich compounds, redox potential, free energy.
14. Enzyme: Enzyme kinetics, catalytic reactions and regulatory properties, inhibitions, iso-enzymes, allosterism, ribozyme and abzymes, vitamins as coenzymes.
15. Chemistry of plant products: structure and properties of carbohydrates, lipids, amino acids, proteins, nucleic acids, secondary metabolites.
16. Photosynthesis: Light reactions, organization of light absorbing system, mechanism of electron and proton transport, Carbon concentrating mechanisms.
17. Energy yielding metabolisms: Paths of energy synthesis through Glycolysis, Citric acid cycle, plant mitochondrial electron transport chain, alternative oxidase, PPP cycle, regulation of respiratory pathways, Lipid metabolism: fatty acid biosynthesis and oxidation.

18. Nitrogen metabolism: Biological and non biological nitrogen fixation, nitrate and ammonium assimilation.
19. Let us sum up
20. Suggested Reading
21. Assignment

1. Introduction

Plant physiology is a subdiscipline of botany concerned with the functioning, or physiology, of plants. Closely related fields include plant morphology (structure of plants), plant ecology (interactions with the environment), phytochemistry (biochemistry of plants), cell biology, genetics, biophysics and molecular biology. Fundamental processes such as photosynthesis, respiration, plant nutrition, plant hormonefunctions, tropisms, nastic movements, photoperiodism, photomorphogenesis, circadian rhythms, environmental stress physiology, seed germination, dormancy and stomata function and transpiration, both parts of plant water relations, are studied by plant physiologists. The field of plant physiology includes the study of all the internal activities of plants-those chemical and physical processes associated with life as they occur in plants. This includes study at many levels of scale of size and time. At the smallest scale are molecularinteractions of photosynthesis and internal diffusion of water, minerals, and nutrients. At the largest scale are the processes of plant development, seasonality, dormancy, and reproductive control. Major subdisciplines of plant physiology include phytochemistry (the study of the biochemistry of plants) and phytopathology (the study of disease in plants). The scope of plant physiology as a discipline may be divided into several major areas of research. Finally, plant physiology includes the study of plant response to environmental conditions and their variation, a field known as environmental physiology. Stress from water loss, changes in air chemistry, or crowding by other plants can lead to changes in the way a plant functions. These changes may be affected by genetic, chemical, and physical factors.

2. Course Objectives

- The objective of this module is not only to develop a better aptitude towards the knowledge of physio-biochemical metabolism of plant system, but also to underline the need for better management with knowledge and skills to handle the task.
- On completion of this study material will be better knowledge –
  - Water potential activity and its significance in plant system
  - Different metabolic analysis of plants
  - Adaptation of plants and regulation of hormonal system
  - Energy conservation, signaling system

3. Water and Plant Cells: Water in Plant life, structure and properties of water, water potential chemical properties of water, water potential of Plant Cells, major factors contributing cell water potential, cell wall and membrane properties.

Water plays a crucial role in the life of the plant. For every gram of organic matter made by the plant, approximately 500 g of water is absorbed by the roots, transported through the plant body and lost tothe atmosphere. Even slight imbalances in this flow of water can cause water deficits and severe malfunctioning of many cellular processes.

Thus, every plant must delicately balance its uptake and loss of water.This balancing is a serious challenge for land plants. To carry on photosynthesis, they need to draw carbon dioxide from the atmosphere, but doing so exposes them to water loss and the threat of dehydration. A major difference between plant and animal cells that affects virtually all aspects of their relation with water is the existence in plants of the cell wall. Cell walls allow plant cells to build up large internal hydrostatic pressures, called **turgor pressure**, which is a result of their normal water balance. Turgor pressure is essential for many physiological processes, including cell enlargement, gas exchange in the leaves, transport in the phloem, and various transport processes across membranes. Turgor pressure also contributes to the rigidity and mechanical stability of non-lignified plant tissues. In this chapter we will consider how water moves into and out of plant cells, emphasizing the molecular properties of water and the physical forces that influence water movement at the cell level. But, first we will describe the major functions of water in plant life

Water in plant life:

Water makes up most of the mass of plant cells, as we can readily appreciate if we look at microscopic sections of mature plant cells: Each cell contains a large water-filled vacuole. In such cells the cytoplasm makes up only 5 to 10% of the cell volume; the remainder is vacuole. Water typically constitutes 80 to 95% of the mass of growing plant tissues. Common vegetables such as carrots and lettuce may contain 85 to 95% water. Wood, which is composed mostly of dead cells, has lower water content; sapwood, which functions in transport into xylem, contains 35 to 75% water; and heartwood has slightly lower water content. Seeds, with a water content of 5 to 15%, are among the driest of plant tissues, yet before germinating they must absorb a considerable amount of water. Plants continuously absorb and lose water. Most of the water lost by the plant evaporates from the leaf as the CO_2 needed for photosynthesis is absorbed from the atmosphere. On a warm, dry, sunny day a leaf will exchange up to 100% of its water in a single hour. During the plant's lifetime, water equivalent to 100 times the fresh weight of the plant may be lost through the leaf surfaces. Such water loss is called **transpiration**.

Transpiration is an important means of dissipating the heat input from sunlight. Heat dissipates because the water molecules that escape into the atmosphere have highert han-average energy, which breaks the bonds holding them in the liquid. When these molecules escape, they leave behind a mass of molecules with lower-than-average energy and thus a cooler body of water. For a typical leaf, nearly half of the net heat input from sunlight is dissipated by transpiration. In addition, the stream of water taken up by the roots is an important means of bringing dissolved soil minerals to the root surface for absorption.

The Polarity of Water Molecules Gives Rise to Hydrogen Bonds:

The water molecule consists of an oxygen atom covalently bonded to two hydrogen atoms. The two O—H bonds form an angle of 105°. Because the oxygen atom is more **electronegative** than hydrogen, it tends to attract the electrons of the covalent bond. This attraction results in a partial negative charge at the oxygen end of the molecule and a partial positive charge at each hydrogen. These partial charges are equal, so the water molecule carries no *net* charge. This separation of partial charges, together with the shape of the water molecule, makes water a *polar molecule*, and the opposite partial charges between neighboring water molecules tend to attract each other. The weak electro static attraction between water molecules, known as a **hydrogen bond**, is responsible for many of the unusual physical properties of water. Hydrogen bonds can also form between water and other molecules that contain electronegative atoms (O or N). In aqueous solutions, hydrogen bonding between water molecules leads to local, ordered clusters of water that, because of the continuous thermal agitation of the water molecules, continually form, break up, and re-form.

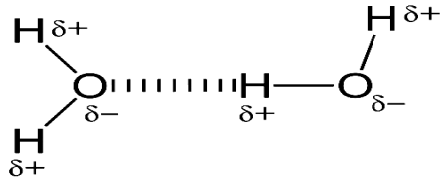


Figure 1. Diagram of the water molecule. The two intramolecular hydrogen–oxygen bonds form an angle of 105°.

The Polarity of Water Makes It an Excellent Solvent

Water is an excellent solvent: It dissolves greater amounts of a wider variety of substances than do other related solvents.This versatility as a solvent is due in part to the small size of the water molecule and in part to its polar nature.The latter makes water a particularly good solvent for ionic substances and for molecules such as sugars and proteinsthat contain polar —OH or —NH_2 groups. Hydrogen bonding between water molecules and ions, and between water and polar solutes, in solution effectively decreases the electrostatic interaction between the charged substances and thereby increases their solubility. Furthermore, the polar ends of water molecules can orient themselves next to charged or partially charged groups in macromolecules, forming **shells of hydration**. Hydrogen bonding between macromolecules and water reduces the interaction between those.

The Cohesive and Adhesive Properties of Water Are Due to Hydrogen Bonding

Water molecules at an air–water interface are more strongly attracted to neighboring water molecules than to the gas phase in contact with the water surface. As a consequence of this unequal attraction, an air–water interface minimizes itssurface area. To increase the area of an air–water interface, hydrogen bonds must be broken, which requires an input of energy. The energy required to increase the surface area isknown as **surface tension**. Surface tension not only influences the shape of the surface but also may create a pressure in the rest of the liquid. As we will see later, surface tension at the evaporative surfaces of leaves generates the physical forces that pull water through the plant's vascular system. The extensive hydrogen bonding in water also gives rise to the property known as **cohesion**, the mutual attractionbetween molecules. Arelated property, called **adhesion**, is the attraction of water to a solid phase such as a cell wall or glass surface. Cohesion, adhesion, and surface tension give rise to a phenomenon known as **capillarity**, the movement of water along a capillary tube.

Water transport processes

When water moves from the soil through the plant to the atmosphere, it travels through a widely variable medium (cell wall, cytoplasm, membrane, air spaces), and the mechanisms of water transport also vary with the type of medium. For many years there has been much uncertainty how water moves across plant membranes. Specifically it was unclear whether water movement into plant cells was limited to the diffusion of water molecules across the plasma membrane's lipid bilayer or also involved diffusion through protein-lined pores. Some studies indicated that diffusion directly across the lipid bilayer was not sufficient to account for observed rates of water movement across membranes, but the evidence in support of microscopic pores was not compelling.

This uncertainty was put to rest with the recent discovery of **aquaporins**. Aquaporins are integral membrane proteins that form water-selective channels across the membrane. Because water diffuses faster through such channels than through a lipid bilayer, aquaporins facilitate water movement into plant cells. Note that although the presence of aquaporins may alter the *rate* of water movement across the membrane, they do not change the direction of transport or the driving force for water movement. Now consider the two major processes in water transport: molecular diffusion and bulk flow.

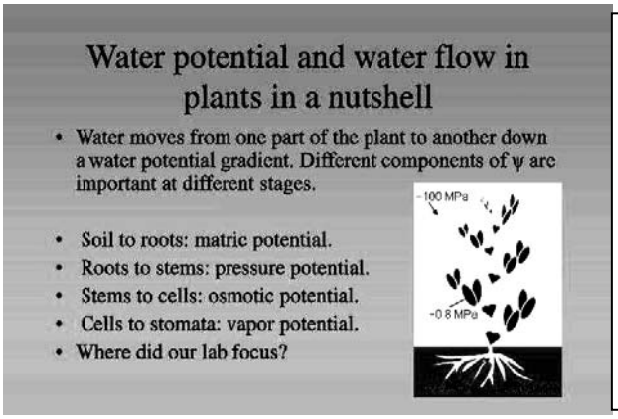


Table 1.: Comparison of units of pressure

1 atmosphere = 14.7 pounds per square inch
= 760 mm Hg (at sea level, 45° latitude)
= 1.013 bar
= 0.1013 Mpa
= $1.013 \cdot 10^5$ Pa

A car tire is typically inflated to about 0.2MPa.

The water pressure in home plumbing is typically 0.2–0.3 MPa.

The water pressure under 15 feet (5 m) of water is about 0.05 MPa.

Osmosis is driven by a water potential gradient

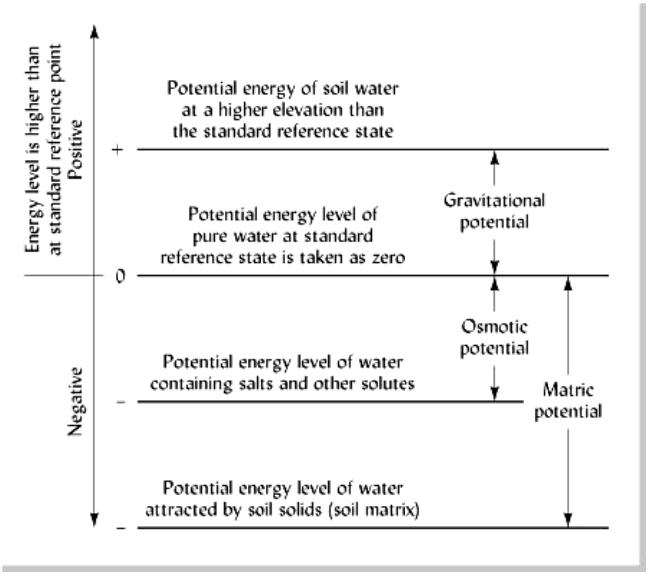
Membranes of plant cells are **selectively permeable**; that is, they allow the movement of water and other small uncharged substances across them more readily than themovement of larger solutes and charged substances. Like molecular diffusion and pressure-driven bulk flow, **osmosis** occurs spontaneously in response to a driving force. In simple diffusion, substances move down a concentration gradient; in pressure-driven bulk flow, substances move down a pressure gradient; in osmosis, bothtypes of gradients influence transport (Finkelstein 1987). The direction and rate of water flow across a membrane are determined not solely by the concentration gradient of water or by the pressure gradient, but by the sum of these two drivingforces.

The Chemical Potential of Water Represents the Free-Energy Status of Water:

All living things, including plants, require a continuousinput of free energy to maintain and repair their highly organized structures, as well as to grow and reproduce. Processes such as biochemical reactions, solute accumulation, and long-distance transport are all driven by an inputof free energy into the plant. The **chemical potential** of water is a quantitative expression of the free energy associated with water. In thermodynamics, free energy represents the potential for performing work. It is expressed as the difference between the potential of a substance in a given state and the potential of the same substance in a standard state. The unit of chemical potential is energy per mole of substance (J mol–1). Thus **water potential**, defined as the chemical potential of water divided by the partial molal volume of water.

Three Major Factors Contribute to Cell Water Potential

The major factors influencing the water potential in plants are *concentration*, *pressure*, and *gravity*. Water potential is symbolized by *Y*w (the Greek letter psi), and the waterpotential of solutions may be dissected into individual components, usually written as the following sum: The terms *Y*s, *Y*p, and *Y*g denote the effects of solutes, pressure, and gravity, respectively, on the free energy of water. The reference state used to define water potential is pure water at ambient pressure and temperature. The term *Y*s, called the **solute potential** or the **osmotic potential**, represents the effect of dissolved solutes on water potential. Solutes reduce the free energy of water by diluting the water. This is primarily an entropy effect; that is, the mixing of solutes and water increases the disorder of the system and thereby lowers free energy.



This means that the osmotic potential is independent of the specific nature of the solute. For dilute solutions of non-disso-ciating substances, like sucrose, the osmotic potential maybe estimated by the **van't Hoff equation**: where *R* is the gas constant (8.32 J mol–1 K–1), *T* is the absolute temperature (in degrees Kelvin, or K), and *c*s is thesolute concentration of the solution, expressed as **osmolality** (moles of total dissolved solutes per liter of water [mol L–1]). The minus sign indicates that dissolved

solutes reduce the water potential of a solution relative to the reference state of pure water.Table 2 shows the values of *RT* at various temperaturesand the *Y*s values of solutions of different solute concentrations. For ionic solutes that dissociate into two or more particles, *c*s must be multiplied by the number of dissociated particles to account for the increased number of dissolved particles.

The term *Y*p is the **hydrostatic pressure** of the solution. Positive pressures raise the water potential; negative pressures reduce it. Sometimes *Y*p is called *pressurepotential*. The positive hydrostatic pressure within cells is the pressure referred to as *turgor pressure*. The value of *Y*p can also be negative, as is the case in the xylem and in the walls between cells, where a *tension*, or *negative hydrostatic pressure*, can develop. As we will see, negative pressures outside cells are very important in moving water long distances through the plant. Hydrostatic pressure is measured as the deviation from ambient pressure. Remember that water in the reference state is at ambient pressure, so by this definition *Y*p = 0 MPa for water in the standard state. Thus the value of *Y*p for pure water in an open beaker is 0 MPa, even though its absolute.

Water can also leave the cell in response to a water potential gradient

Water can also leave the cell by osmosis. If, in the previous example, we remove our plant cell from the 0.1 *M* sucrose solution and place it in a 0.3 *M* sucrose solution, *Y*w (solution) (–0.732 MPa) is more negative than *Y*w(cell) (–0.244 MPa), and water will move from the turgid cell to the solution. As water leaves the cell, the cell volume decreases. As the cell volume decreases, cell *Y*p and *Y*w decrease also until *Y*w (cell) = *Y*w (solution) = –0.732 MPa. From the water potential equation, we can calculate that at equilibrium, *Y*p = 0 MPa. As before, we assume that the change in cell volume is small, so we can ignore the change in *Y*s. If we then slowly squeeze the turgid cell by pressing it between two plates, we effectively raise the cell *Y*p, consequently raising the cell *Y*w and creating a *Y*w such that water now flows *out* of the cell. If we continue squeezing until half the cell water is removed and then hold the cell in this condition, the cell will reach a new equilibrium. However, the components of the cell *Y*w will be quite different. Because half of the water was squeezed out of the cell while the solutes remained inside the cell (the plasma membrane is selectively permeable), the cell solution is concentrated twofold, and thus *Y*s is lower (–0.732= –1.464 MPa). Knowing the final values for *Y*w and *Y*s, we can calculate the turgor pressure, using Equation 3.6, as *Y*p = *Y*w – *Y*s = (–0.244) – (–1.464) = 1.22 MPa. In our example we used an external force to change cell volume without a change in water potential. In nature, it is typically the water potential of the cell's environment that changes, and the cell gains or loses water until its *Y*w matches that of its surroundings. One point common to all these examples deserves emphasis: *Water flow is a passive process. That is, water moves in response to physical forces, toward regions of low water potential or low free energy.*

The water potential concept helps us evaluate the water status of a plant

The concept of water potential has two principal uses: First, water potential governs transport across cell membranes, as we have described. Second, water potential is often used as a measure of the *water status* of a plant. Because of transpirational water loss to the atmosphere, plants are seldom fully hydrated. They suffer from water deficits that lead to inhibition of plant growth and photosynthesis, as well as to other detrimental effects. Figure 3 lists some of the physiological changes that plants experience as they become dry. The process that is most affected by water deficit is cell growth. More severe water stress leads to inhibition of cell division, inhibition of wall and protein synthesis, accumulation of solutes, closing of stomata, and inhibition of photosynthesis. Water potential is one measure of how hydrated a plant is and thus provides a relative index of the *water stress* the plant. In leaves of well-watered plants, *Y*w ranges from –0.2 to about –1.0 MPa, but thel eaves of plants in arid climates can have much lower values, perhaps –2 to –5 MPa under extreme conditions. Because water transport is a passive process, plants can take up water only when the plant *Y*w is less than the soil *Y*w. As the soil becomes drier, the plant similarly becomes less hydrated (attains a lower *Y*w). If this were not the case, the soil would begin to extract water from the plant.

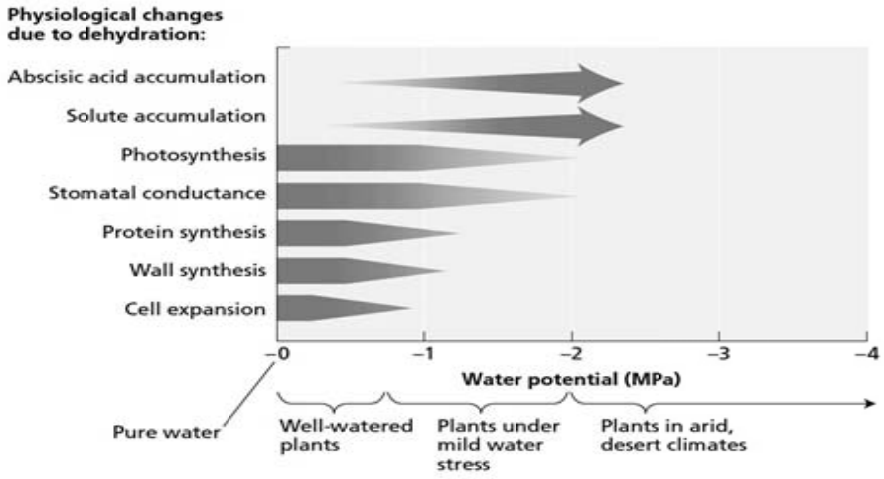


Figure 3: Water potential of plants under various growing conditions, and sensitivity of various physiological processes to water potential. (After Hsiao 1979.)

4. Water balance of Plants: Water in the Soil, Water absorption by roots, Water transport through Xylem, Water movement from leaf to atmosphere, Soil-Plant-atmosphere continuum.

Role of Water in Plant Physiology:

Of all the vital substances essential for plant growth and development, water is needed in enormous amounts. It is present throughout the plant body. Water constitutes more than 70% of the fresh weight of a plant and in some of the growing cells its level may be up to 90%. Water supply affects the growth rate of plants considerably.

(i) It is a major component of the plant body.

(ii) What is an essential solvent in which mineral nutrients are dissolved and translocated from the roots to the apex of the plant body. Minerals are also absorbed through water.

(iii) Large number of metabolic reactions take place in the water medium.

(iv) It maintains the structure of nucleic acids, proteins by supplying hydrogen bonding.

(v) Several processes like photosynthesis use water as a reactant of raw material. Thus formation of complex carbohydrates from the simple ones also involves the removal of water while the reverse reaction requires water as a reactant.

(vi) This essential component is required to maintain the turgidity of the cell. Thus, it helps the cells to retain their tensile strength and provides proper shape to the cells.

(vii) Water also acts as a temperature buffer since it has an exceptionally high heat capacity for specific heat.

(viii) Water molecules have the unique property of adhesion and cohesion and thus these processes keep the water molecules together. This property helps in upward movement of water in the plant body.

(ix) The elongation phase of cell growth is mostly dependent on water absorption.

(x) Water is also a metabolic end product of respiration.

(xi) Plans absorb enormous quantities of water and simultaneously lose greater amounts of water through transpiration.

Water in soil

Though the soil particles often lie close to each other yet some angular space is always present there. All such spaces in the soil are collectively called pore space. The pore space comprises a fairly constant volume in the soil (40-60% of total soil volume) which remains filled with water and gases in

varying proportions. In a “dry” soil, water occupies a very small proportion of this space. In a “wet” soil, it is water that occupies most of this space. The soil, in fact, acts as a water reservoir for plants.

The water stored in the soil may be classified into the following four groups :

Different forms of water:

Water is mainly obtained through rain. Some of the water goes into the reservoirs. This is called run off or Run away water. Rest of the water enter into the land.

Water present in soil is following type:

(a) Gravitational water: Form of water, which reaches at the soil water table due to the gravitational force after the rainfall. This form is not available to plants but available by mechanical methods or by tube well irrigation.

(b) Hygroscopic water: Thin film of water is tightly held by the soil particles is called hygroscopic water. This water is also not available to the plants.

(c) Chemically combined water: The amount of water present in the chemical compounds, which are present in the particles of soil. This is not available to the plants.

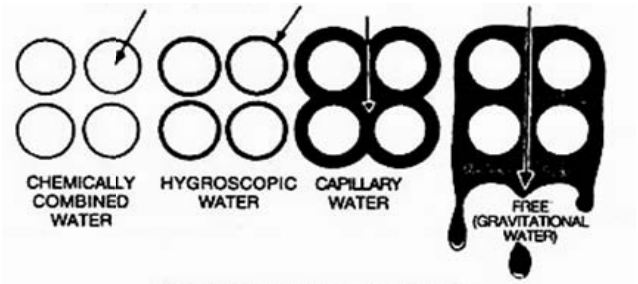


Fig. 2.1 Different types of soil water.

(d) Capillary water: Water exists between soil particles in small capillary pores is called Capillary water. It is the most common available form of water for absorption.

(e) Atmospheric humidity: This is water vapor present in air, which can be absorbed by hanging roots of the epiphytes due to presence of spongy velamen tissue and hygroscopic hairs.

Field Capacity or Water Holding Capacity of the Soil:

After heavy rainfall or irrigation of the soil, some water is drained off along the slopes while the rest percolates down in the soil. This amount of water retained by the soil after the drainage of gravita¬tional water has become very slow is called as field capacity or the water holding capacity of the soil. The field capacity is affected by soil profile, soil structure and temperature. For instance a fine textured soil overlying a coarse textured soil will have a higher field capacity than a uniformly fine textured soil. Similarly, the field capacity increases with decreasing temperature and vice versa.

Permanent Wilting Percentage or Wilting Coefficient:

The percentage of the soil water left after the plant growing in that soil has permanently wilted is called as permanent wilting percentage or the wilting coefficient. The permanent wilting percentage can be determined by growing the seedlings in small containers under conditions of adequate water supply till they develop several leaves. The soil surface is then covered and the water supply is cut until wilting occurs. The containers are now transferred to humid chamber.If the plants do not recover, they are considered to be permanently wilted. Otherwise, they are again transferred to normal atmospheric conditions. This process is repeated till they are permanently wilted. The percentage of the soil water is determined at this point after removing the plants from the containers and shaking off as much soil from their roots as possible. Thus the different plants if grown in the same soil wilt at different times depending upon their osmotic potential after the water supply to the soil is stopped.

Soil Texture in Relation to Water Absorption:

The texture of a soil depends upon the proportion of different sized soil particles in that soil and is a very important factor for the absorption of water in plants.

Depending upon their diameters the soil particles are classified as below:

Sandy Soils: Such soils are very rich in sand particles and though well aerated them have poor water holding capacity. Sandy soils are, therefore, not good for water absorption.

Clayey Soils: These are rich in clay particles and are poorly aerated. Such soils often become water-logged and are, therefore, neither good for water absorption nor for normal growth of the plants.

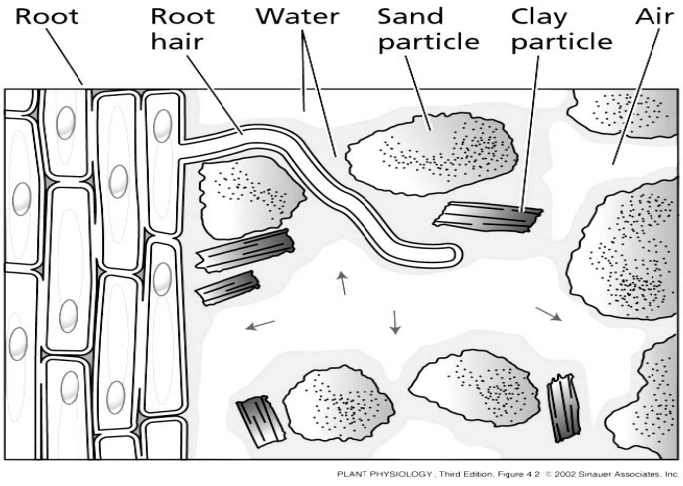
Loam: Such soils contain almost equal proportion of the different sized soil particles. They are sufficiently aerated and have good water holding capacity. Therefore, they are very good for water absorption and growth. The loam soil in which the proportion of sand is slightly higher is called as sandy loam while a loam soil in which clay particles predominate, is called as clayey loam.

Water absorption by roots:
Water is an important constituent of soil because all land plants depend upon it for their requirement of water. Deep in the soil and above the impermeable stratum, water occurs freely in the previous rocky matter. It is called ground water. Ground water bearing pervious straturn is known as aquifer.

The upper layer of ground water is called water table. In the region of water table soil is completely saturated with water. Air is excluded. The plants which do so, are called phreatophytes, e.g., *Populus deltoides*, *Alhagi pseudalhagi*, *Tamarix*, *Prosopis cineraria* etc. Phreatophytes have been used successfully to locate under-ground water since the period of Varahmihira. Soil water, important to most plants, is the one present in 1-2m of soil because their roots are generally restricted to this region.

Water absorption from soil:

- Water clings to the surface of soil particles.
- As soil dries out, water moves first from the center of the largest spaces between particles.
- Water then moves to smaller spaces between soil particles.
- Root hairs make intimate contact with soil particles – amplify the surface area for water absorption by the plant.



PLANT PHYSIOLOGY, Third Edition, Figure 4.2 © 2002 Sinauer Associates, Inc.

Water Moves through soil by bulk flow
Bulk flow:
Concerted movement of groups of molecules in mass, most often in response to a **pressure gradient**.

- Dependant on the radius of the tube that water is traveling in.
- This is the main method for water movement in **Xylem, Cell Walls** and in the **soil**.
- **It is Independent** of solute concentration gradients – to a point In addition, **diffusion** of water vapor accounts for some water movement.
- As water moves into root – less in soil near the root
- Results in a **pressure gradient** with respect to neighboring regions of soil.
- So there is a reduction in yp near the root and a **higher** yp in the neighboring regions of soil.
- Water filled pore spaces in soil are interconnected, water moves to root surface by bulk flow down the pressure gradient
- As water moves from soil into root the spaces fill with air. This **reduces** the flow of water

Mechanism of Absorption of Water:

In higher plants water is absorbed through root hairs which are in contact with soil water and form a root hair zone a little behind the root tips (Fig. 4.1). Root hairs are tubular hair like prolongations of the cells of the epidermal layer (when epidermis bears root hairs it is also known as piliferous layer) of the roots. The walls of root hairs are permeable and consist of pectic substances and cellulose which are strongly hydrophilic (water loving) in nature. Root hairs contain vacuoles filled with cell sap.

Mechanism of water absorption is of two types:

(1) Active Absorption of Water:

In this process the root cells play active role in the absorption of water and metabolic energy released through respiration is consumed.

Active absorption may be of two kinds:

(a) Osmotic absorption i.e., when water is absorbed from the soil into the xylem of the roots according to the osmotic gradient.

(b) Non-osmotic absorption i.e., when water is absorbed against the osmotic gradient.

(2) Passive Absorption of Water:

It is mainly due to transpiration, the root cells do not play active role and remain passive.

(1a) Active Osmotic Absorption of Water:

First step in the osmotic absorption of water is the imbibition of soil water by the hydrophilic cell walls of root hairs. Osmotic Pressure (O.P.) of the cell-sap of root hairs is usually higher than the O.P. of the soil water. Therefore, the Diffusion Pressure Deficit (D.P.D.) and the suction pressure in the root hairs become higher and water from the cell walls enters into them through plasma-membrane (semi-permeable) by osmotic diffusion. Now, the cortical cells adjacent to root hairs have higher O.P., suction pressure and D.P.D. in comparison to the root hairs. Therefore, water is drawn into the adjacent cortical cells from the root-hairs by osmotic diffusion.

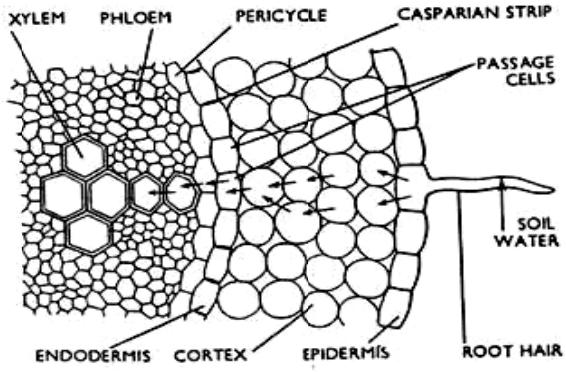


Fig. 4.2. A part of T.S. of typical dicot root. The arrows indicate the path of water.

Water from endodermal cells is drawn into the cells of pericycle by osmotic diffusion which now becomes turgid and their suction pressure is decreased. In the last step, water is drawn into xylem from turgid pericycle cells. (In roots the vascular bundles are radial and protoxylem elements are in contact with pericycle). It is because in absence of turgor pressure of the xylem vessels (which are

non-elastic), the suction pressure of xylem vessels become higher than the suction pressure of the cells of the pericycle. When water enters into xylem from pericycle, a pressure is developed in the xylem of roots which can raise the water to a certain height in the xylem. This pressure is called as root pressure.

(1b) Active Non-Osmotic Absorption of Water:

Sometimes, it has been observed that absorption of water takes place even when the O.P. of the soil water is higher than the O.P. of cell-sap. This type of absorption which is non- osmotic and against the osmotic gradient requires the expenditure of metabolic energy probably through respiration.

Following evidences support this view:

(i) The factors which inhibit respiration also decrease water absorption.

(ii) Poisons which retard metabolic activities of the root cells also retard water absorption.

(iii) Auxins which increase metabolic activities of the cells stimulate absorption of water.

(2) Passive Absorption of Water:

Passive absorption of water takes place when rate of transpiration is usually high. Rapid evaporation of water from the leaves during transpiration creates a tension in water in the xylem of the leaves. This tension is transmitted to water in xylem of roots through the xylem of stem and the water rises upward to reach the transpiring surfaces.

As a result, soil water enters into the cortical cells through root hairs to reach the xylem of roots to maintain the supply of water. The force for this entry of water is created in leaves due to rapid transpiration and hence, the root cells remain passive during this process.

During absorption of water by roots, the flow of water from epidermis to endodermis may take place through three different pathways:

(i) Apoplastic pathway (cell walls and intercellular spaces),

(ii) Trans-membrane pathway (by crossing the plasma membranes) and

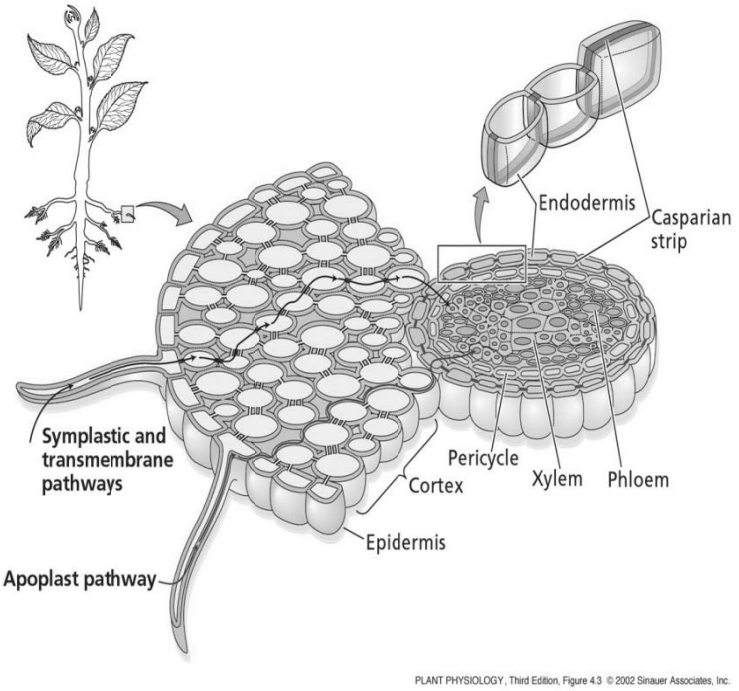
(iii) Symplast pathway (through plasmodesmata).

Combination of these three pathways is responsible for transport of water across the root.The mechanism of water absorption described earlier, in-fact belongs to the second category. The relative importance of these three pathways in water absorption by roots is not clearly established. However,

Movement of water and nutrients in the roots

Negative water potential continues to drive movement once water (and minerals) are inside the root; Ψ of the soil is much higher than Ψ or the root, and Ψ of the cortex (ground tissue) is much higher than Ψ of the stele (location of the root vascular tissue). Once water has been absorbed by a root hair, it moves through the ground tissue through one of three possible routes before entering the plant's xylem:

- **The symplast**: "sym" means "same" or "shared," so symplast is shared cytoplasm. In this pathway, water and minerals move from the cytoplasm of one cell in to the next, via plasmodesmata that physically join different plant cells, until eventually reaching the xylem.
- **The transmembrane pathway**: in this pathway, water moves through water channels present in the plant cell plasma membranes, from one cell to the next, until eventually reaching the xylem.
- **The apoplast**: "a" means "outside of," so apoplast is outside of the cell. In this pathway, water and dissolved minerals never move through a cell's plasma membrane but instead travel through the porous cell walls that surround plant cells.



Water and minerals that move into a cell through the plasma membrane has been "filtered" as they pass through water or other channels within the plasma membrane; however water and minerals that move via the apoplast do not encounter a filtering step until they reach a layer of cells known as the **endodermis** which separate the vascular tissue (called the stele in the root) from the ground tissue in the outer portion of the root. The endodermis is exclusive to roots, and serves as a checkpoint for materials entering the root's vascular system. A waxy substance called suberin is present on the walls of the endodermal cells. This waxy region, known as the **Casparian strip**, forces water and solutes to cross the plasma membranes of endodermal cells instead of slipping between the cells. This ensures that only materials required by the root pass through the endodermis, while toxic substances and pathogens are generally excluded.

Aquaporins:

In recent years some integral membrane proteins have been discovered which form water selective channels in cell membranes (lipid bilayers) and facilitate faster movement of water across the membranes into the plant cells. These channels have been called as aquaporins (Fig. 4.4). The direction of water transport across the membranes however, is not affected by aquaporins.

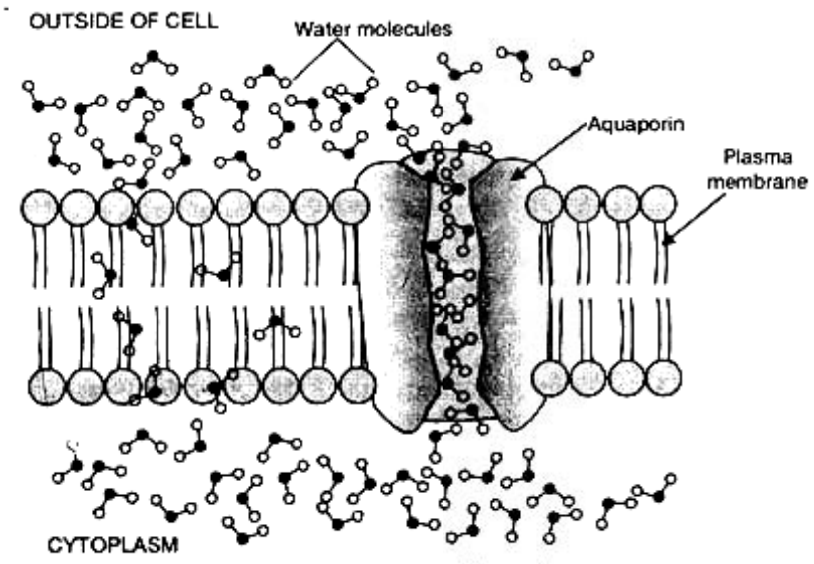


Fig. 4.4. Bulk flow of water across the cell membrane through aquaporin.

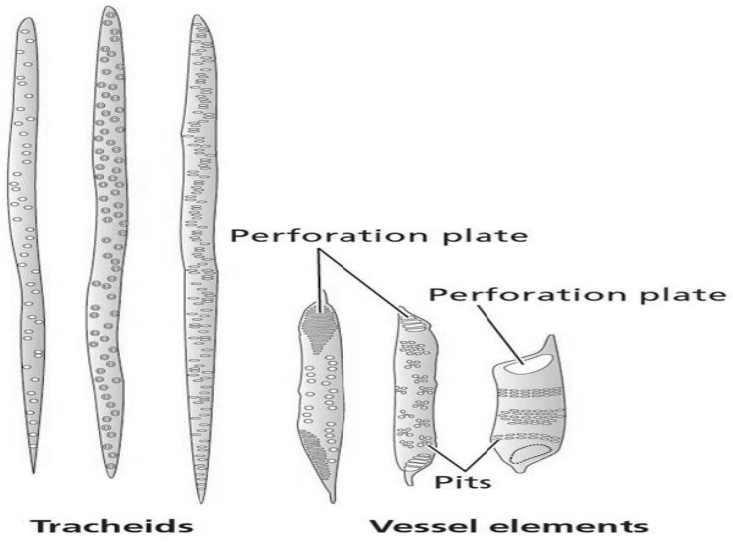
Aquaporin's are found in both plant and animal membranes but they are

relatively abundant in plants. The aquaporin's satisfactorily account for the observed rate of water movement across the membranes which could not be explained earlier simply by direct diffusion of water through lipid bilayer as the latter does not allow bulk flow of water across it. According to Tyerman et al (2002), expression and activity of aquaporin's appear to be regulated probably by protein phosphorylation in response to availability of water.

Water transport through xylem

Xylem:

- Tracheids: Elongated spindle-shaped cells –arranged in overlapping vertical files.
- Water flows between them via pits – areas with no secondary walls and thin porous primary walls
- Vessel elements: Shorter & wider. The open end walls provide an efficient low-resistance pathway for water movement.
- Perforation plate forms at each end – allow stacking end on to form a larger conduit called a vessel.



Among the reasons for this recognition of primary functions of xylem are the following:

(1) The anatomy of xylem obviously and clearly fits it for conducting purposes and it is also true that among the vascular tissues, xylem vessels have the right cross-sectional area to allow the upward transport of the large amounts of water required by plants;

(2) In the classical ringing experiments, it is observed that the removal of a ring of tissue external to xylem from the stem does not interfere significantly with upward movement of water to the organs situated above the ring whereas the removal of a cylinder of xylem from the stem certainly seriously disturbs or even completely stops conduction of water. The net result in many cases is almost complete wilting of leaves attached to the stem above the ring;

(3) Upward movement of water continues for some time even in shoots cut from plant, when placed in water. The rate of water movement through xylem vessels varies greatly from almost imperceptibly slow, to speeds as high as 75 cm per min.

Ringing Experiments:

Most land plants obtain the necessary water for normal growth and development from the soil. By far, the largest proportion of absorbed water is lost as vapour in the process of transpiration from the aerial parts.

Much smaller quantities are utilised for growth and for various other metabolic processes of plants. Water, therefore, must move continuously through the intervening tissues and organs from the absorbing regions of roots to the tissues in which it is utilised or from which, it escapes as vapour, the leaves.

In small herbs and shrubs, the distance to be traversed by water on its onward journey from the root tips to the leaves, is usually not more than a few feet. But even in such plants, they may

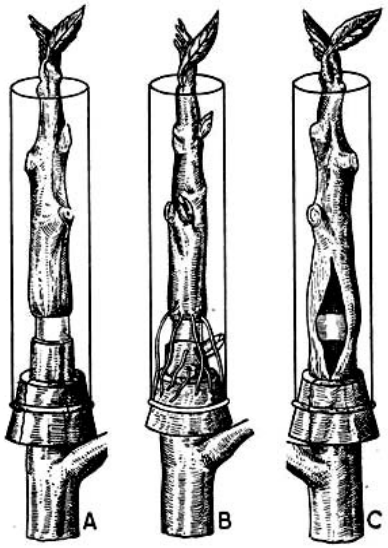


FIG. 686. Ringing experiments; A & B, all tissues external to xylem removed; C, only xylem removed.

have such deep-seated root system, that the ascending water has to cover distances as great as 6-10 metres (m) before even reaching the surface of the soil. It is in tall trees, however, in which the most striking illustrations of upward conduction of water occur. The tallest trees in record are a species of redwood in U.S.A., which attain immense heights of 90-120 m. The heights of our own eucalyptus and firs range between 60-90 m. Since in all trees, the root systems also penetrate at least a few feet into the ground, the actual vertical distance through which the absorbed water must be conducted in order to reach the topmost leaves, may easily be 120 m or even more!

A simple calculation reveals that the force required to lift water to the top of tallest trees must be enormous indeed. For example, in case of a 90 m tree, a pressure of 10 atm. is just sufficient to support the column of water and according to all available experimental evidence; another 10 atm. is needed to overcome resistance due to friction.

So altogether a minimum of 20 atm. is necessary if the water is actually moving up through the xylem vessels. The mechanism by which this feat is accomplished against the force of gravity has been the subject of much speculation and it has been one of the most fascinating problems of plant physiology which has intrigued generations of investigators, not to speak of laymen. This has been termed, ascent of sap.

We know that water enters the land plant entirely through the root hairs and after crossing the cells of the cortex, the peculiarly thick-walled endodermis and pericycle, finally reach the xylem .vessels or tracheids of the roots.

At this point, its upward movement begins. We know that xylem vessels form a continuous conducting system, just like a pipeline, from the root tips through the main tissues of the roots and through the stems, the petioles of the leaves and ultimately through veins and veinlets, ending up in the surrounding mesophyll tissue of the leaves. In the mesophyll cells, water moves onward from cell to cell and eventually the mesophyll cell walls, losing most of it by evaporation into the intercellular spaces, from where it escapes into the external air through stomata. It was generally agreed, that xylem vessels are the main pathway of the upward conduction of water. A cell-to-cell lateral movement of water in a radial direction undoubtedly occurs along the vascular rays in the stems of most species of plants.

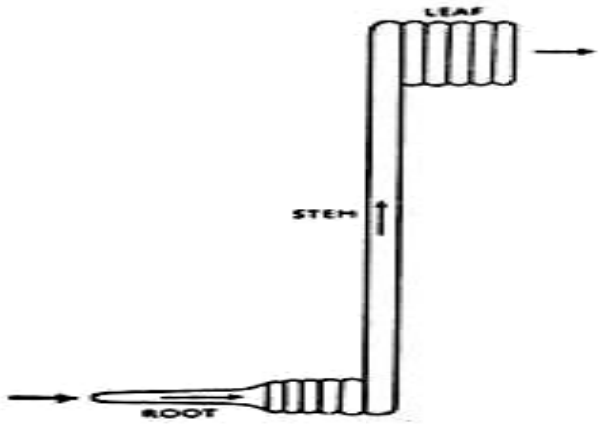


Fig. 685. Diagrammatic path of absorbed water from the root to the leaf and to external air.

Ascent of Sap:

The mechanism of water moving vertically in plants against the force of gravity is still a classical problem of plant physiology. Since diffusion is much too slow to account for the rates that commonly occur in plants, mass movement of water must be envisaged; the whole column must move simultaneously instead of molecular movement.

A number of different theories of the mechanism by which ascent of water are brought about in plants has been suggested and the present state of our knowledge justifies a discussion of only three possible mechanisms:

(1) That the ascent of water is caused by the activity of the living cells - vital theories;

(2) That upward movement occurs as a result of root pressure;

(3) That ascent of water has its explanations in a set of purely physical principles, supplemented by cohesion of water theory, proposed by Dixon.

How is water transported up a plant against gravity, when there is no “pump” to move water through a plant’s vascular tissue? There are three hypotheses that explain the movement of water up a plant

against gravity. These hypotheses are not mutually exclusive, and each contributes to movement of water in a plant, but only one can explain the height of tall trees:

1. **Root pressure** pushes water up
2. **Capillary action** draws water up within the xylem
3. **Cohesion-tension** pulls water up the xylem

We'll consider each of these in turn.

Vital Theories:

As living cells are more or less in intimate contact with the dead xylem elements through which upward conduction of water occurs; suggestions have often been made that the motive power of ascent of sap is provided by the vital activity of living cells. Our own J. C. Bose was the most ardent advocate of the vital theory of ascent of sap. Bose's idea was essentially an elaboration of the theory proposed by Godlewski in 1884. According to Godlewski, ascent of water resulted from periodic changes in the osmotic pressure of the living cells—wood-ray cells in the xylem. It visualised a repetitive process of alternate increase and decrease in the osmotic pressure of the wood-ray cells, resulting in driving water out and into the xylem vessel. Thus the movement of water was supposed to be due to an alternate contraction and expansion of wood-ray cells, which also supplied necessary energy for the mechanism, perhaps energy released in cellular respiration.

The concepts of Bose and Molisch have been destructively criticised by a number of investigators, including Smith, Benedict, MacDougal, and others.

Elaborate experiments carried out by these investigators, however, showed conclusively that water would continue to ascend for some time through the plant after all the living cells present in a woody stem were killed either by soaking the cut end into boiling water or by dipping it into a strong solution of picric acid.

Root Pressure:

Root pressure relies on positive pressure that forms in the roots as water moves into the roots from the soil. Water moves into the roots from the soil by osmosis, due to the low solute potential in the roots (lower Ψs in roots than in soil). This intake of water in the roots increases Ψp in the root xylem, driving water up. In extreme circumstances, root pressure results in **guttation**, or secretion of water droplets from stomata in the leaves. However, root pressure can only move water against gravity by a few meters, so it is not strong enough to move water up the height of a tall tree.

Capillary action:

Capillary action or capillarity is the tendency of a liquid to move up against gravity when confined within a narrow tube (capillary). Capillarity occurs due to three properties of water:

1. **Surface tension**, which occurs because hydrogen bonding between water molecules is stronger at the air-water interface than among molecules within the water.
2. **Adhesion**, which is molecular attraction between "unlike" molecules. In the case of xylem, adhesion occurs between water molecules and the molecules of the xylem cell walls.
3. **Cohesion**, which is molecular attraction between "like" molecules. In water, cohesion occurs due to hydrogen bonding between water molecules.

On its own, capillarity can work well within a vertical stem for up to approximately 1 meter, so it is not strong enough to move water up a tall tree.

Cohesion Tension Theory:

The **cohesion-tension** hypothesis is the most widely-accepted model for movement of water in vascular plants. Cohesion-tension essentially combines the process of capillary action with **transpiration**, or the evaporation of water from the plant stomata. Transpiration is ultimately the main driver of water movement in xylem. The cohesion-tension model works like this:

1. **Transpiration** (evaporation) occurs because stomata are open to allow gas exchange for photosynthesis. As transpiration occurs, it deepens the meniscus of water in the leaf, creating negative pressure (also called tension or suction).
2. The **tension** created by transpiration "pulls" water in the plant xylem, drawing the water upward in much the same way that you draw water upward when you suck on a straw.
3. **Cohesion** (water sticking to each other) causes more water molecules to fill the gap in the xylem as the top-most water is pulled toward the stomata.

In the application of physical principles for an explanation of the mechanism of ascent of sap in plants, atmospheric pressure naturally comes first to mind. But it is at once apparent that atmospheric pressure can only account for a rise of about 10 m. Suggestions were then put forward that water rose by imbibitional forces through the thick walls of the xylem vessels.

Physicists have shown that the forces of imbibition are very great, ranging from 100-1000 atm. and this at first sight would seem more than adequate for carrying water to any required height.

However, the rate of movement of water through imbibing colloids is extremely slow compared with known rates of water conduction in the xylem. Moreover, it was soon shown that water actually moves not through the cell walls, which would be required for an imbibitional transport, but through the lumen or cavities of the xylem vessels. We have seen that negative pressure (or tension) generally exists in the xylem during rapid transpiration from leaves.

This can be shown by the use of a very sensitive instrument known as dendrograph which measures diameters of the tree trunk and such measurements definitely show, at times at least, a contraction of trunk-diameter during the day (caused by tension or pull due to transpiration) and expansion at night (tension less, due to stopping of transpiration). Thus the ascent of sap is usually associated with a pull from above rather than a push from below (positive root pressure). Forces developing in aerial parts of the plants, especially in the leaves, cause the rise of water through the plant. It is easy to calculate mathematically whether this can be explained by a capillary pull in the vessels.

A vessel diameter of 0·1 mm would account for a rise of only 30 cm. But it must be understood clearly that it is not in the xylem vessels themselves that the main capillary pull occurs. Since this is clearly a surface tension phenomenon, the pull must occur at the water surface due to transpiration.

Thus the transpiration pull, it seems, is responsible not only for the movement of water within the plant, but also of absorption of water from the external root medium, i.e., soil solution.

The following three stages in the process then may be distinguished:

(1) Transpiration from the surface of microcapillaries,

(2) Capillary rise of fine threads of water due to the force of adhesion between water and cell wall, and

(3) The whole column of water moves all together and resists breaking because of the force of cohesion between the water molecules—similar particles always have tendency to stick to each other.

Of course, these three stages occur simultaneously. This concept is known as Dixon's transpiration-cohesion-tension theory of ascent of sap in plants.

Objections:

(i) The gases dissolved in sap shall form air bubbles under tension and high temperature. Air bubbles would break the continuity of water column and stop ascent of sap due to transpiration pull,

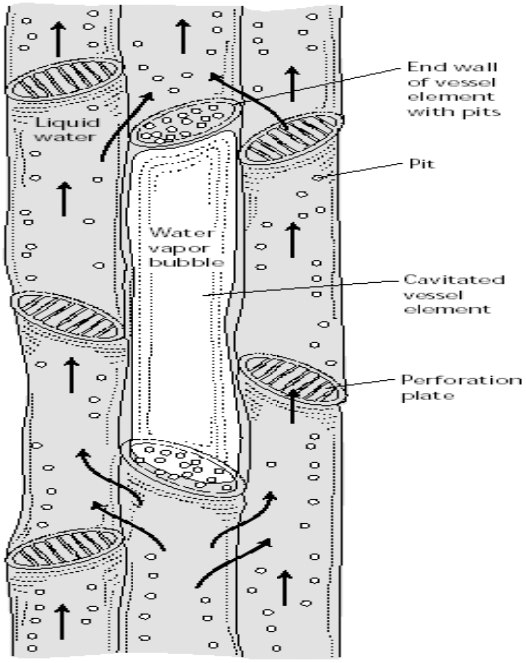
(ii) A tension of up to 100 atm has been reported in the xylem sap by Mac Dougal (1936) while the cohesive force of sap can be as low as 45 atm.

(iii) Overlapping cuts do not stop ascent of sap though they break the continuity of water column.The cohesion-tension theory of sap ascent is shown. Evaporation from the mesophyll cells produces a negative water potential gradient that causes water to move upwards from the roots through the xylem.

Transpiration Pull:

It is the pulling force responsible for lifting the water column. As water is lost in form of water vapour to atmosphere from the mesophyll cells by transpiration, a negative hydrostatic pressure is created in the mesophyll cells which in turn draw water from veins of the leaves.

The negative tension is then gradually transmitted downwards via xylem tissues of the leaf, stem and finally to the roots. Thus the transpiration pull acts as pull from above on the-whole of water column of the plant which pushes the water column of xylem vessels of roots lowers leaves i.e. in an upward direction. This is how ascent of sap is affected in plants. The whole process can be compared to a person (transpiration pull) pulling a bucket full of water with a steel rope (unbroken and continuous water column)



The Significance of Transpiration:

Although transpiration has been studied extensively, very little is known concerning the significance of transpiration in the life of the plant. There is even now some controversy whether transpiration is a necessary and unavoidable evil or whether it is in some way essential for the welfare of the plant.

In the first category, opinions prevail that transpiration brings nothing but harm to the plant, and the harmful effects ascribed to transpiration far outweigh its supposed beneficial effects, while in the second, there are opinions which consider transpiration as important as photosynthesis and respiration.

From the former point of view the primary function of stomata is to permit exchange of CO_2 and O_2 between the plant and the atmosphere in the process of photosynthesis and respiration and water is lost through the open stomata, because there is no stopping the passage of water vapour, while permitting CO_2 and O_2 exchange.

Thus the primary function of stomata is not to be thought of as one permitting water loss any more than the function of a main door in a busy office building is to let heat escape from the building in winter.

Just as loss of heat cannot be avoided if the door must be kept open to continuous streams of persons who enter and leave the building during business hours, similarly loss of water vapour from the leaf is inevitable when stomata are open to permit exchange of CO_2 and O_2between the plant and the atmosphere.

Regulation or Control of Water Loss by Stomata:

The general view came to be held that the stomata by opening and closing control or regulate the transpirational water loss from the plant.

The earlier idea was that stomata could close in anticipation of wilting and thus conserve the water supply of the leaf and prevent it from wilting. When later it was found that the stomata do not close until the leaf has wilted to some extent, it was then realised that the regulation, if any, was passive since under such conditions, the stomatal closure is not due to any special activity of the guard cells but to a decrease in their turgour caused by loss of water from the leaf cells.

The rate of transpiration is generally higher than the absorption of water by roots from soil during the day and the reverse is true at night. The water content of a leaf may vary over a considerable range during a 24-hour period without any visible signs of wilting of the aerial organs.

We have discussed before that no correlation generally exists between the number of stomata per unit area of the leaf surface, and the rate of transpiration. The general view now held by most investigations is that when the stomata are fully open or nearly so, the transpiration rate is primarily determined by the same physical factors which control evaporation from a free water surface.

Transpiration may actually increase when stomata begin to close and may decrease when they open wider. This is sometimes due to the fact, as we have seen before, that when the diameters of the stomatal pores are about 1 /10th to 1 /20th the distance between the two pores, no further opening of the pore can affect the rate of diffusion of water vapour through them. As the stomata gradually close, influence of physical factors of evaporation is lessened until at 50% opening of stomatal pore, variable atmospheric conditions become the primary factors in controlling transpiration, physiological regulation by the stomata, of the water loss, taking only a subsidiary role. Only when the pores are almost closed, the guard cells begin to exert a controlling influence on the water loss regardless of evaporation and climatic factors prevailing at the time.

Mechanism of Water Loss in Transpiration:

In order to form vapours, water present inside the exposed parts of the plant requires a source of heat energy. It is the radiant energy during the day and heat energy from the transpiring organ during the night. In both the cases the temperature of the transpiring organs comes to lie 2-5 °C below that of the atmosphere.

The atmosphere is rarely saturated with water vapours. The dry air of the atmosphere has a high DPD (or low water potential)-13.4 atm at 99% relative humidity or RH, 140 atm at 90% RH, 680 atm at 60% and 2055 atm at 20% RH.

Such a high DPD or low water potential can overcome various types of resistances water molecules have to meet in chang-ing from liquid phase to vapour phase and the movement of water vapours out of the transpiring organ.

The intercellular spaces of the transpiring organ is almost saturated with water vapours. When the stomata are open, the water vapours are drawn from the sub-stomatal cavities to the outside air due to high DPD of the latter. This increases the DPD of the sub-stomatal air which draws more water vapours from the intercellular spaces.

The latter in turn get water vapours from the wet walls of mesophyll cells. Stomatal transpiration will continue till the stomata are open. Mechanism of lenticular transpiration is similar to that of stomatal transpiration.

Cuticle is not much permeable to water. However, its molecules absorb water from the epidermal cells by imbibition. The imbibed water is slowly lost to the atmosphere which has a high DPD. Imbibition flow is reduced by the thickness of cuticle.

Therefore, a thick cuticle does not allow transpiration to occur through it. Cuticle is shrunken and thicker during the day but at night it expands and becomes loose. Therefore, cuticular transpiration can be more at night. Mechanism of bark transpiration is similar to that of cuticular transpiration**.**

Soil-plant-atmosphere continuum:

The soil-plant-atmosphere continuum (SPAC) is the pathway for water moving from soil through plants to the atmosphere. Continuum in the description highlights the continuous nature of water connection through the pathway. The low water potential of the atmosphere, and relatively higher (i.e. less negative) water potential inside leaves, leads to a diffusion gradient across the stomatal pores of leaves, drawing water out of the leaves as vapour. As water vapour transpires out of the leaf, further water molecules evaporate off the surface of mesophyll cells to replace the lost molecules since water in the air inside leaves is maintained at saturation vapour pressure. Water lost at the surface of cells is replaced by water from the xylem, which due to the cohesion-tension properties of water in the xylem of plants pulls additional water molecules through the xylem from the roots toward the leaf.

5. Cell wall structure, biogenesis; membrane transport processes; solute transport and photo assimilate translocation

Structure of Cell Wall:

A cell wall can have upto three parts— middle lamella, primary wall and secondary wall.

Middle Lamella It is a thin, amorphous and cementing layer between two adjacent cells. Middle lamella is the first layer which is depos-ited at the time of cytokinesis. It is just like brick work of the common wall be-tween two adjacent rooms.

Middle lamella is absent on the outer side of surface cells. It is made up of calcium and magnesium pectates. The softening of ripe fruits is caused by partial solubilisation of pectic compounds to produce jelly-like consistency.

Primary Wall:

It is the first formed wall of the cell which is produced inner to the middle lamella. The primary wall is com-monly thin (0.1-3.0 μm) and capable of exten-sion. It grows by intussusceptions or addition of materials within the existing wall. Some cells possess only primary wall, e.g., leaf cells, fruit cells, of cortex and pith.

Primary wall consists of a number of micro fibrils embedded in the amorphous gel like matrix or ground substance. In the majority of plants, the micro fibrils are formed of cellulose. They are synthesized at plasma membrane by particle rosettes (terminal com-plexes) having cellulose synthetize enzyme.

The wall is made of a polymer of P, 1-4 acetyl glucosamine or fungus cellulose in many fungi. Fungus cellulose is similar to chitin present in the exoskeleton of insects. Micro fibrils are oriented variously according to the shape and thickening of the wall. Usually they are arranged in a loose network due to incomplete cross-linking.

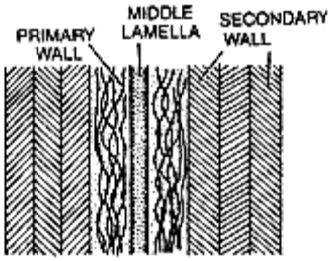


Fig. 8.14. Arrangement of microfibrils in the common wall between two adjacent cells as seen in L.S.

The matrix of the wall consists of water, pectin, hemicelluloses and glycoproteins. Pectin is the filler substance of the matrix. Proteins are structural and enzymatic. Protein expansin (Me Queen-Mason et al, 1992) is involved in loosening and expansion of cell wall through incorporation of more cellulose. Hemicellulose binds micro fibrils with matrix.

Secondary Wall:

It is produced in some mature cells when the latter have stopped growth, e.g., tracheids, vessel elements, fibres, collenchyma's. Secondary wall is laid inner to the primary wall by accretion or deposition of materials over the surface of existing structure. It is thick (3—10 μm) and made up of at least three layers, sometimes more (e.g., latex tube of Euphorbia milli). They are named as S1, S2, S3, Sx, etc.

The innermost layer of the secondary wall is sometimes distinct both chemically as well as in staining properties due to the presence of xylans. It is then called tertiary wall, e.g., tension wood in gymnosperms. Secondary wall may be absent, irregularly deposited or formed uniformly in the cells. This results in differentiation of cells— parenchyma, collenchyma, sclerenchyma, tracheids and vessels.

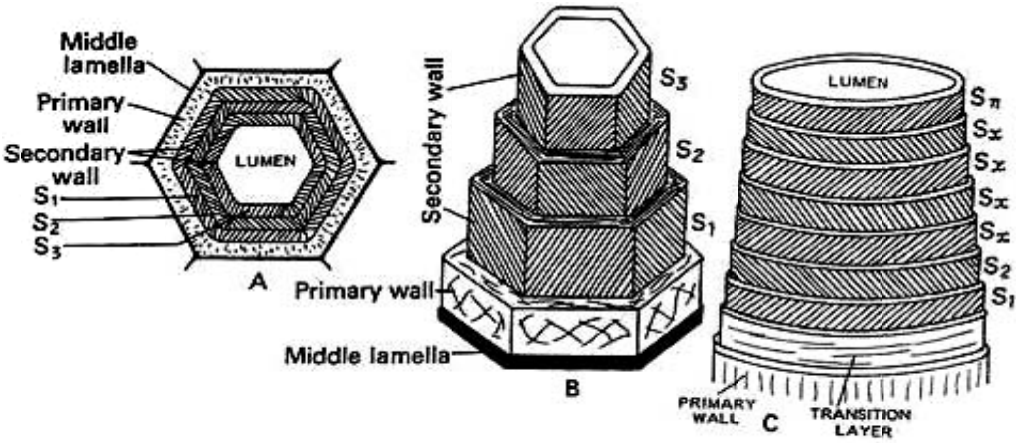


Fig. 8.15. Parts and layers of cell wall. A, a cell in T.S. showing parts of cell wall. B, typical wood fibre cut at various levels to show parts and layers of the wall. C, latex tube of *Euphorbia milli* (= *E. splendens*) cut at various levels to show parts.

The composition of secondary wall is basically similar to the primary wall in having cellulose micro-fibrils embedded in a matrix of pectin and hemicelluloses. Cellulose mi-cro-fibrils of the secondary wall lie close, parallel and at an angle to the longitudinal axis of the cell. Their orientation is different in the different layers of the secondary wall. A number of different materials may be deposited in the wall.

The important ones are:

(a) Lignin:

It reduces the water content of the wall matrix and increases its hardness. However, water permeability is not affected. The characteristic of lignification's (and cutinisation) has evolved with the evolution of land plants.

(b) Suberin:

The wall of cork and endodermal cells contains a special fatty substance called suberin. Suberin makes the walls impermeable.

(c) Cutin:

The epidermal cells possess another fatty substance called cutin. Cutin is also laid as a distinct layer on the outside of the epidermal cell walls. It is known as cuticle. Cutin reduces the rate of epidermal or surface transpiration. Other substances which can be deposited in the cell wall are silica (e.g., grasses), minerals, waxes, tannins, resins, gums, etc.

Plasmodesmata:

Plasmodesmata (singular- plasmodesma; Tangl, 1879; Strasburger, 1901) are cytoplasmic bridges between adjacent plant cells which develop in the minute pores of their walls. They form a protoplasmic continuum called symplast. Cell wall and intercellular spaces form a non-living component of the plant body called apoplasm. A plasmoderma is 40—50 nm in diameter. It may be simple or branched.

Plasmodesma is lined by plasma membrane. It encloses tubular extention of endoplasmic reticulum called desmotubule The space between desmotubule and plasma membrane contains 8-10 microchannels. Plasmodesmata

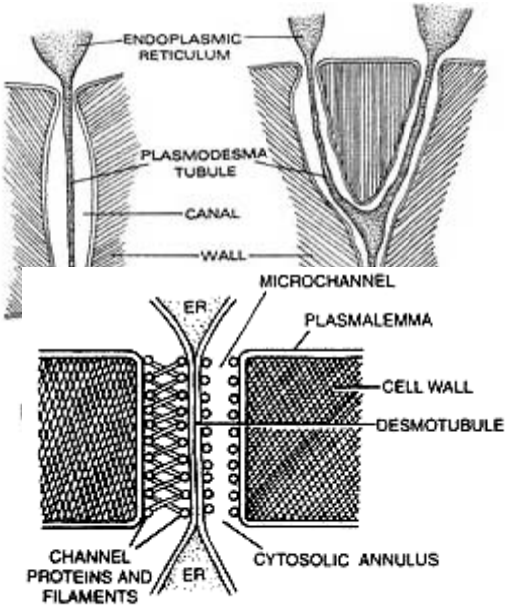


Fig. 8.17. Components of plasmodesmata.

form channels for controlled passage of small sized particles between adjacent cells as well as transfer of some specific signals.

Pits:

Pits are un-thickened areas in the secondary walls of plant cells. They, therefore, appear as depressions. Pits generally occur in pairs on the wall of two adjacent cells. A pit has a cavity or pit chamber and a pit membrane.

The pit membrane consists of primary wall and middle lamella. Pits are of two types, simple and bordered. Simple pit has uniform width of the pit chamber. In bordered pit, the pit chamber is flask-shaped because the secondary wall overarches its mouth.

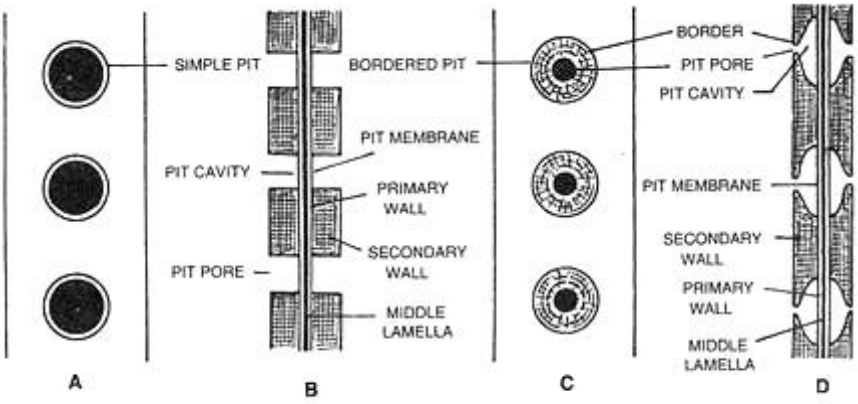


Fig. 8.18. Pits. A, surface view of simple pits. B, simple pit pairs in section. C, surface view of bordered pits. D, bordered pit pairs in section.

Pit membrane is permeable. It may have minute sub-microscopic pores. Therefore, pits help in rapid translocation between two adjacent cells.

Cell wall biogenesis:

The middle lamella is laid down first, formed from the cell plate during cytokinesis, and the primary cell wall is then deposited inside the middle lamella. The actual structure of the cell wall is not clearly defined and several models exist - the covalently linked cross model, the tether model, the diffuse layer model and the stratified layer model. However, the primary cell wall, can be defined as composed of cellulose microfibrils aligned at all angles. Cellulose microfibrils are produced at the plasma membrane by the cellulose synthase complex, which is proposed to be made of a hexameric rosette that contains three cellulose synthase catalytic subunits for each of the six units. Microfibrils are held together by hydrogen bonds to provide a high tensile strength. The cells are held together and share the gelatinous membrane called the middle lamella, which contains magnesium and calcium pectates (salts of pectic acid). Cells interact though plasmodesmata, which are inter-connecting channels of cytoplasm that connect to the protoplasts of adjacent cells across the cell wall.

In some plants and cell types, after a maximum size or point in development has been reached, a secondary wall is constructed between the plasma membrane and primary wall. Unlike the primary wall, the cellulose microfibrils are aligned parallel in layers, the orientation changing slightly with each additional layer so that the structure becomes helicoidal. Cells with secondary cell walls can be rigid, as in the gritty sclereid cells in pear and quince fruit. Cell to cell communication is possible through pits in the secondary cell wall that allow plasmodesmata to connect cells through the secondary cell walls.

Passive and active transport

According to Fick's first law, the movement of molecules by diffusion always proceeds spontaneously, down a gradient of concentration or chemical potential, until equilibrium is reached. The spontaneous "downhill" movement of molecules is termed **passive transport**. At equilibrium, no

further net movements of solute can occur without the applicationof a driving force. The movement of substances against or up a gradient of chemical potential (e.g., to a higher concentration) is termed **active transport**. It is not spontaneous, and itrequires that work be done on the system by the application of cellular energy. One way (but not the only way) of accomplishing this task is to couple transport to the hydrolysis of ATP. The driving force for diffusion, or, conversely, the energy input necessary to move substances against a gradient, by measuring the potential-energy gradient, which is often a simple function of the difference in concentration. Biological transport can be driven by four major forces: concentration, hydrostatic pressure, gravity, and electric fields. The **chemical potential** for any solute is defined as the sum of the concentration, electric, and hydrostatic potentials (and the chemical potential under standard conditions.

Transport of ions across a membrane barrier

If the two KCl solutions in the previous example are separated by a biological membrane, diffusion is complicated by the fact that the ions must move through the membrane as well as across the open solutions. The extent to which a membrane permits the movement of a substance is called **membrane permeability**. As will be discussed later, permeability depends on the composition of the membrane, as well as on the chemical nature of the solute. In a loosesense, permeability can be expressed in terms of a diffusion coefficient for the solute in the membrane. However, permeability is influenced by several additional factors, suchas the ability of a substance to enter the membrane, that are difficult to measure. Despite its theoretical complexity, we can readily measure permeability by determining the rate at which a solute passes through a membrane under a specific set of conditions.Generally the membrane will hinder diffusion and thus reduce the speed with which equilibrium is reached.The permeability or resistance of the membrane itself, however, cannot alter the final equilibrium conditions. Equilibrium occurs when $m\sim j = 0$.In the sections that follow we will discuss the factors that influence the passive distribution of ions across amembrane. These parameters can be used to predict there lationship between the electrical gradient and the concentration gradient of an ion.

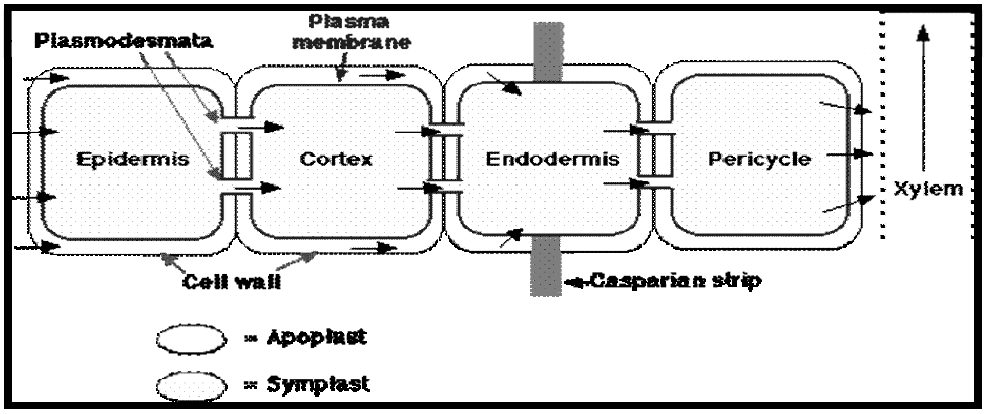


Figure 6: Three classes of membrane transport proteins: channels, carriers, and pumps. Channels and carriers can mediate the passive transport of solutes acrossmembranes

Carriers Bind and Transport Specific Substances

Unlike channels, **carrier** proteins do not have pores thatextend completely across the membrane. In transport mediated by a carrier, the substance being transported is the pore at the center. The pore-forming regions of the four subunits dip into the membrane, with a K+ selectivity finger region formed at the outer (near) part of the pore. (B) Side view of the inward rectifying K+ channel, showing a polypeptide chain of one subunit, with six membrane-spanning helices. The fourth helix contains positively-charged amino acids and acts as a voltage-sensor. The pore-forming region is a loop between helices 5 and 6. (A after Leng et al. 2002; B after Buchanan et al. 2000.) initially bound to a specific site on the carrier protein. This requirement for binding allows carriers to be highly selective for a particular substrate to be transported. Carriers therefore specialize in the transport of

specific organic metabolites. Binding causes a conformational change in the protein, which exposes the substance to the solution on the other side of the membrane. Transport is complete when the substance dissociates from the carrier's binding site.

Because a conformational change in the protein is required to transport individual molecules or ions, the rate of transport by a carrier is many orders of magnitude slower than through a channel. Typically, carriers may transport 100 to 1000 ions or molecules per second, which is about 106 times slower than transport through a channel. The binding and release of a molecule at a specific site on a protein that occur in carrier-mediated transport are similarto the binding and release of molecules from an enzyme in an enzyme-catalyzed reaction. Carrier-mediated transport (unlike transport through channels) can be either passive or active, and it can transport a much wider range of possible substrates. Passive transporton a carrier is sometimes called **facilitated diffusion**, although it resembles diffusion only in that it transports substances down their gradient of electrochemical potential, without an additional input of energy. (This term might seem more appropriately applied to transport through channels, but historically it has not been used in this way.)

Primary Active Transport Is Directly Coupled to Metabolic or Light Energy

To carry out active transport, a carrier must couple the uphill transport of the solute with another, energy-releasing, event so that the overall free-energy change is negative.**Primary active transport** is coupled directly to a source of energy other than *m*~j, such as ATP hydrolysis, an oxidation–reduction reaction (the electron transport chain of mitochondria and chloroplasts), or the absorption of light by the carrier protein (in halobacteria, bacteriorhodopsin).

The membrane proteins that carry out primary active transport are called **pumps**. Most pumpstransport ions, such as H^+ or $Ca2^+$. However, as we will see later in the chapter, pumps belonging to the "ATPbindingcassette" family of transporters can carry large organic molecules.Ion pumps can be further characterized as either electrogenicor electroneutral. In general, **electrogenic transport** refers to ion transport involving the net movement ofcharge across the membrane. In contrast, **electroneutral transport**, as the name implies, involves no net movementof charge. For example, the Na+/K+-ATPase of animal cells pumps three Na+ ions out for every two K+ ions in, resulting in a net outward movement of one positive charge. The Na+/K+-ATPase is therefore an electrogenic ion pump. In contrast, the H+/K+-ATPase of the animal gastric mucosa pumps one H+ out of the cell for every one K+ in, so thereis no net movement of charge across the membrane. Therefore, the H+/K+-ATPase is an electroneutral pump. In the plasma membranes of plants, fungi, and bacteria, as well as in plant tonoplasts and other plant and animal endomembranes, H+ is the principal ion that is electrogenically pumped across the membrane. The **plasma membrane H+-ATPase** generates the gradient of electrochemicalpotentials of H+ across the plasma membranes, while the **vacuolar H+-ATPase** and the **H+-pyrophosphatase** (**H+-PPase**) electrogenically pump prot.

In the plasma membranes of plants,fungi, and bacteria,as well as in plant tonoplasts and other plant and animal endomembranes, H+ is the principal ion that is electrogenicallypumped across the membrane. The **plasmamembrane**. **H+-ATPase** generatesthe gradient of electrochemical potentials of H+ across the plasmamembranes, while the **vacuolar H+-ATPase** and the (**H+-PPase**) electrogenically pump protons into the lumenof the vacuole and the Golgi cisternae.In plant plasma membranes, the most prominent pumpsare for H+ and Ca2+, and the

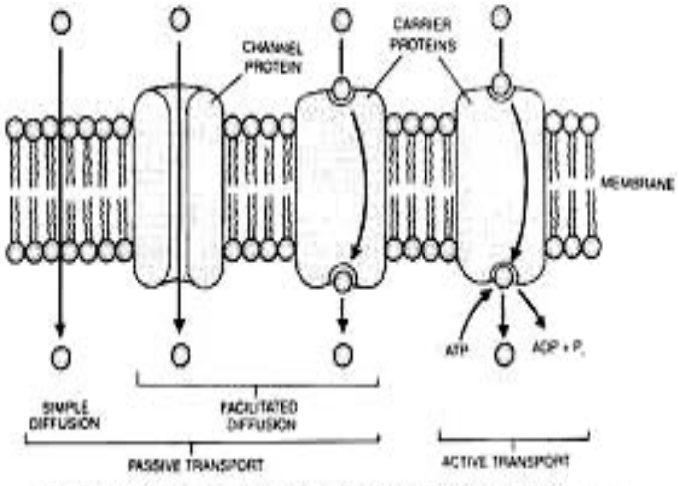


Fig. 11.1. Three ways of exchange of ions and solutes across membranes.

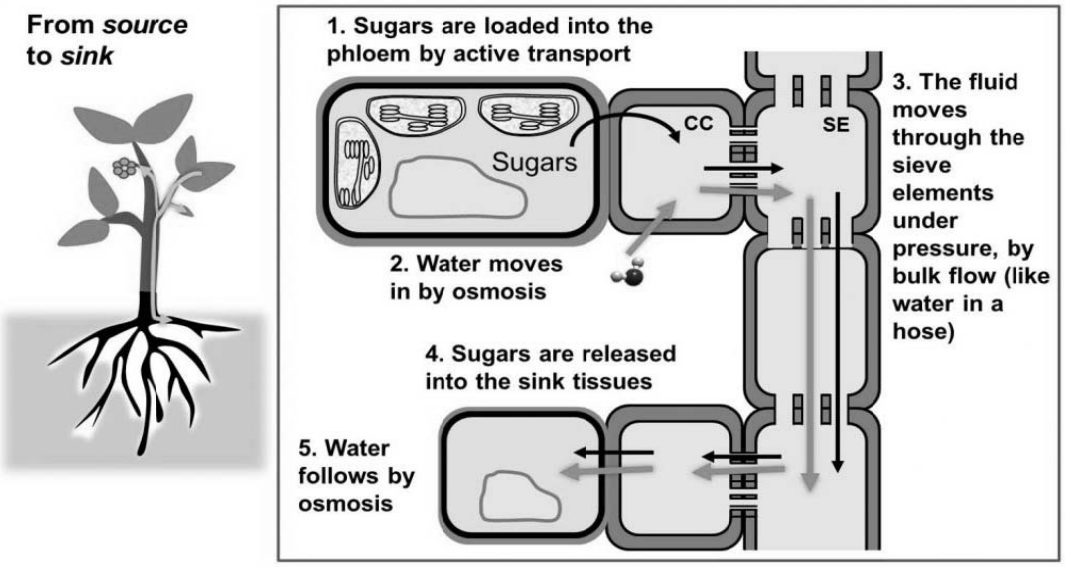
direction of pumping is outward. The other important way that solutes can be actively transported across a cmembrane against their gradient of electrochemical potential is by coupling of the uphill transport of one solute to the downhill transport of another. This type of carrier mediated co-transport is termed **secondary active transport**,

Secondary Active Transport Uses the Energy Stored in Electrochemical-Potential Gradients

Protons are extruded from the cytosol by electrogenic H+- ATPases operating in the plasma membrane and at the vacuole membrane. Consequently, a membrane potential and a pH gradient are created at the expense of ATP hydrolysis. This gradient of electrochemical potential for H+, m_{H^+},or (when expressed in other units) the **proton motive force** (**PMF**), or p, represents stored free energy in the form of the H+ gradient. The proton motive force generated by electrogenic H+ transport is used in secondary active transport to drive the transport of many other substances against their gradient of electrochemical potentials. Figure 6.9 shows how secondary transport may involve the binding of a substrate (S) and an ion (usually H+) to a carrier protein, and a conformational change in that protein. There are two types of secondary transport: symport and antiport. The **symport** (and the protein involved is called a *symporter*) because the two substances are moving in the same direction through the membrane. **Antiport** (facilitated by a protein called an *antiporter*) refers to coupled transport in which the downhill movement of protons drives the active (uphill) transport of a solute in the opposite direction.In both types of secondary transport, the ion or solutebeing transported simultaneously with the protons is moving against its gradient of electrochemical potential, so its transport is active. However, the energy driving this transport is provided by the proton motive force rather than

Solutes Move through Both Apoplast and Symplast

Thus far, our discussion of cellular ion transport has not included the cell wall. In terms of the transport of small molecules, the cell wall is an open lattice of polysaccharides through which mineral nutrients diffuse readily. Because all plant cells are separated by cell walls, ions can diffuse across a tissue (or be carried passively by water flow) entirely through the cell wall space without ever entering a living cell. This continuum of cell walls is called the *extracellular space*, or *apoplast*. We can determine the apoplastic volume of a slice of plant tissue by comparing the uptake of ^{3}H-labeled water and ^{14}C-labeled mannitol. Mannitol is a non-permeating sugar alcohol that diffuses within the extracellular space but cannot enter the cells. Water, on the other hand, freely penetrates both the cells and the cell walls. Measurements of this type usually show that 5 to 20% of the plant tissue volume is occupied by cell walls.



Ions Moving through the Root Cross Both Symplastic and Apoplastic Spaces

Ion absorption by the roots is more pronounced\in the root hair zone than in the meristem and elongation zones. Cells in the root hair zone have completed their elongation but have not yet begun secondary growth. The root hairs are simply extensions of specific epidermal cells that greatly increase the surface area available for ion absorption. An ion that enters a root may immediately enter the symplast by crossing the plasma membrane of an epidermal cell, or it may enter the apoplast and diffuse between the epidermal cells through the cell walls. From the apoplast of the cortex, an ion may either cross the plasma membrane of a cortical cell, thus entering the symplast, or diffuse radially all the way to the endodermis via the apoplast. In all cases, ions must enter the symplast before they can enter the stele, because of the presence of the Casparian strip. The apoplast forms a continuous phase from the root surface through the cortex. At the boundary between the vascular cylinder (the stele) and the cortex is a layer of specialized cells, the endodermis. A suberized cell layer in the endodermis, known as the Casparian strip, effectively blocks he entry of water and mineral ions into the stele via the apoplast. Once an ion has entered the stele through the symplastic connections across the endodermis, it continues to diffuse from cell to cell into the xylem. Finally, the ion reenters the apoplast as it diffuses into a xylem tracheid or vessel element. Again, the Casparian strip prevents the ion from diffusing back out of the root through the apoplast. The presence of the Casparian strip allows the plant to maintain a higher ionic concentration in the xylem than exists in the soil water surrounding the roots.

Xylem Parenchyma Cells Participate in Xylem

The movement of molecules and ions from one location to another is known as transport. Plants exchange solutes and water with their environment and among their tissues and organs. Both local and long-distance transport processes in plants are controlled largely by cellular membranes. Forces that drive biological transport, which include concentration gradients, electric-potential gradients, and hydrostatic pressures, are integrated by an expression called the electrochemical potential. Transport of solutes down a chemical gradient (e.g., by diffusion) is known as passive transport. Movement of solutes against a chemical potential gradient is known as active transport and requires energy input. The extent to which a membrane permits or restricts the movement of a substance is called membrane permeability. The permeability depends on the chemical properties of the particular solute and on the lipid composition of the membrane, as well as on the membrane proteins that facilitate the transport of specific substances. When cations and anions move passively across a membrane at different rates, the electric potential that developsis called the diffusion potential. For each ion, the relationship between the voltage difference across the membrane and the distribution of the ion at equilibrium is described by the Nernst equation. The Nernst equation shows that atequilibrium the difference in concentration of an ion between two compartments is balanced by the voltage difference between the compartments. That voltage difference, or membrane potential, is seen in all living cells because of the asymmetric ion distributions between the inside and outside of the cells. The electrical effects of different ions diffusing simultaneously across a cell membrane are summed by the Goldman equation. Electrogenic pumps, which carry out active transport and carry a net charge, change the membrane potential from the value created by diffusion. Membranes contain specialized proteins—channels, carriers, and pumps—that facilitate solute transport. Channels are transport proteins that span the membrane, forming pores through which solutes diffuse down their gradient of electrochemical potentials. Carriers bind a solute on one side of the membrane and release it on the other side. Transport specificity is determined largely by the properties of channels and carriers. A family of H+-pumping ATPases provides the primary driving force for transport across the plasma membrane of

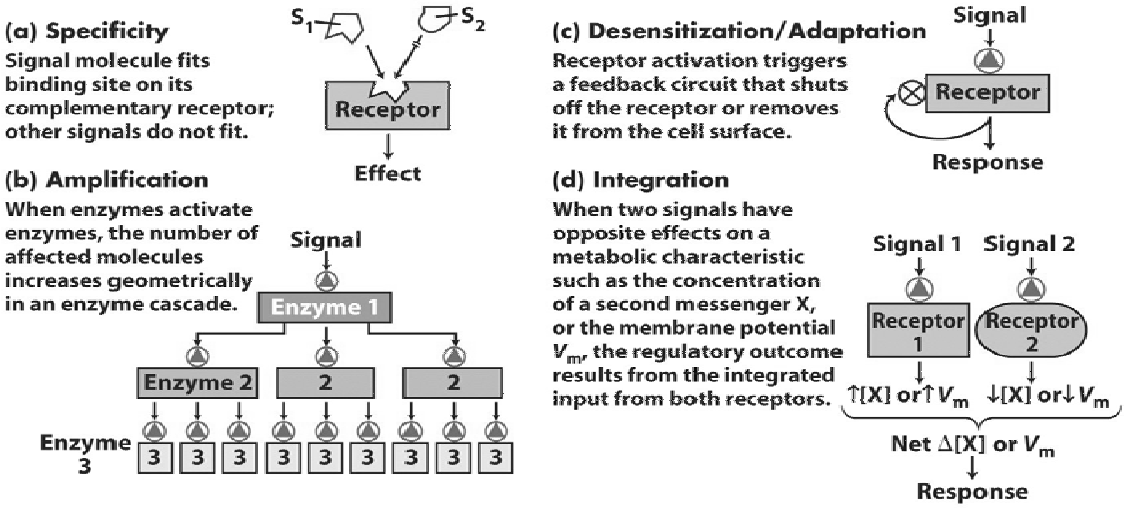
plant cells. Two other kinds of electrogenic proton pumpsserve this purpose at the tonoplast. Plant cells also have calcium- pumping ATPases that participate in the regulation of intracellular calcium concentrations, as well as ATP binding cassette transporters that use the energy of ATP to transport large anionic molecules. The gradient of electrochemical potential generated by H+ pumping is used to drive the transport of other substances in a process called secondary transport. Genetic studies have revealed many genes, and their corresponding transport proteins, that account for the versatility of plant transport. Patch clamp electrophysiology provides unique information on ion channels, and it enables measurement of the permeability and gating of individual channel proteins .Solutes move between cells either through the extracellular spaces (the apoplast) or from cytoplasm to cytoplasm(via the symplast). Cytoplasms of neighboring cells are connected by plasmodesmata, which facilitate symplastic transport. When an ion enters the root, it may be taken up into the cytoplasm of an epidermal cell, or it may diffuse through the apoplast into the root cortex and enter the symplast through a cortical cell. From the symplast, the ion is loaded into the xylem and transported to the shoot.

6. Signal transduction: Signal transduction in eukaryotes.

Signal transduction is the process by which a chemical or physical signal is transmitted through a cell as a series of molecular events, most commonly protein phosphorylation catalyzed by protein kinases, which ultimately results in a cellular response. Proteins responsible for detecting stimuli are generally termed receptors, although in some cases the term sensor is used. The changes elicited by ligand binding (or signal sensing) in a receptor give rise to a biochemical cascade, which is a chain of biochemical events as a signaling pathway.

When signaling pathways interact with one another they form networks, which allow cellular responses to be coordinated, often by combinatorial signaling events. At the molecular level, such responses include changes in the transcription or translation of genes, and post-translational and conformational changes in proteins, as well as changes in their location. These molecular events are the basic mechanisms controlling cell growth, proliferation, metabolism and many other processes. In multicellular organisms, signal transduction pathways have evolved to regulate cell communication in a wide variety of ways.

Characteristics of Signal Transduction:



1. RECEPTORS:

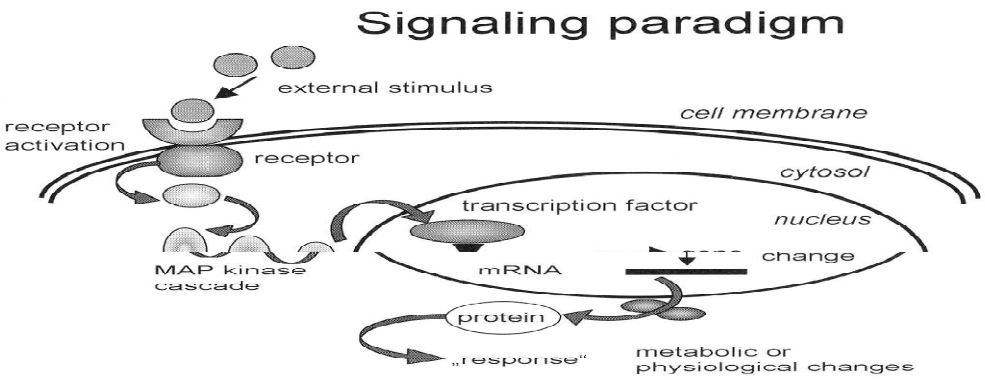


Fig. 6.1 Visualization of the signaling paradigm (for description, see text). The receptor is stimulated by a ligand or another kind of signal, and it changes its own state from susceptible to active. The active receptor initiates the internal signaling cascade, including a series of protein phosphorylation state changes. Subsequently, transcription factors are activated or deactivated. The transcription factors regulate the transcription rate of a set of genes. The absolute amount or the relative changes in protein concentrations alter the state of the cell and trigger the actual response to the signal.

Simple receptor activation

• The simplest mechanism is the reversible binding of the ligand L to the receptor R to form the complex RL $R + L = \leftrightarrow RL$

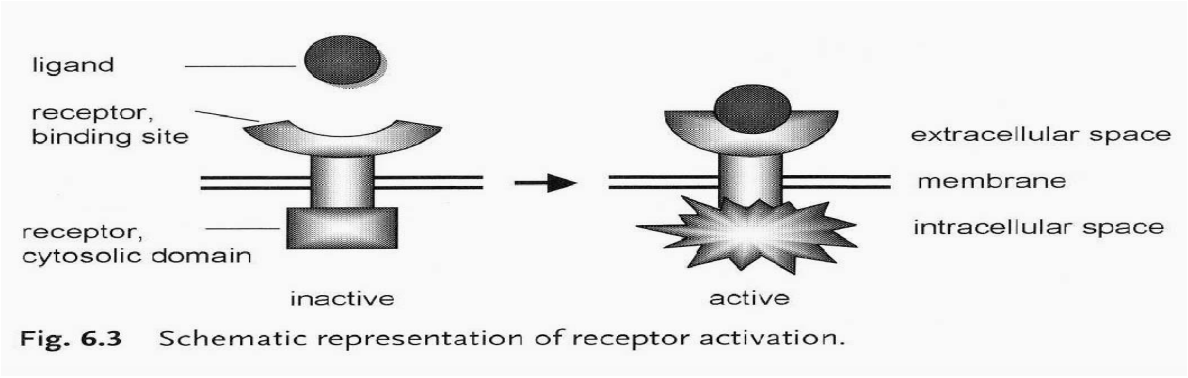


Fig. 6.3 Schematic representation of receptor activation.

The Signalling System:

Signalling system is very complex which may be compared to electronic circuits. You know that electronic system is such that can integrate, modulate and amplify inputs and generate output signals when switched on or switched off after getting suitable signals.

The signalling systems include few basic type of modules. There are four main processes, but the signalling system uses the one or more processes.

The types of signalling modules used in intracellular signalling are:

(a) Receptor kinases (e.g. tyrosine kinase, serine kinase, histidine kinase),
(b) Receptor non-kinases (e.g. serpentine, cytokine, His-Asp phosphorelay),
(c) Protein kinase (intracellular enzymes e.g. cycline families, Asp kinase),
(d) Lipid modifying intracellular enzymes (e.g. p13K, p15K, PLC),
(e) Cyclic nucleotides (e.g. cAMP, cGMP), and
(f) Metal ions (e.g. Ca^{++}).

The four main processes are
These are:
(a) Protein phosphorylation by kinases,
(b) Small molecule and protein interaction, often involving phosphates,
(c) Protein-protein interaction, often mediated by common motifs (specific protein sequences) which frequently results in membrane recruitment when one component is tethered to a membrane, and
(d) Protein and DNA interaction that promotes gene expression or gene inhibition.
When many signals are interconnected in a series or parallel, the intracellular signalling becomes complex. General principles of these modules, the way of processing and onward transmission of signals in prokaryotes and eukaryotes are the main topics of discussion.

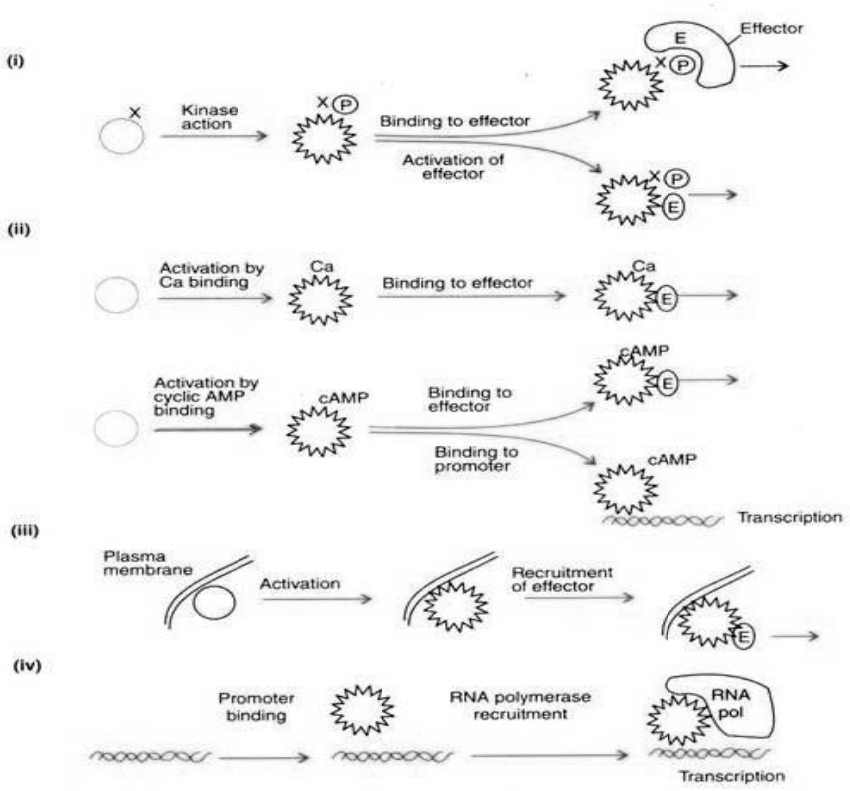


Fig. 27.8: Signalling molecules that use different types of interactions. *(i)* protein phosphorylation, where X=Tyr, Ser, Thr, His or Asp, *(ii)* interaction between small molecules and proteins, e.g. Ca^{++}, or cAMP, *(iii)* interaction between protein and protein, and *(iv)* interaction between protein and DNA which regulates transcription.

The Basic Building Blocks used in Signalling:
(a) Protein Phosphorylation:
Protein phosphorylation is closely linked to cellular signaling. It exits in all signalling modules. The terminal y-phosphate is directly transferred from ARP (in some cases) to an acceptor protein by a protein kinase. The activity of the acceptor is modified example mitogen-activated protein (MAP) kinases in eukaryotes and histidyl-aspartyl phosphorelay in bacteria.
In some cases, indirect phosphorylation of protein also occurs (e.g. in G protein when binding of GTP activates their function, while GDP binding inactivates). There are secondary messengers which are used in intracellular signalling such as phosphorylated inositol's or cyclic nucleotides (cAMP, cGMP).
Kinases are regulated by any of a number of mechanisms: threonine and/or tyrosine phosphorylation, legend occupancy resulting in autophosphorylation or interaction with small molecules (e.g. cAMP or Ca^{++}).

i. Histidine Kinases:

These are found in bacteria, lower eukaryotes and plants as transmembrane protein. They are stimulated to undergo self-phosphorylation by ligand occupancy.

ii. Protein Phosphatases:

Proteins which remove phosphate groups from proteins are called protein phosphatases. Protein kinases add phosphate group to proteins and play a key role in activation of signals.

Specific phosphatases e.g. dephosphorylate phosphotyrosine and phosphoserine/phosphothreonme play a key role in control of proliferation, differentiation and cell cycle Phosphoproteins take part in signalling. They moderate the phosphorylation status by regulating the balance of phosphatases and kinases.

(b) Nucleotide-Binding Proteins:

The three nucleotides (GTP, cGMP and cAMP) play a major role in the intracellular signalling.

iii. GTP-Binding Proteins:

There is a set of eukaryotic proteins (G proteins) that show GTPase activity. They bind to GTP and remove the terminal phosphate of GTP and produce GDP bound to G protein. This cycle operates similar to ATP and ADP cycles. When GDP dissociates from the G protein and GTP binds again, the cycle is repeated. G proteins are of two type: the heterotrimeric G proteins (the dominating proteins), and the small G proteins or membrane of Ras super family (the intermediate member of the signalling pathways).

The heterotrimeric G proteins consist of three different subunits- α, β and y subunits The a-subunit has GTP-binding domain; hence Gβ has a role in signal transduction.

The βy subunits transmit signals by non-covalent interaction with effector molecules. Activation of G proteins and Association of α-subunit from βy subunits are given in Fig. 27.9. GTPase activity results in after binding the Gα subunit with GDP and subsequent association with Gβy and down regulation.

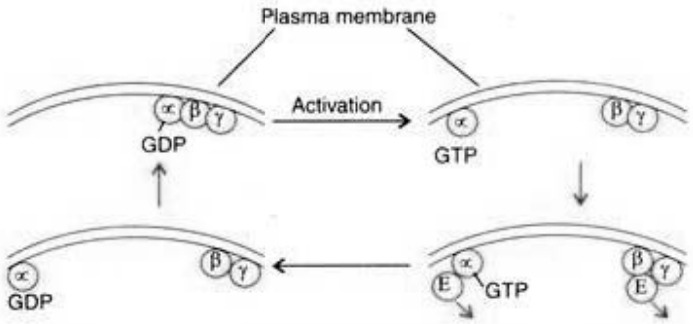


Fig. 27.9 : The function of membrane-bound heterotrimeric G proteins having α, β and γ subunits.

The small G proteins (Ras super family or p21 family) play a key role in many cellular functions such as proliferation and differentiation (Ras family), cytoskeletal organisation (Rho) and nuclear membrane transport (Ran). The activity of small G proteins is modulated after interaction with several classes of proteins. GDP-dissociation inhibitors (GDI) inhibit the loss of bound GDP and keep the G proteins in an inactive form to attenuate signalling from the activated G proteins. GTPase activity IS stimulated by GTPase-activating proteins (GAP). The removal of the bound GDP IS helped by guanine nucleotide exchange factors (GEF) which enable the GTP to bind and activate G proteins. Some of these factors have shown to be proto-oncogenes.

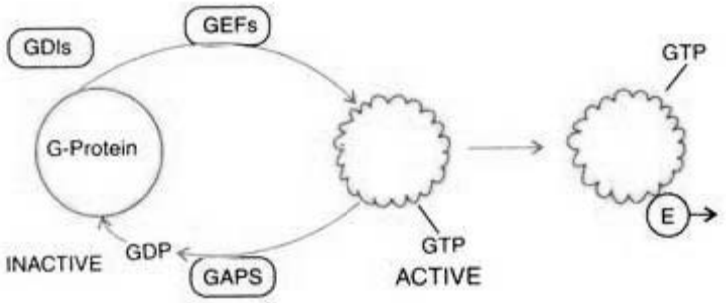


Fig. 27.10 : Functioning of small G proteins.

iv. Cycline Nucleotide-Binding Proteins:

In 1950s, cAMP was identified as the first intracellular signalling molecules. It mediates hormone action and acts as molecules transmitting the primary signal that has been received at the cell membrane). The cAMP mediates the response to chemo-attractants. The adenylate cyclase and guanylate cyclase regulate the concentration of cAMP and cGMP, respectively. The soluble bacterial adenylate cyclases produce cAMP which binds to c AMP receptor protein (CRP) and activate them. CRP is a transcription factor. The cAMP influences the expression of many of genes. Consequently bacteria become able to express metabolic enzymes which are required during growth. The cAMP also regulates the expression of the other genes which can cause pathogenesis. In eukaryotes heterotrimeric G proteins regulate the membrane-bound adenylate cyclases which produce cAMP. G proteins are coupled to transmembrane receptors. The cAMP-dependent protein kinases (protein kinase A) are the main effects of the cAMP signals. While in the inactive form, protein kinase A consists of a dimer of regulatory (A) subunits and two catalytic (C) sub- units. The molecules of cAMP binds to reach R subunits and induce conformational changes. Consequently activated C subunits are released. This activated protein kinase A phosphorylates many substrates on serine or threonine (Fig. 27.11).

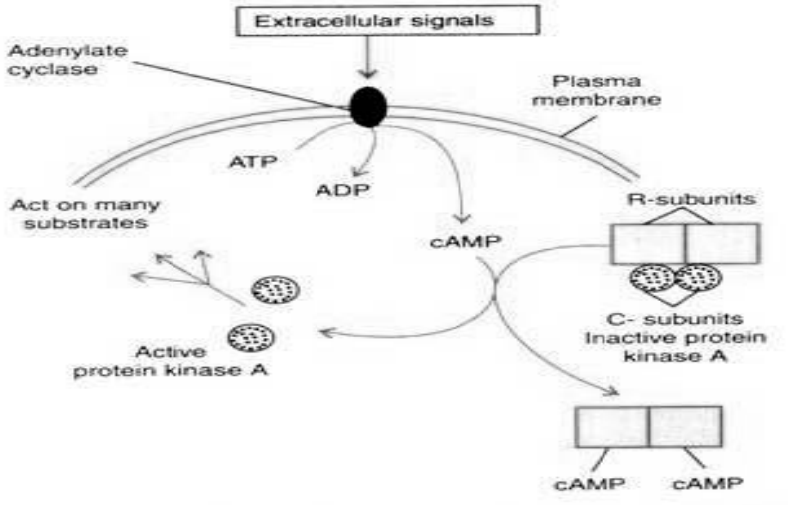


Fig. 27.11 : Production of cAMP and activation of protein kinase A.

Both the cycles work in eukaryotes by direct binding to proteins which form cation channels. Binding events result in opening of the channel. The G-protein-linked cell surface receptor generates small intracellular mediators thought cAMP pathways (Fig. 27.12).

(c) Role of Intracellular Concentration of Ca^{++} in Cell Signalling:

Calcium is found in Cytoplasm and maintained in a very low concentration (10-100 nM). But its concentration varies with cell cycle, exogenous source or release from the stores. It gets complexes in membrane bound vesicles acting as stores. A highly specific protein calmodulin (CaM) binds to Ca^{++} and transmit the signal. Ca-binding to CaM brings about changes in conformation of CaM.

Consequently CaM interacts with many effectors including CaM-modulated kinase. The most extensively studied CaM is the phosphatase calcineurin which is associated with several cellular activities such as NO synthesis, apoptosis, and induction of T lymphocytes. In eukaryotic cells Ca^{++} acts as a second messenger. Fig. 27.12 shows the two major pathways by which G-protein-linked cell surface receptors generate small intracellular mediators.

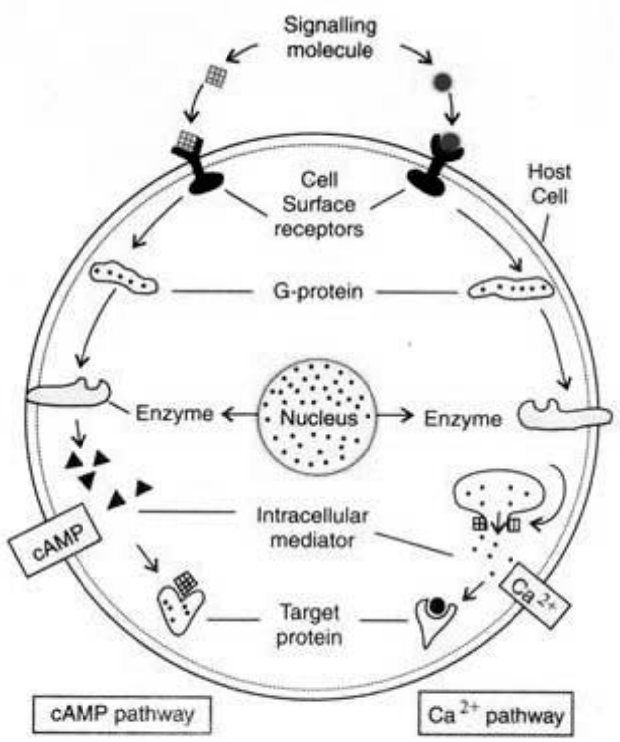


Fig. 27.12 : Generation of small intracellular mediators by G-protein-linked cell.

(d) Role of Phosphorylated Lipids in Cell Signalling:

In eukaryotes lipids are involved in signalling process. Cellular phospholipases attack the lipid moieties of the membrane to produce different types of signalling molecules. For example, phosphatidylinositol lipids play a role in cellular stimulation. They have inositol as head, the six-membered carbon ring with a -OH group on each carbon. On the basis of phosphorylation status of inositol head group, several phosphatidylinositols are found in the cells. The activity of three enzymes triggers their signalling role. These are: phosphoinositide 5′-kinase (P15p, phosphoinositide 3′-kinase (P13K), and phospholipase C (PLC). Extracellular signals regulate all these enzymes.

(e) Regulation of Transcription:

Both types of cells are able to respond to any signal by changing their gene expression. In a signalling pathway the end point acts as signal. Regulators causing changes in expression of many genes in bacteria are called 'global regulators'. In prokaryotes post-transcriptional events regulate expression of many of the transcriptional factors for example cAMP-mediated CRP-DNA interactions. In prokaryotes, phosphorylation or protein-protein interactions regulate the control of transcriptional factors and also select the other factors to the promotors. Besides, some other factors also get translocated from cytoplasm to the nucleus and regulate transcription.

(f) Role of Cell Membrane in Signalling:

Cell membrane acts as boundary of the cell through which extracellular signal has to enter. In bacteria histidine kinases act as receptor and directs signals across the membrane. Besides, there are many signal molecules which are associated with cell membrane because the end effect is membrane-associated. The components can be well organised in three-dimensional way in cell membrane. The signalling components recruit the other molecules to the membrane where they interact with other factors. For example, GTP-bound Ras activates Raf kinase to recruit Raf to the membrane where the membrane-bound kinase activates it through phosphorylation.

3. Prokaryotic Signalling Mechanisms:

Intracellular signalling is very complex like electronic circuit. Genome size of different bacteria varies and those organisms work according to genes present in them.

In bacteria the generic mechanism of regulation is called signalling systems which include:

(a) The histidyl-aspartae phosphorelay systems (the main module of bacteria used to receive and process incoming signals such as chemotaxis, response to osmolarity, oxygen and phosphate, and virulence system),

(b) The cAMP and CRP (involved in regulation of hundreds of genes. The cAMP is controlled at transcriptional and post-transcriptional levels. Binding of CRP- cAMP complex induces gene expression).

4. Eukaryotic Signalling Pathway:

Earlier it was thought that signalling process in eukaryotes was very complex to understand in molecular terms. Fragmented understanding about individual components could be known.

The knowledge of signalling expanded with the development of new techniques such as genome sequencing, increasing number of reagents (isolated components, specific probes e.g. antibodies for individual components and selective inhibitors).

In spite of all these, no pathway has been fully elucidated. The best characterised pathway is the Ras activation and MAP kinases of which several details are unclarified. They are interconnected and cannot work without reference to others.

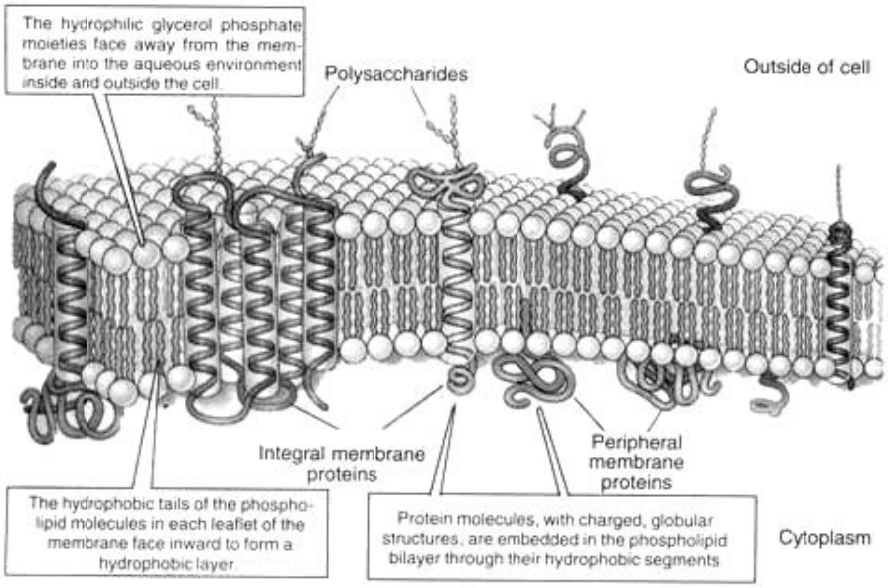
(a) The Ras/MAP Kinase Pathway:

In this pathway the ligand occupancy leads to receptor transphosphorylation, membrane recruitment of GrB2, Sos (son-of-sevenless) where sevenless is the Drosophila (gene), Ras activation and activation of the Ras kinase, and stimulation by phosphorylation of the MAP kinase pathway resulting in various sequels in the cells.

(b) The Phospholipase C/Inositol Triphosphate Pathway:

The phospholipase C, beta or gamma is activated by membrane signalling events and cleaves PIP2 to produce diacylyglycerol (DAG) and inositol triphosphate (IP_3). These activates the release of Ca^{++}ions

and results in activation of protein kinase C (PKC), which phosphorylates many additional protein substrates.



Bacterial cell membrane structure : Extracellular signal has to enter via cell membrane which acts as boundary of the cell.

(c) The Adenylate Cyclase, cAMP and Protein Kinase A Pathway:

Adenylate cyclase is activated at the membrane by interaction with the activated heterotrimeric G protein G_5. The cAMP is generated and binds to and activates protein kinase A (PKA), which phosphorylates many substrates.

(d) Integrin's, the Rho Family and Organisation of Cytoskeletal:

The integrins are the signalling molecules that interact with the extracellular matrix on the outside of the cell and various proteins-linked to actin on the cells interior. The proteins involved include α-actin, lalin, tensin, vinculin and pavilin.

A local adhesion is formed upon activation that includes focal adhesion kinase (FAK). The Src kinase is recruited and several proteins in the complex are activated by phosphorylation by Src and FAC. These signals lead to the Ras/Raf, Rho signalling pathway and to cytoskeletal rearrangement.

In eukaryotes, the central role of signalling pathway of a cell is to define its phenotype and function. The increasing novel knowledge about the components of signalling pathways and the types of genes which they interact are already being applied in new strategy to combat the cancer. For example, genetically engineered viruses are attempted to grow in such cells that lack functional p53 and kill these cells.

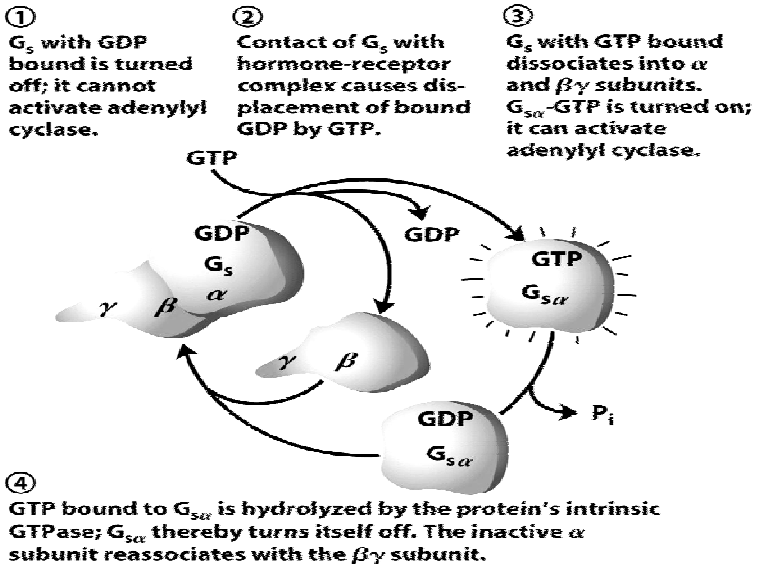
There are about 2000-5000 signal transduction proteins in mammalian cells. Bacteria have capacity to utilise eukaryotic signalling pathway during the process of infection. These findings make a line between the signalling pathways involved in infection and the other responsible for the pathology in diseases such as cancer and inflammation.

STRUCTURAL COMPONENTS OF SIGNALING:

- **G protein cycle**
- **MAP kinase cascade**
- **G-Protein mediated Signaling:**

Guanosine nucleotide-binding protein (G protein)

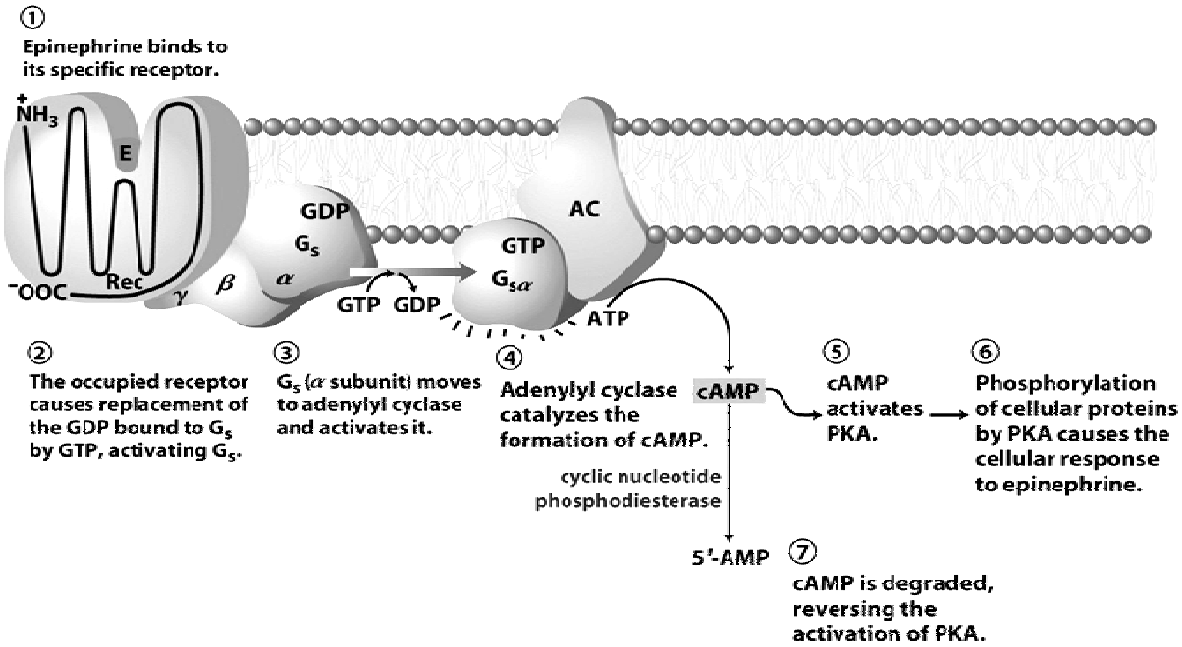
• The **G protein** is an heterotrimer, consisting of 3 different subunits (alpha, beta and gamma)
• The alpha subunit bound to GDP (guanosine di-[two] phosphate) has high affinity for the beta and gamma subunits
• When it is stimulated by the activated receptor, the alpha subunit exchanges bound GDP for GTP (guanosine tri-[three] phosphate)
• The GTP-bound alpha subunit dissociates from the beta/gamma components, and it binds to a nearby enzyme, altering its activity
• GTP is then hydrolysed to GDP (looses a phosphate group) by the intrinsic GTPase activity of the alpha subunit, that regains its affinity for the beta/gamma subunits



The G-Protein cycle

G protein Coupled Receptors

• The human genome encodes more than 1000 Gprotein Coupled Receptors (GPCR), that transduce messages as diverse as light, smells, taste, and hormones
• An example is the beta-adrenergic receptor, that mediates the effects of epinephrine on many Tissues



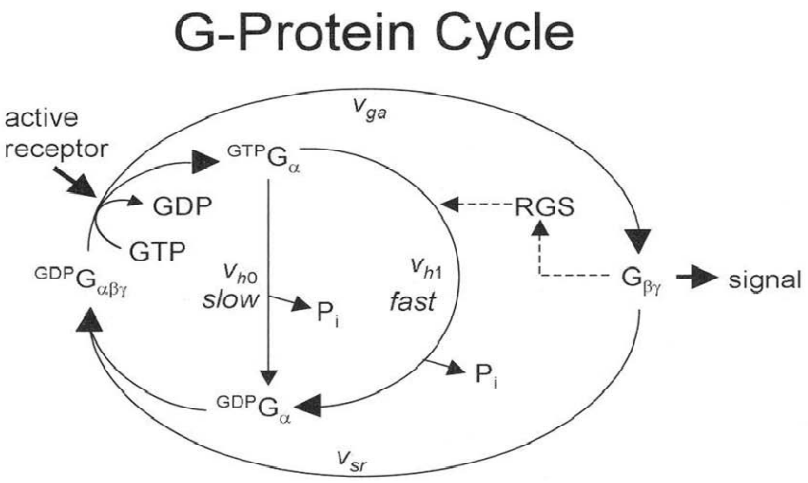


Fig. 6.6 Activation cycle of G protein. Without activation, the heterotrimeric G protein is bound to GPD. Upon activation by the activated receptor, an exchange of GDP with GTP occurs and the G protein is divided into GTP-bound Gα and the heterodimer Gβγ. Gα-bound GTP is hydrolyzed, either slowly in reaction v_{h0} or fast in reaction v_{h1}, supported by the RGS protein. GDP-bound Gα can re-associate with Gβγ (reaction v_{sr}).

Ras Protein and MAPK Pathway

The gene family ras encodes small GTPases that are involved in cellular signal transduction. Ras the super-family of proteins regulates diverse cell behaviors such as cell growth, differentiation and survival. Since Ras communicates signals from outside the cell to the nucleus, mutations in ras genes can permanently activate it and cause inappropriate transmission inside the cell even in the absence of extracellular signals. Because these signals result in cell growth and division, disregulated Ras signaling can ultimately lead to oncogenesis and cancer.

Ras proteins function as binary molecular switches that control intracellular signaling networks. Ras-regulated signal pathways control processes such as actin- cytoskeletal integrity, proliferation, differentiation, cell adhesion, apoptosis, and cell migration. Ras and Ras-related proteins are often deregulated in cancers, leading to increased invasion and metastasis, and decreased apoptosis. Activated Ras activates the protein kinase activity of RAF kinase. RAF kinase phosphorylates and activates MEK. MEK phosphorylates and activates a mitogen-activated protein kinase (MAPK).

Mitogen-activated protein (MAP) kinases are serine/threonine-specific protein kinases that respond to extracellular stimuli (mitogens, osmotic stress, heat shock and pro-inflammatory cytokines) and regulate various cellular activities, such as gene expression, mitosis, differentiation, proliferation, and

Tab. 6.1 Names of the components of MAP kinase pathways in different organisms and different pathways.

Organism	*Budding yeast*		*Xensopus oocytes*	*Human, cell cycle regulation*		
	HOG pathway	Pheromone pathway			p38 pathway	JNK pathway
MAPKKK	Ssk2/Ssk22	Ste11	Mos	Rafs (c-, A- and B-),	Tak1	MEKKs
MAPKK	Pbs2	Ste7	MEK1	MEK1/2	MKK3/6	MKK4/7
MAPK	Hog1	Fus3	p42 MAPK	ERK1/2	p38	JNK1/2

cell survival/apoptosis. MAPK pathways are activated within the protein kinase cascades called “MAPK cascade”. Each one consists of three enzymes, MAP kinase, MAP kinase kinase (MKK, MEKK, or MAP2K) and MAP kinase kinase kinase (MKKK or MAP3K) that are activated in series. A MAP3K that is activated by extracellular stimuli, which phosphorylates a MAP2K on its serine and threonine residues and this MAP2K activates a MAP kinase through phosphorylation on its serine and

tyrosine residues. This MAP kinase signaling cascade has been evolutionary well-conserved from yeast to mammals. Cascades convey information to effectors, coordinates incoming information from other signaling pathways, amplify signals, and allow for a variety of response patterns. Down-regulation of MAP kinase pathways may occur through dephosphorylation by serine/threonine phosphatases, tyrosine phosphatases, or dual-specificity phosphatases and through feedback inhibitory mechanisms that involve the phosphorylation of upstream kinases. Drugs that selectively down-regulate MAP kinase cascades could prove to be valuable as therapeutic agents in the control of malignant disease. Mitogen-activated protein kinases (MAPKs)

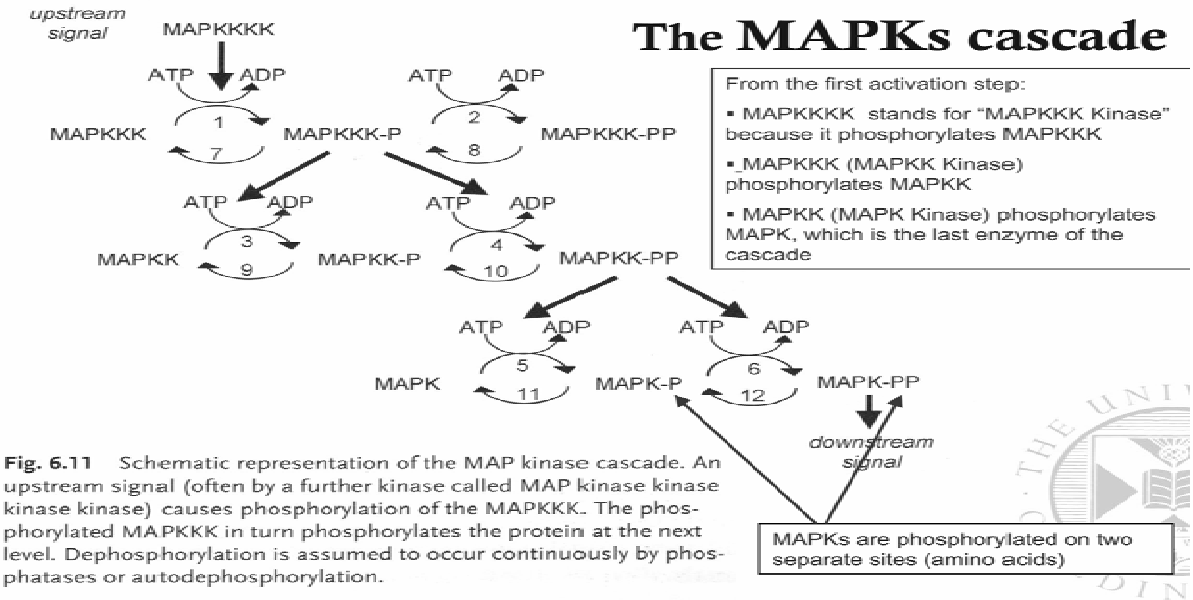


Fig. 6.11 Schematic representation of the MAP kinase cascade. An upstream signal (often by a further kinase called MAP kinase kinase kinase kinase) causes phosphorylation of the MAPKKK. The phosphorylated MAPKKK in turn phosphorylates the protein at the next level. Dephosphorylation is assumed to occur continuously by phosphatases or autodephosphorylation.

•MAPKs belong to a family of serine/threonine kinases (kinases add phosphate groups to/phosphorylate other proteins)

•They participate in cell growth, differentiation, transformation, apoptosis (controlled cell death) and others

•They are conserved from yeast to mammal, but their names differ in different species:

Single Transduction Pathways in Plants:

Plant cells, due to their sessile nature, are able to interact with its surrounding environment. Plants use various environmental signals to alter their mode of developmental morphology. Throughout their life cycle, plant and plant cells respond to both internal and external signals, such as nutrients, organic metabolites, water availability, light, temperature, germination, growth and flowering. Sometimes plants respond to harsh environmental stresses at cellular and molecular level, as well as at physiological levels to confer tolerance of the stress and ensure better survivality. The genome sequences of Arabidopsis and rice have now been determined and have revealed the presence of complex gene families that encode signalling molecules and transcription factors (TFs). There are as many as 1800 genes that encode transcription factor, more than 600 genes that encode protein kinases and major junk of 600 genes that encode F-box proteins particularly in Arabidopsis genome. The participation and stability of signalling factors and TFs is indispensable for the regulation of signal pathways. In addition, post transcriptional regulation at RNA level also leads to various other signalling pathways (Fig. 4.1).

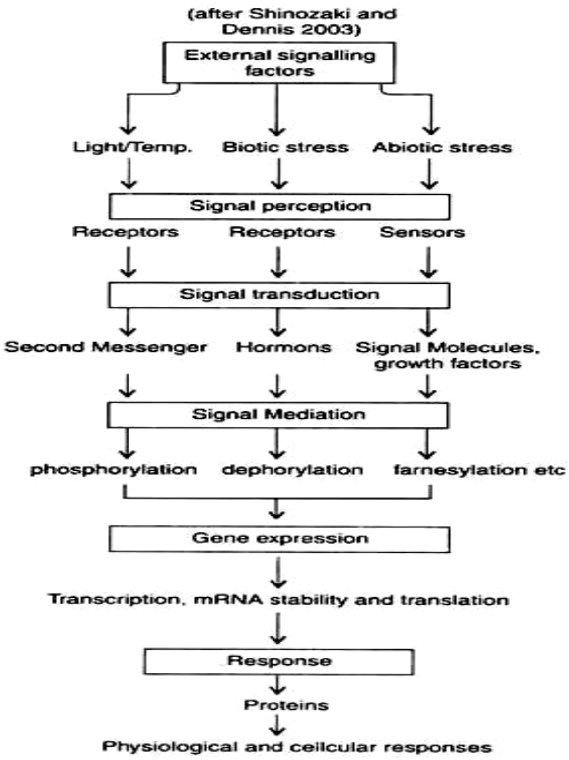


Fig. 4.1 Signal transduction pathways in plants

Mechanism of Signal Transduction:

Generally, signal transduction is initiated by sensing of signal by a receptor. These receptors are either located in the plasma membrane or in the cytoplasm or restricted to cellular compartments. The receptors happen to be a protein. The plasma membrane by virtue of its membrane potential can act as receptor by employing proteinaceous pores, called channels, to control in and out flux of ions through the cell. As a consequence of alteration in membrane potential opens a group of voltage gated channel that allow Ca^{2+} to enter and initiate transduction sequence. Several signals such as light wavelength (red < blue), fungal elicitors or growth regulators can modify membrane potential. Several unique receptors have been characterized in the cells. ome transmembrane protein receptors are phosphorylated by protein kinases. In plants, receptor-like protein consists of a large extra cytoplasmic domain with active site of a protein kinase involves in signal transduction process. Binding of ligand leading to dimerization of the receptor and brings protein domains into very close proximity of the cytoplasm. This receptor complex is then activated by phosphorylation. The active RLK complex interacts with membrane bound or soluble transduction proteins to initiate the signal transduction in a different direction. Several RLKs have been characterised in plant cells including protein kinases which are implicated in incompatibility process and precludes fertilization.

Calcium Signalling in Plants:

The Ca^4 ion is being established as a signalling molecule in plants. Several plant signal transduction processes have been shown to employ Ca^{2+} as an integral signalling molecule. In plants, Ca^+ ion acts as second messenger, a term often used to describe readily diffusible molecules, conveys information from outside to the largest enzymes within the cell.

The cytosolic level of Ca^{2+} plays a significant role in understanding signalling. Several Ca^{2+} signalling mediated responses have been observed in plants. In plants, Ca^{2+} ions in cytosol are maintained at many orders of magnitude lower than in the cell wall. During signalling, elevation of Ca^{2+} level takes place which is associated with initiation of responses. The calcium act on several proteins involved in signalling of which protein kinasesis prominent. Among Ca^{2+} mediated signal responses, prominent ones are initiation of Stomatal aperture closure in guard cell, direction of growth in tubes and wall thickening in seedling in response to wind.

In stomatal guard cell, abscisic acid induces elevation in [Ca^{2+}] and cytochromes were found to be unevenly distributed. The Ca^{2+}signalling in plants is in the form of a Ca^{2+} waves. Increase in Ca^{2+}elevation after exposure to hypo-osmotic stress leads to clustering of Ca^{2+} and is thaliana guard cells showed that SIP modules guard cell turgor by affecting the activities of the plasma membrane K^+ channels and slow anion channels. Sphingosine-1-phosphate mediated changes in guard cell turgor can be transduced via the second messenger Ca^{2+} and through a protein a subunit (GPA1) (Fig. 4.5).

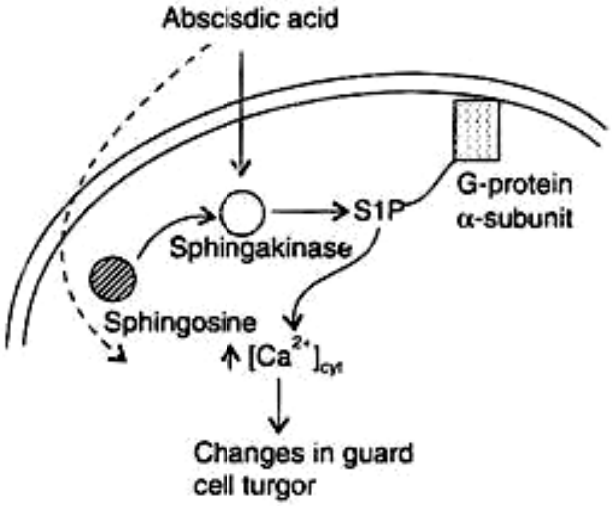


Fig. 4.5 Sphingolipid-mediated signalling in plants

Other Plant Signaling Molecules:

Jasmonate:

Jasmonates are biologically, active signalling molecules controlling metabolic, development and defence response in plants. Jasmonic acid (JA) is synthesised at its terminal end product in octadocanoid pathways, and several intermediates in this pathway for JA synthesis, also act as signalling molecule which affect a variety of plant processes including fruit ripening, production of viable pollen, root growth and biotic as well as abiotic stress, particularly in defence response against insect and pathogen attack.

In addition to JA, its precursor 12- oxophytodienoic acid (OPDA) and other oxylipins act as signal molecule for defence suggests that plant response to pathogen attack may be regulated by a complex mix of signals, otherwise termed as oxylipin signature.

The production of JA leads to the induction of many genes such as vegetative storage protein and a plant defence in. It also facilitates transcription of genes that regulate JA synthesis. Microarray analysis confirmed that at least five out of 41 Jasmonate responsive genes are involved in JA biosynthesis.

In tomato plant, systemic induction of JA response occurs through system in signal pathway. System in, an 18-aminoacid polypeptide acts as primary signal for the activation of defence genes in leaves of wounded tomato plants. System in causes a cascade of intracellular signalling events leading to the release of linolenic acid from plasma membrane. Conversion of linolenic acid into oxylipin signals the expression of defense genes.

Plant Hormone Signaling:

1. Cytokinin Signalling:

Cytokininis have been registered as one of the key plant hormones involved in differential development and other physiological process in plants, such as cell division, root and shoot growth, chlorophyll development, leaf senescence and including biotic and abiotic stress response.

Comprehensive progress has been made in analysing cytokinin perception and signalling. Several painful efforts resulted in the identification of three cytokinin receptor proteins such as GK11, a receptor histidine kinase, CRE_1/AHK_4 histidine kinase and AHK_2/AHK_3 histidine kinase receptor in Arabidopsis due to the availability of its genome sequence.

In addition cytokinis down-stream transmitters like AHPs (Arabidopsis Histidine Phosphotransfer protein) and ARR (Arabidopsis Responsive Regulator) have been characterized. Expression of cytokinin receptors takes place mainly in the roots whereas remaining two is expressed in all other major organs.

Three cytokinin receptors contain two to three trans membrane domains at the N-tranasminal part, transmitter (histidin Kinase) and two receiver domains. The extracellular ligand-binding regions of 210 amino acid long, have drawn special attention as they are the main recognition sites for cytokinins.

These domains are present in variety of functionally diverse membrane receptor proteins that recognise cytokinin like adenine derivations or peptide ligands. This domain has been coined as the CHASE domain (cyclases/histidine-kinase-associated sensory extracellular domain). This domain is specific for AHK_2, AHK_3 and CRE_1/AHK_4 receptors in Arabidopsis.

Cytokinin signal-transduction pathway consists of Arabidopsis five histidine phospho-transmitters (AHPs) and 22 responsive regulators (ARRs). Five AHPs genes encodes proteins of 12 kDa, transmit the signal from the receptor, which is localised in the plasma membrane to ARRs, which are probably present in the nucleus. The ARRs are divided into two major classes, such as A and B-type.

There are 22 predicted responsive regulator (ARR) genes. Presence of receiver domain is the characteristic feature of type A-ARR genes. By contrast, the type B-ARR contains a C-terminal output domain in addition to the receiver domain. Transcription of A- type ARR increases rapidly within 10 min in response to cytokinin. When cytokinin binds to the receptor, it undergoes dimerization and autophosphorylation. Following activation of receptor complex, phosphoryl group is transferred to AHPs which transport the signal from the cytoplasm to type B ARRs in the nucleus.

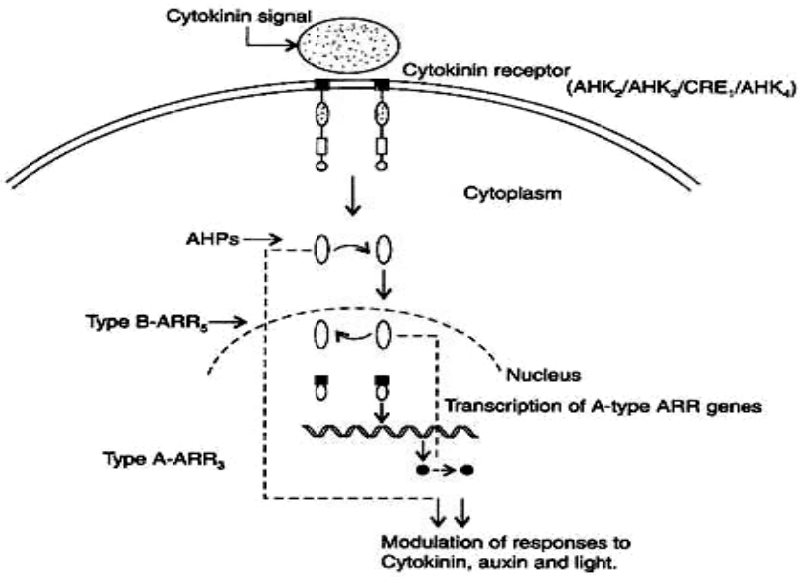


Fig. 4.2 Hypothetical depiction for cytokinin signal transduction. (Reproduced with permission from Elsevier. Copyright 2003. Current Opinion in Plant Biology.)

Following transmission of signal inside the nucleus type-B response regulators transcribe target genes, such as type-A ARR genes. In this signal process, type-A response regulations may down regulate the primary cytokinin signal response via a negative feedback process (Fig. 4.2).

2. Auxin Signalling:

Indole 3 acetic acid (IAA) is a naturally occurring auxin known to involve in cell division elongation or expansion, besides participation in other morphogenesis. During past several decades intensive research have been done in the identification of receptors for the auxin signal. The possible auxin receptor known as auxin-binding protein (ABP_1), has recently been characterized.

In addition, several other auxin binding proteins such as glutathione S-transferase, β-glucanase, and a cytokinin glucosidase are known to express in stressed plants. ABP_1 is a small family of 23 kDa protein that bind to auxins like Indole-3-acetic acid (IAA) and napthalene-1-acetic acid (NAA). ABP_1 contains a C-terminal HDEL endoplasmic reticulum retention domain.

Over expression of ABP_1 in transgenic tobacco evidenced its role of controlling cell expansion. Recent studies have established that AtP_1N_1, a 67 kDa protein is a trans membrane component involved in polar auxin transport. Similarly another member of the auxin efflux carrier family AtP_1N_2 is probably involved in controlling movement of auxin in elongation zone.

3. Gibberellin Signalling:

Gibberellins (GA) are associated with plant growth, seed germination, stem elongation, flowering and fruit development. Besides, it is also involved in the regulation of gene expression in the cereal aleurone layer.

Recent findings on the GA signalling pathways shows GAMYB is a GA-regulated MYB transcription factor, which is involved in the activation of a-amylase expression in barley aleurone cells and anther development. In addition, GAMYB (GA regulated MYB transcription factor) interacts with KGM (KINASE-associated with GAMYB). KGM is a member of protein kinase sub group, represses GAMYB functions in barley aleurone.

Recent studies on GA signalling shows that PHORI (photoperiod-Responsive 1) acts as positive regulator in GA signalling particularly in the expression of ent-Kaurene oxidase gene. When GA binds to an unidentified GA receptor (s), activates G proteins (D_1) that enhance the GA signal.

Enhanced GA signal then mediates PHORI transport into the nucleus, where it acts as a positive regulation by GA signalling. Meanwhile nucleus located protein kinases and GID_1 (GA insensitive dwarf 1) are also activated by GA signal, which in turn trigger GID_2/SLY_1-mediated degradation of DELLA proteins (SLR/RGA). These DELLA proteins directly or indirectly inhibit the expression of

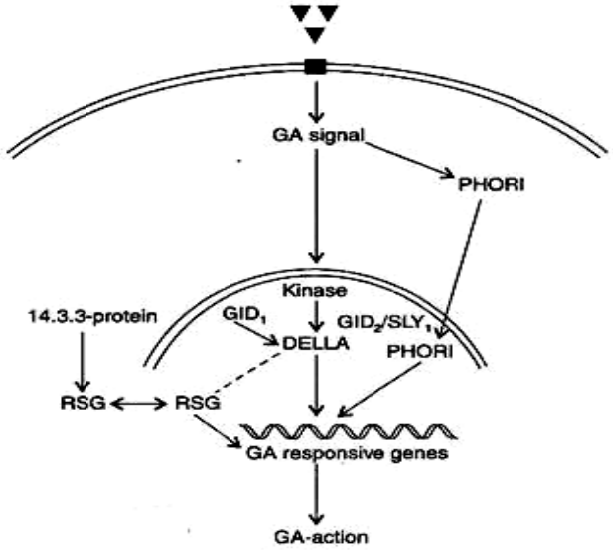


Fig. 4.3 GA signalling shows positive and negative regulation of GA responsive Genes. (After Gomi and Matsuoka, 2003 with permission from Elsevier Copyright 2003.)

GA-induced genes in the absence of GA in cells. Therefore in presence of GA, PHORI regulates expression of GA-responsive genes. Similarly 14.3.3 proteins regulates the sub-cellular localisation of RSG (Repression of Shoot Growth), which in turn controls the expression of the GA-response genes and GA mediated action take

lecules are known to be involved in signalling pathway. These specialised photoreceptors are involved in bundantly synthesised in the dark as inactive form and are degraded upon exposure to light.

Phytochrome undergoes inter convertable form from inactive red (R) light absorbing Pfr form into active far red (FR) absorbing Pfr form after absorption of a light photon. Phytochrome in this active status in turn can be transformed back into the Pr form after absorption of FR. There are five distinct phytochromes in Arabidopsis designated phy A to phy E and play a key role in different photomorphog PIF_3(Phytochrome Interacting Factor). In this process, phy B can bind specifically to PIF_3 that is already bound to light responsive G-box DNA sequence site (CACGTG) and facilitates transcriptional activation of specific gene (Fig. 4.4).

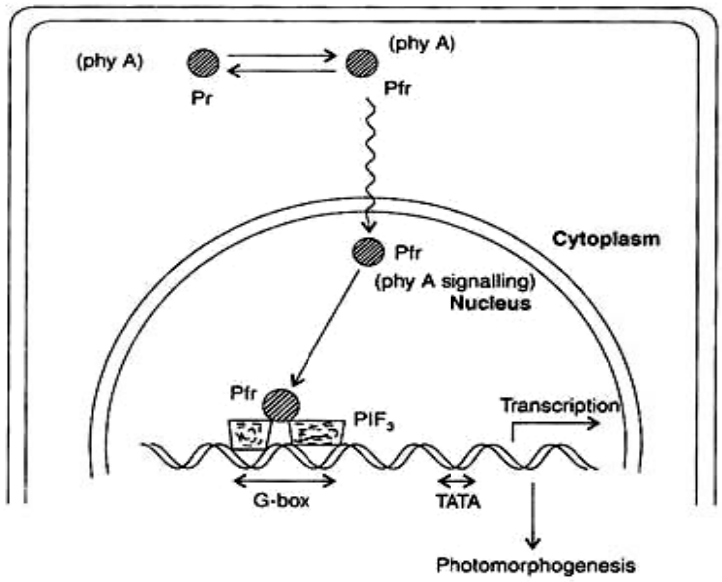


Fig. 4.4 Phytochrome signalling and control of gene expression. (After Wang and Deng, 2003. With permission from Elsevier Copyright 2003. Trends in Plant Science.)

7. Sensory photobiology: Light control of plant development; phytochrome: properties, phytochromeinduced response, phytochrome signaling pathways, blue light responses.

Growth and development of plants are influenced by several environmental factors including light. However, light causes several responses other than photosynthesis. These responses greatly influence the course of plant development and the final plant appearance. They are photomorphogenetic responses. For example, the seeds of many plants do not germinate unless they are exposed to light. Besides photosynthesis in which light is harvested by green plants and is converted- into chemical energy, there are numerous other plant responses to light such as phototropism, germination of some light sensitive seeds e.g. lettuce, de-etiolation of monocot and dicot seedlings etc., which are quite independent of photosynthesis and in which light just acts as environmental signal to bring about the particular photo-response.

Most of these photo-responses control genetically defined structural development or morphogenesis (i.e., origin of form) of plants. The role of light in regulating morphogenesis is known as photo-morphogenesis. In plants, red and blue light are especially effective in inducing a photo-morphogenetic response. The effect of light in controlling morphogenesis can best be demonstrated by comparing a monocot (maize) or dicot (bean) seedling grown in light with one grown in darkness both of which have been reared from genetically identical seeds. It can easily be noticed that dark grown seedling has become etiolated (i.e., pale and weak) while the one grown in light has stockier and green appearance with short stem and large leaf area (Fig. 25.1). Since both etiolated and light grown seedlings were reared from genetically identical seeds, light must have altered the gene expression during germination so that the appearance or form of etiolated and light grown seedlings looks different.

De-etiolation of light grown seedling can be done in very short period (hours) by placing it even in dim light. During de-etiolation, marked reduction in the rate of stem elongation, straightening of apical hook and development of green pigments can easily be noticed. The etiolated form of the seedling is thus gradually transformed to stockier green appearance and is the result of photo-morphogenesis. The development of seedling in darkness is called as skoto-morphogenesis (from Greek word Skotos = darkness).

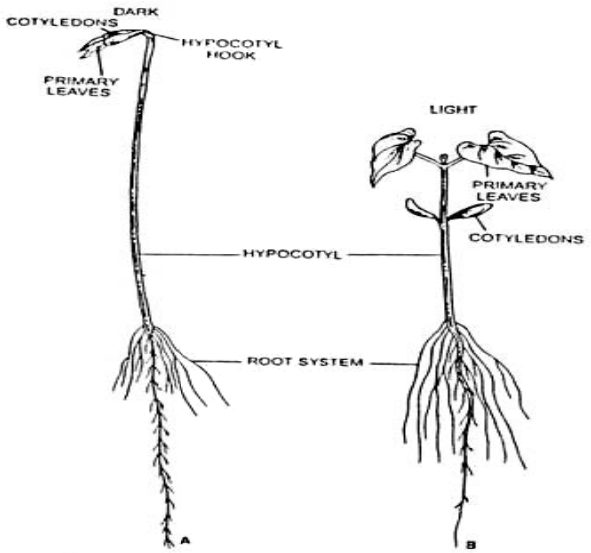


Fig. 25.1. Effect of light on seedling development in bush bean (*Phaseolus vulgaris*)

According to Hans Mohr (1983), there are two important stages of photo-morphogenesis:
(i) Pattern specification, in which cells and tissues develop specific ability or competence to respond to light during certain developmental stage and
(ii) Pattern realization, during which time the photo-response occurs

Photoperiodism:
The discovery of photoperiodism, i.e., the growth response to the length of light and dark periods by Garner and Allard, completely changed the concept of flower initiation. It was demonstrated by experiments that the length of the dark period rather than the light period is the critical factor in the photoperiod response.

As per the result of investigations, It became apparent that a majority of plants fell into one of three categories:

(i) **Short day** (ii) **Long day** (iii) **Day-neutral plants**.

Short-day plants require a dark period exceeding some critical length to flower, and cannot flower under continuous illumination (light). Long-day plants are inhibited from flowering when the dark period exceeds some critical length, and they can flower under continuous illumination.

Day-neutral plants can flower under any night length. In some plants, the leaves need only to be exposed to one light-dark cycle of the proper day length to cause flower initiation, whereas most plants require several or many such cycles. It has been shown that photoperiodic responses may be altered by brief exposure to low light intensity. For example, interruption of the dark period in short-day plants has shown that it is red light (wave length 660 mμ) which is effective. Similarly it has been proved experimentally that, far-red light (wave length 730 mμ) reverses the red light effect, i.e., if exposure to red light is followed by exposure to far-red, the result is as if there had been no exposure at all. If repeated alternating exposures to red and far-red are given, the final exposure determines the response.

Light and Plant Development

Light provides the signal for photomorphogenesis.

Plants detect parts of the light spectrum that are relevant for photosynthesis.

Classes of major plant photoreceptors:

1) Phytochromes: detect red light
2) Cryptochromes: detect blue light
3) Phototropins: detect blue light

Phytochromes

Discovery of Phytochrome:

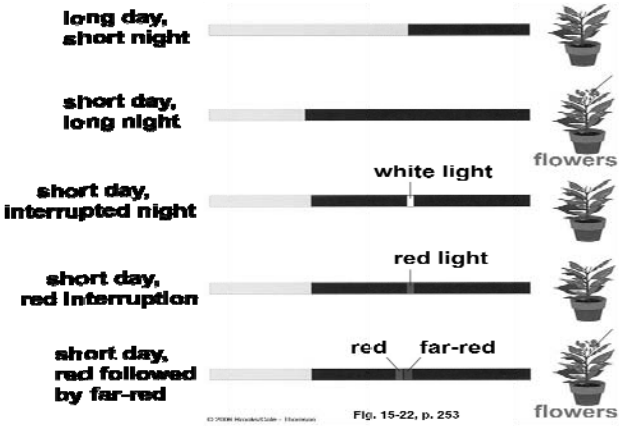
Phytochrome is a blue protein pigment responsible for the perception of light in photo-physiological processes. It is possibly the only photoreceptor in photoperiodism and the flowering process. The discovery of phytochrome is closely associated with studies on flowering. However, many other light controlled plant responses other than photosynthesis, collectively called photo-morphogenesis, are the effects of phytochrome action. In 1932, Beltsville research group of the USDA headed by Borthwick and Hendricks showed that red light (630 to 680 nm) elicits the germination of lettuce seeds, whereas far-red light (710 to 740 nm) inhibits the process.

The phytochrome involvement in the flowering process was envisioned when in 1952. Borthwick. Hendricks and Parker demonstrated that red light inhibition of flowering in Xanthium could be reversed by a subsequent far-red light treatment. The action spectra for inhibition and promotion of flowering shows that the red light near 660 nm and far-red light near 730 nm respectively, are maximally effective.

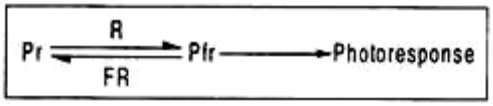
From these observations, Borthwick and his associates concluded that phytochrome, the photoreceptor, exists as two inter-convertible forms, one absorbing red radiation (Pr or P_{660}), the other with an absorption maximum in the far-red region of the spectrum (Pfr or P_{730}).

The Pr form of pigment is converted by red light (R) to the Pfr form, conversely, far-red light (PR) changes the Pfr form of pigment to Pr. The effect of natural white light is like red light (R). Pfr form

of phytochrome, produced by exposure to R, is thought to be the physiologically active form since relatively small amount of Pfr brings about a response.



A simple model of phytochrome action can be represented as follows:



In 1959, Butler and his associates first extracted phytochrome from etiolated oat coleoptiles. It occurs as a chromo-protein in which the chromophore is a linear tetrapyrrole similar to C-phycocyanin.

Phytochrome is widely distributed in the plant kingdom. Although green leaves are the organs, which perceive day light most effectively, their phytochromecontent is very low. For this reason, most studies with phytochrome have used etiolated seedlings from which phytochrome has been obtained in highly purified form. The absorption spectra of the two forms of phytochrome, i.e., P660 and P730 overlap considerably (Fig. 14.1). The overlap is the reason why total photochemical conversion is not possible when irradiated with either red or far-red.

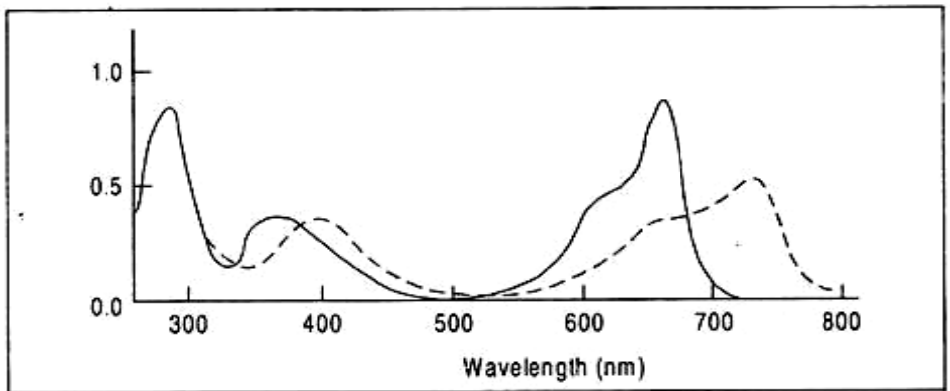
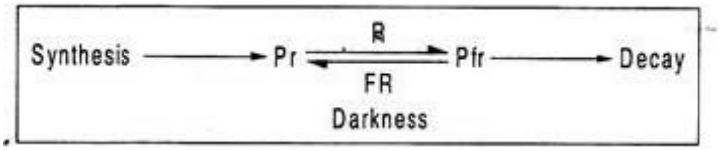


Fig. 14.1 : Absorption spectra of Pr (—) and Pfr (---) froms of isolated phytochrome

Besides photochemical conversions, non-photochemical reactions also occur in vivo. Thus, Pfr may undergo dark reversion to Pr. Since natural white light acts like R, phytochrome will remain mainly in the Pfr form at the end of the day. It has been observed that after several hours of .darkness, plants become sensitive to R indicating that Pfr is present in a large amount. Thus, it is inferred that Pfr is

converted spontaneously to Pr in darkness. Phytochrome decay or destruction is also a dominant irreversible process in a seedling, which is the thermo-chemical transformation of Pfr to an inactive form.

Thus, the model of phytochrome action including synthesis, dark reversion and decay can be presented as:



Structure and Biosynthesis of Phytochrome:

Phytochrome is a soluble chromo-protein with a molecular mass of 250 kDa it occurs as a dimer made up of two subunits, each of 125 kDa. Each subunit consists of two components - light- absorbing pigment molecule, chromophore and a polypeptide chain, Apo protein. The Apo protein monomer has a molecular mass of 125 kDa. Apo protein and chromophore together make up the holochrome. The chromophore is a linear tetrapyrrole similar to phycocyanin termed phytochromobilin and it is a ring attached to the protein through thioether-linkage to a cysteine residue.

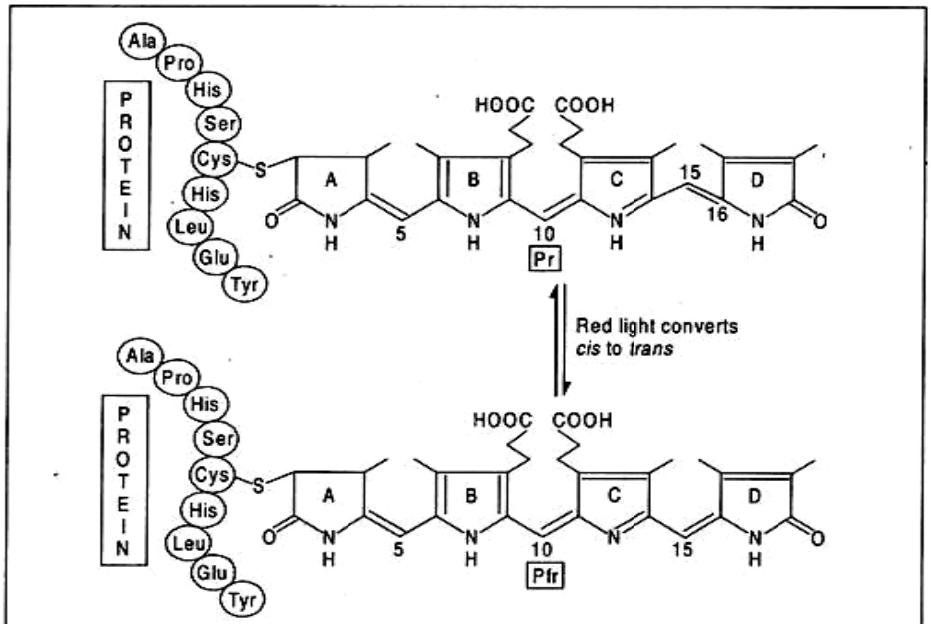


Fig. 14.2 : Structure of the phytochrome chromophore and its binding to the apoprotein. The chromophore is covalently linked to the protein at cysteine residue via a thioether bond. The chromophore undergoes *cis-trans* isomerization at carbon 15 in response to red and far-red light

The principal difference between the Pr chromophore and the Pfr chromophore appears to be a cistrans isomerization of the methane-bridge between rings C and D. The absorption of red light provides the energy required to overcome a high activation energy for rotation around the double bond. There is further evidence that the protein also undergoes photo chemically induced conformational changes.

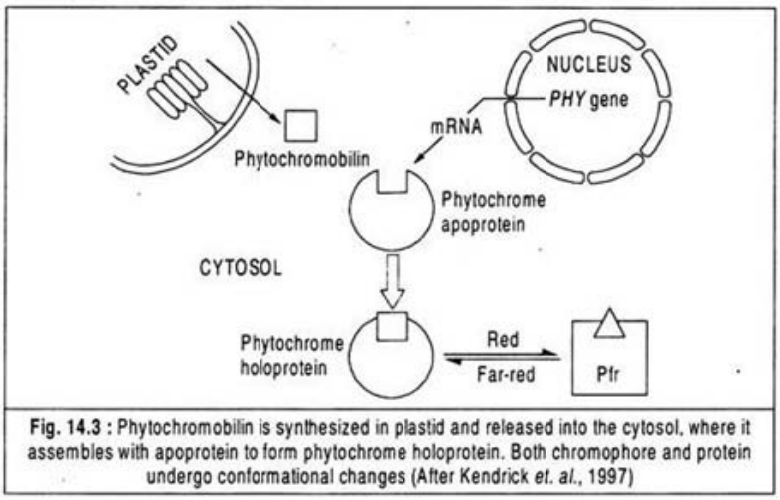


Fig. 14.3 : Phytochromobilin is synthesized in plastid and released into the cytosol, where it assembles with apoprotein to form phytochrome holoprotein. Both chromophore and protein undergo conformational changes (After Kendrick *et. al.*, 1997)

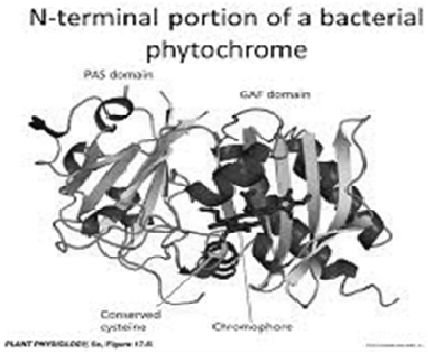
Phytochromobilin is Synthesized in Plastids:

Phytochrome Apo protein alone cannot absorb red or far-red light. Light can be absorbed only when the polypeptide is covalently linked with phytochromobilin to form the holoprotein. Phytochromobilin is synthesized within plastids. After synthesis, it leaks out of the plastid into the cytosol. Assembly of Apo protein with chromophore is autocatalytic, that is, it occurs spontaneously when purified Apo protein is mixed with purified chromophore in test tube, for which no cofactors are necessary. Assembly in vivo of these two components is also autocatalytic (Fig. 14.3).

(a) Phytochrome is encoded by a Multi-gene Family:

Complementary DNA (cDNA) copies of mRNAs were isolated from oat and zucchini (*Cucurbita pepo*) seedlings. Using these clones as probe, five structurally related phytochrome genes were identified in Arabidopsis. This gene family is known as PHY, and its 5 individual members are PHYA, PHYB, PHYC, PHYD and PHYE. The Apo protein without chromophore is also called PHY, and the holoprotein with chromophore is called phy. Phytochrome sequences from other higher plants are named according to their homology with Arabidopsis PHY genes.

- The phytochrome apoprotein alone cannot absorb red or far-red light
- Light can be absorbed only when the polypeptide is covalently linked with phytochromobilin to form the holoprotein.



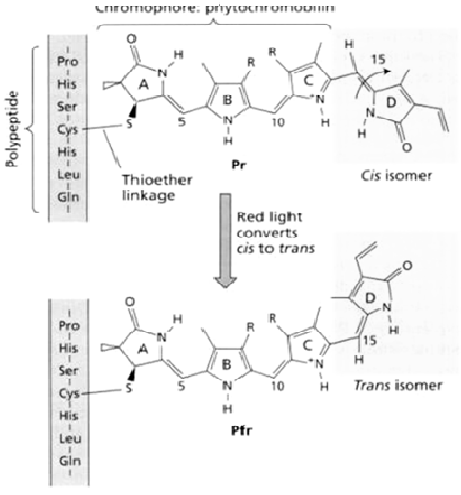
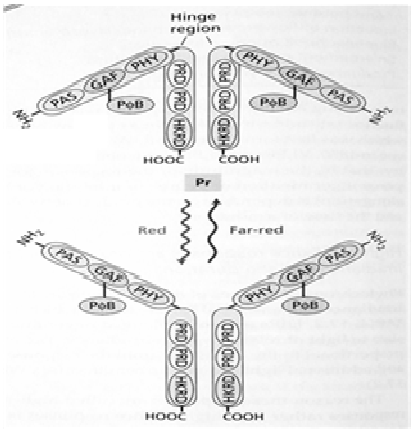


Figure: Structure of Pr and Pfr forms of chromophore (Phytochromobilin) and the peptide region bound to the chromophore through a thioether linkage. The chromophore undergoes a cis-trans isomerization at carbon 15 in response to red and far-red light

Phytochrome structure and function



- Phytochrome functions as a dimer. Each subunit consist of a light-absorbing chromophore covalently linked to a polypeptide chain, the apoprotein. Only the holoprotein can absorb red and far-red light.

There are several conserved domains within the phytochromes:

- **PAS:**(N-terminal domain) The term PAS comes from the first letter of each of the three founding member proteins of the family-PER,ARNT and SIM
- **GAF** (contains bilin-lyase domain): is necessary for autocatalytic assembly of the chromophore with the peptide
- **PHY:** stabilizes phytochrome in the Pfr form.
- A hinge region separates the N-terminal and C-terminal halves and plays a critical role in conversion of PR to Pfr
- **PRD** (PAS-related domain): these domains mediate phytochrome dimerisation.
- **HKRD** (HIS kinase-related domain): This C-terminal domain is essential for autophosphorylation .
- **PφB**, phytochromobilin.

(b) Phytochrome Controlled Responses:

Phytochrome is the photoreceptor involved in many developmental responses of plants to light. It is involved as a light detector and also in the measurement of light duration. The regulatory effects of light on plant growth and development are visualized most prominently at two stages in the life cycle of the plant — firstly, at the stage of seed germination and seedling development, and secondly, at the stage of transition from the vegetative to the flowering phase.

Type I: Fast Responses:

The type I responses include those processes in which the quantum energy absorbed by the plant is transduced to another form of energy. Examples of this type of essentially energy-transducing responses include leaf movement of Mimosa and chloroplast movement in Mougeotia, Other

examples are surface potential changes, membrane potential changes and ion fluxes. These phenomena are relatively rapid, occurring on a time scale of seconds and minutes.

Type II: Slow Responses:

The rates and activation of certain aspects of growth and development are switched on or modulated under the influence of the quality of light (red or far-red). Examples of type II responses include stem elongation, seed germination, hook opening, leaf expansion, flower initiation and pigment biosynthesis.

Type II responses are relatively slow responses occurring on a time scale of hours and days. The phytochrome molecule is thought to act as a photo chrome sensor that controls the photo-morphogenetic machinery of plants.

(c) Variation in Lag Time, Escape Time and Light Quanta for Phytochrome Responses:

Morphological responses to the photo-activation of phytochrome may be visually observed after a lag time ranging from a few minutes (chloroplast rotation in green alga Mougeotia) to as long as a few weeks (flower initiation). It has further been established that red-light induced effects are reversible by far-red light only for a limited period of time after which the response escapes from the photo-reversible control.

Not only the lag and escape times differ in diverse phytochrome responses, but different amounts of light (fluence) are required to induce them. The amount of light is termed **"fluence"** which is defined as the number of photons per unit surface area. Each phytochrome response is characterized by a specific range of light fluences over which the magnitude of response is proportional to the fluence.

These responses can be categorized into three major groups based on their sensitivities to fluence, viz., (a) very low fluence response (VLFR), (b) low fluence response (LFR) and (c) high irradiation response.

(i) Very Low Fluence Responses (VLFRs):

Examples can be provided by Arabidopsis seeds, which can germinate with very low red light. The reciprocal relationship between fluence and time, known as the Bunsen-Roscoe law of reciprocity is valid in case of VLFRs, which, however, fail to show reversal control by light.

This means that a response can be induced either by brief pulse of red light that is quite bright or by a very dim light for a longer duration. Another point of interest is that far-red cannot reverse VLFRs. The reason is that about 3% of the total phytochrome remaining after far-red exposure is sufficient to induce VLFRs.

(ii) Low Fluence Responses (LFRs):

They exhibit characteristic induction with red light and reversion with far-red light. The law of reciprocity, i.e., light-induced response is a function of total fluence (fluence rate x irradiation time) and independent of the fluence rate or irradiation time holds for LFRs. Such responses include classic red/far-red photo-reversible responses, such as promotion of lettuce seed germination and leaf movement.

(iii) High Irradiation Responses (HIRs):

Some photo-morphogenetic responses require prolonged or continuous exposure to light of high irradiance and are proportional to irradiance, but the reciprocity law is not followed here.

Examples of HIRs are:

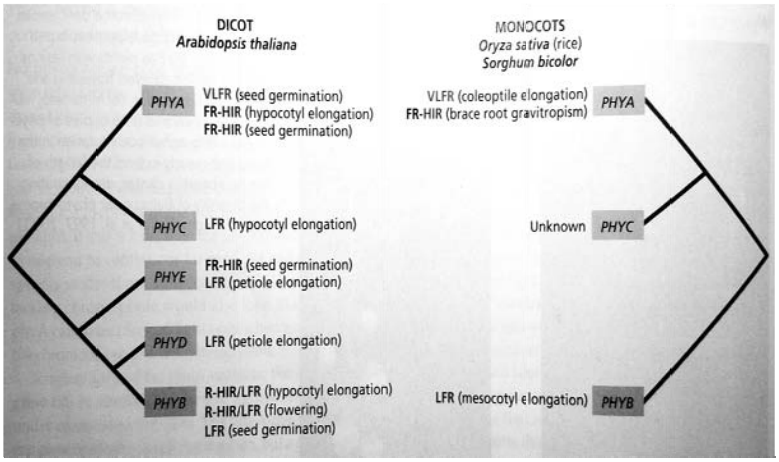
(i) Anthocyanin synthesis in dicot seedlings and apple skin

(ii) Inhibition of seedling elongation (hypocotyl)

(iii) Flower induction
(iv) Plumular hook opening
(v) Cotyledon expansion in mustard
(vi) Ethylene production to Sorghum
(vii) De-etiolation of seedlings. However, the effect is not photo-reversible. The reason that these responses are called high irradiance responses (HIR) rather than high fluence responses (HFR) is that they are proportional to irradiance (i.e., brightness of source) rather than to fluence.

Genetic Analyis of Phytochrome Function

- Phytochrome A mediates responces to continuous far-red light but also mediated red-light responses. It also controls flowering in Arabidopsis and rice
- Phytochrome B mediates responses to continuous red or white light, and regulates responses to shade such as accelerated flowering and increased elongation.
- Phytochromes C, D, and E also have specific developmental roles that may be partially redundant with those of phytochrome A and B.
- The signaling pathway components downstream from phytochromes vary and can lead to different responses in different organs or taxa.



Mode of Action in Phytochrome:
Pfr is regarded as the physiologically active form of phytochrome. Conversion of Pr to Pfr by light will produce a particular response depending on the localization of phytochromeand the state of differentiation of the responding cells. It is also possible that the photo stationary state ratio Pfr/P_{total} acts as a signal perceived by the plant under certain conditions. For example, the HIR response for the inhibition of hypocotyl growth in lettuce can be explained in term of the ratio Pfr/P_{total} = 0.03, i.e., 3% Pfr level is necessary for the HIR response. First step is the absorption of light by the pigment. Then the absorbed light alters the molecular properties of phytochrome, which induces a sequence of cellular events ultimately leading to a change in growth, development or position of an organ. Generally, two consistent lines of evidences are sought to explain the effects of phytochrome. One is concerned with the Pfr effect on changes in the properties of cellular membranes. The second theme is that Pfr regulates gene expression.

Cryptochromes

Apart from phytochrome mediated photo-responses, large number of photo-responses in plants are known which are controlled by blue light and are believed to be mediated through a group of yet unidentified pigments called crypto chrome (crypto from cryptogams), the latter acting as photoreceptor in such responses. Blue light responses have been reported in algae, fungi, ferns and higher plants.

Some of the typical and most commonly known blue-light responses in plants are:

(i) Phototropism
(ii) Stomatal opening
(iii) Inhibition of hypocotyl elongation
(iv) Sun tracking by leaves
(v) Phototaxis
(vi) Movements of chloroplasts within the cells and
(vii) Stimulation of synthesis of carotenoids and chlorophylls etc.

Crypto chrome absorbs light rays mostly in violet-blue region of the spectrum (400 – 500 nm). It also absorbs long wave ultraviolet rays in UV-A region (320 to 400 nm). However, most photo-responses of plants caused by crypto chrome result from absorption in violet-blue region of the spectrum but they are simply called as blue-light responses. Although phytochrome and some other photoreceptors also absorb blue light, but the typical blue-light morphogenetic responses differ from photo-responses mediated by them in being insensitive to red light and there is no red/far- red reversibility.

i. The action spectra of many blue-light responses in higher plants such as phototropism, stomatal movement, inhibition of hypocotyl elongation etc. are similar and characteristic. They show three peaks in blue region (400 – 500 nm) of the spectrum of visible light. This three peaked, action spectrum is also known as three fingers action spectrum (because of its resemblance in shape with three fingers) and is typical of most blue light responses (Fig. 25.3). Three fingers action spectrum is not observed in phytochrome mediated photo-responses or photo-responses mediated by other photoreceptors other than crypto-chrome.

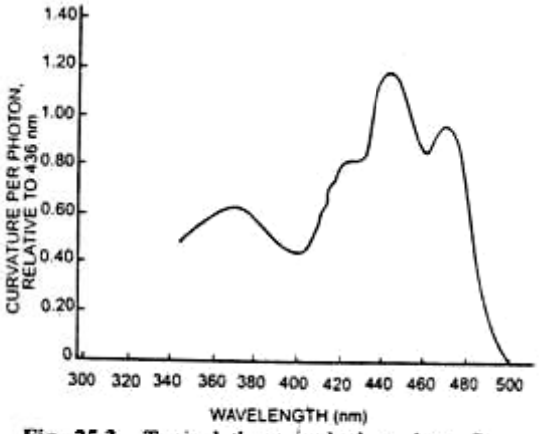


Fig. 25.3. Typical three peaked or three fingers action spectrum for blue light stimulated phototropism in ***Avena*** coleoptile. (After Thimann and Curry, 1960).

ii. Scientists have implicated roles of yellow pigment carotenoids or flavins as photoreceptors in blue-light responses of plants for a long time. However, the spectroscopy of blue-light responses is complex and it is not easy to distinguish between these two types of pigments by comparing available action and absorption spectra.

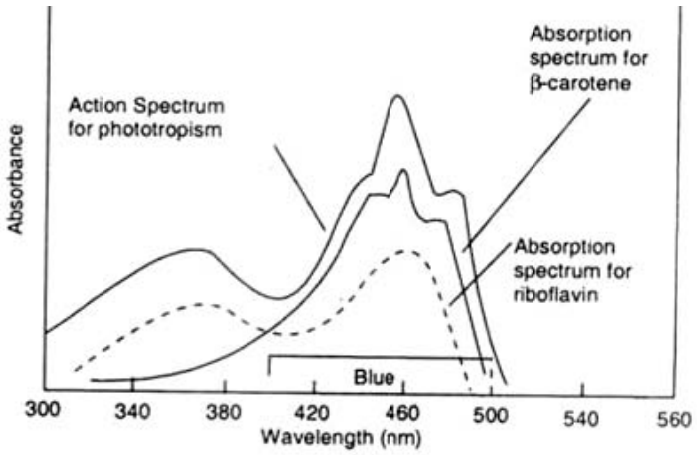


Fig. 25.4. Action spectrum for phototropism compared with the absorption spectra of riboflavin and carotene.

Schmidt (1984) has summarised arguments in favour of flavins or carotenoids as photoreceptor pigments in blue light responses of plants as follows:

(a) Arguments in Favour of Flavins:

(i) Action spectra show UV maximum between 350-400 nm.

(ii) Primary steps of the blue light response are dependent on presence of O_2.

(iii) Flavin reactions are often redox reactions.

(iv) Light can be substituted by oxidants while reductants suppress the blue light reaction.

(v) Blue light reaction is inhibited by flavin inhibitors such as KI.

(vi) Blue light action spectra resemble low temp, spectra of flavins.

(vii) Neurospora mutant which is free of carotenoids shows blue light response.

(viii) Half life of carotenoids in first excited singlet state is very short (10^{-13} seconds)

(b) Arguments in Favour of Carotenoids:

(i) Three peaked (three fingers) action spectra resemble absorption spectra of carotenoids.

(ii) Small or no UV maximum in some action spectra.

(iii) Energy transfer from UV absorbing pigment to carotenoids is feasible.

(iv) Carotenoids from diatom mutant do not show blue light response.

Earlier evidences suggested crypto chrome to be one or both of the yellow pigments, carotenoids (such as β-carotene, zeaxanthin) and/or flavins (such as riboflavin, FAD) which mediate blue-light responses in plants.

However, with extensive researches done with mutants and transgenic plants and over expression studies beginning in early 1990s, the vexed problem of identification of blue-light receptors in plants has gradually been resolved now.

The term crypto chrome is now applied specifically to flavoprotein photoreceptor that mediates inhibition of hypocotyl (stem) elongation caused by blue-light. Blue-light photoreceptor in phototropism and chloroplasts movements in plants is phototropin which is also a flavoprotein. The carotenoid zeaxanthin is blue-light photoreceptor involved in stomatal opening.

Cryptochrome Structure:

The first protein with characteristics of blue-light receptor was isolated in 1993 from Arabidopsis. It was found that hy4 mutant of Arabidopsis had lost the capacity to respond specifically to blue-light in that it showed an elongated hypocotyl even on irradiation with blue-light (In the wild type, blue-light causes inhibition of hypocotyl elongation).

Isolation of the hy4 gene (later named as cryl) showed that it encoded a 75 kDa protein called crypto-chrome 1 (CRY1) with remarkable sequence similarity (homology) to DNA photolyase in having two chromophores: a flavin adenine dinucleotide (FAD) and a pterin attached to the apoprotein (Fig. 25.5). This led to the establishment of cryptochrome to be a flavoprotein that was involved in inhibition of hypocotyl elongation in response to blue-light. (The structure of pterin is given in figure 25.6. For structure of FAD).

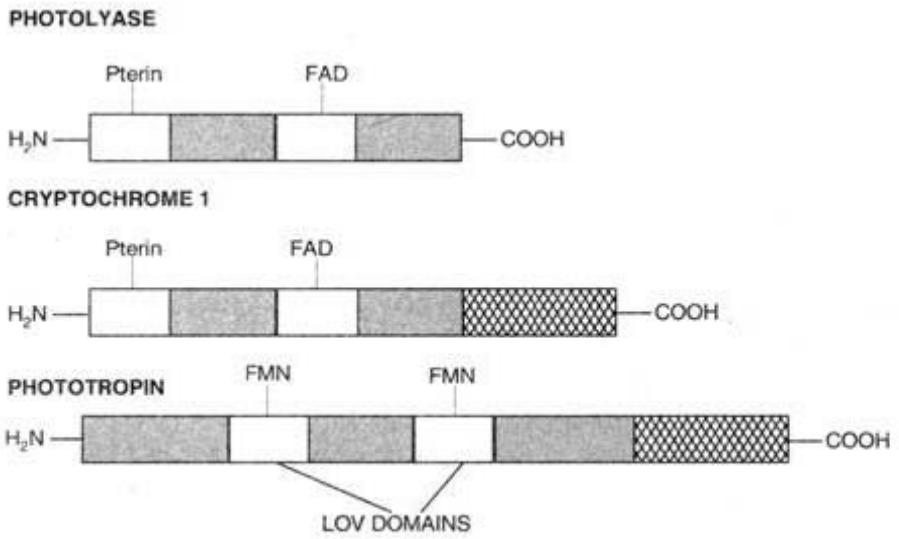


Fig. 25.5. Diagrammatic representation of the chromophore binding domains of microbial photolyase, cryptochrome 1 (cry1) and phototropin. Cross-hatched blocks represent carboxy-terminal domains with kinase activity.

(DNA photolyase is a blue-light activated flavoenzyme which repairs UV-induced damage to microbial DNA. Cryptochrome differs from photolyase mainly in two respects. Firstly, the cryptochrome does not show photolyase activity and secondly, unlike photolyase it has an extended carboxy-terminal domain (Fig. 25.5) with kinase activity).

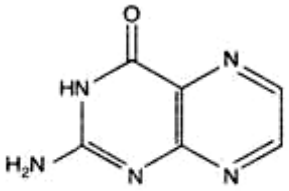


Fig. 25.6. Structure of pterin (fully oxidised)

A second cryptochrome 2 (CRY2) also with two chromophores like CRY1, has also been isolated from Arabidopsis (Lin 2000). CRY2, mediates blue-light stimulated inhibition of hypocotyl elongation, increase in cotyledon expansion and anthocyanin production. It also has a role in determining flowering time. Both CRY1 and CRY2 appear to be ubiquitous in plant kingdom, but while CRY1 is stable in light grown seedling, CRY2 is rapidly degraded in light.

Mechanism of action of cryptochrome:

The mechanism of action of crypto chrome remains elusive so far. The flavins are known to participate in oxidation-reduction reactions and photolyases repair damaged DNA (as a result of UV-radiations) by transferring electrons to pyrimidine dimers. Crypto chromes may act probably in a similar way through some electron transfer mechanism.

Phototropins:

Phototropins are blue-light receptors that mediate phototropism and chloroplasts movements in plants. In late 1980s, it was found that blue-light stimulated phosphorylation of a 120 kDa protein located on plasmamembrane of actively growing regions of etiolated seedlings. These regions were also most responsive to phototropic stimulus. Extensive biochemical and physiological studies showed this protein to be a kinase autophosphorylating in blue-light and which could be the photoreceptor for phototropism.

Later on, a mutant nph1 (won phototropic hypocotyl 1) was isolated from Arabidopsis which lacked phototropic response in the hypocotyl and also the 120 kDa membrane protein. It was genetically independent of the hy4 mutant as it showed blue-light induced inhibition of hypocotyl elongation.

The nph1 gene was cloned and it was found (as postulated) to encode a 120 kDa protein nph1. The nph1 gene was renamed as phot1 and the protein encoded by it was named phototropin (Briggs and Christie, 2002).

Phototropin is also a flavoprotein with two flavin mononucleotide (FMN) chromophores. The protein has a carboxy-terminal domain with a serine/threonine kinase activity. In the amino-terminal half, there are two domains called LOV domains (of about 100 amino acids each) to which are attached the chromophores (Fig. 25.5). (LOV domains are so called because they are characteristics of microbial proteins which regulate response to light, oxygen and voltage).

Recent spectroscopic studies done by Swartz et al, 2000) have shown that in dark, FMN molecules remain non covalently bound to LOV domains, but on irradiations with blue-light they become covalently bound to cysteine residues of the apoprotein through a sulphur atom forming a cysteine-flavin covalent adduct. The reaction is reversed in dark. A second gene called phot 2 has also been isolated from Arabidopsis which is related to phot 1. It is believed that phototropic response involves both phot 1 and phot 2.

Mechanism of action of phototropins:

The mechanism of action of phototropins is not clear. It has been observed that blue-light causes a transient increase in cytosolic calcium concentration and there are indications that phototropin signalling chain may partly involve regulation of cytoplasmic calcium concentration.

Zeaxanthin:

The carotenoid zeaxanthin has been shown to be blue-light receptor in guard cells that plays central role in blue-light stimulated stomatal opening. (See chapter 17 for structure of zeaxanthin).

Following evidences strongly support role of zeaxanthin in stomatal opening:
(i) The absorption spectrum of zeaxanthin closely resembles the action spectrum of blue- light stimulated stomatal opening.
(ii) During stomatal opening in intact leaves, the incident radiation, zeaxanthin concentration in guard cell, and stomatal apertures have been found to be directly correlated.
(iii) Blue-light sensitivity of guard cells increases with an increased concentration of zeaxanthin in guard cells.
(iv) There is complete inhibition of blue-light stimulates stomatal opening by 3mM conc. of dithiothreitol (DTT) which is a potent inhibitor of the enzyme that converts violaxanthin to zeaxanthin.
(v) In facultative CAM plant species such as MeSembryanthemum crystallinum, there is a shift from C_3 to CAM mode of carbon metabolism in response to accumulation of salts. In C_3 mode, the guard cells accumulate zeaxanthin and exhibit blue-light response. But, in CAM mode, neither there is accumulation of zeaxanthin in guard cells nor they respond to blue- light. (In CAM plants, stomata remain closed during the day).

8. Plant hormones and growth regulators: Chemistry, biosynthesis, physiological effects, and signal transduction pathways of auxins, gibberellins, cytokinin, ethylene, abscisic acid, brassinosteroids, polyamines, jasmonates.

In higher plants, regulation and coordination of metabolism, growth, and morphogenesis often depend on chemical signals from one part of the plant to another. This idea originated in the nineteenth century with the German botanist Julius von Sachs (1832–1897).
Sachs proposed that chemical messengers are responsible for the formation and growth of different plant organs. He also suggested that external factors such as gravity could affect the distribution of these substances within a plant. Although Sachs did not know the identity of these chemical messengers, his ideas led to their eventual discovery.
Plants produce signaling molecules, called *hormones* that have profound effects on development at vanishingly low concentrations. Until quite recently, plant development was thought to be regulated by only five types of hormones: auxins, gibberellins, cytokinins, ethylene, and abscisic acid. However, there is now compelling evidence for the existence of plant steroid hormones, the brassinosteroids, that have a wide range of morphological effects on plant development.

Biosynthesis and metabolism of auxin:

Went's studies with agar blocks demonstrated unequivocally that the growth-promoting "influence" diffusing from the coleoptile tip was a chemical substance. The fact that it was produced at one location and transported in minute amounts to its site of action qualified it as an authentic plant hormone.
In the years that followed, the chemical identity of the "growth substance" was determined, and because of its potential agricultural uses, many related chemical analogs were tested. This testing led to generalizations about the chemical requirements for auxin activity. In parallel with these studies, the agar block diffusion technique was being applied to the problem of auxin transport. Technological advances, especially the use of isotopes as tracers, enabled plant biochemists to unravel the pathways of auxin biosynthesis and breakdown.

Multiple Pathways Exist for the Biosynthesis of IAA

IAA is structurally related to the amino acid tryptophan, and early studies on auxin biosynthesis focused on tryptophan as the probable precursor. However, the incorporation of exogenous labeled tryptophan (e.g., [3H]tryptophan) into IAA by plant tissues has proved difficult to demonstrate.

Nevertheless, an enormous body of evidence has now accumulated showing that plants convert tryptophan to IAA by several pathways, which are described in the paragraphs that follow.

The IPA pathway. The **indole-3-pyruvic acid** (**IPA**) pathway (see Figure 19.6C), is probably the most common of the tryptophan-dependent pathways. It involves a deamination reaction to form IPA, followed by a decarboxylation reaction to form indole-3-acetaldehyde (IAld). Indole-3- acetaldehyde is then oxidized to IAA by a specific dehydrogenase.

The TAM pathway. The **tryptamine** (**TAM**) **pathway** (see Figure 19.6D) is similar to the IPApathway, except that the order of the deamination and decarboxylation reactions is reversed, and different enzymes are involved. Species that do not utilize the IPApathway possess the TAM pathway. In at least one case (tomato), there is evidence for both the IPAand the TAM pathways (Nonhebel et al. 1993).

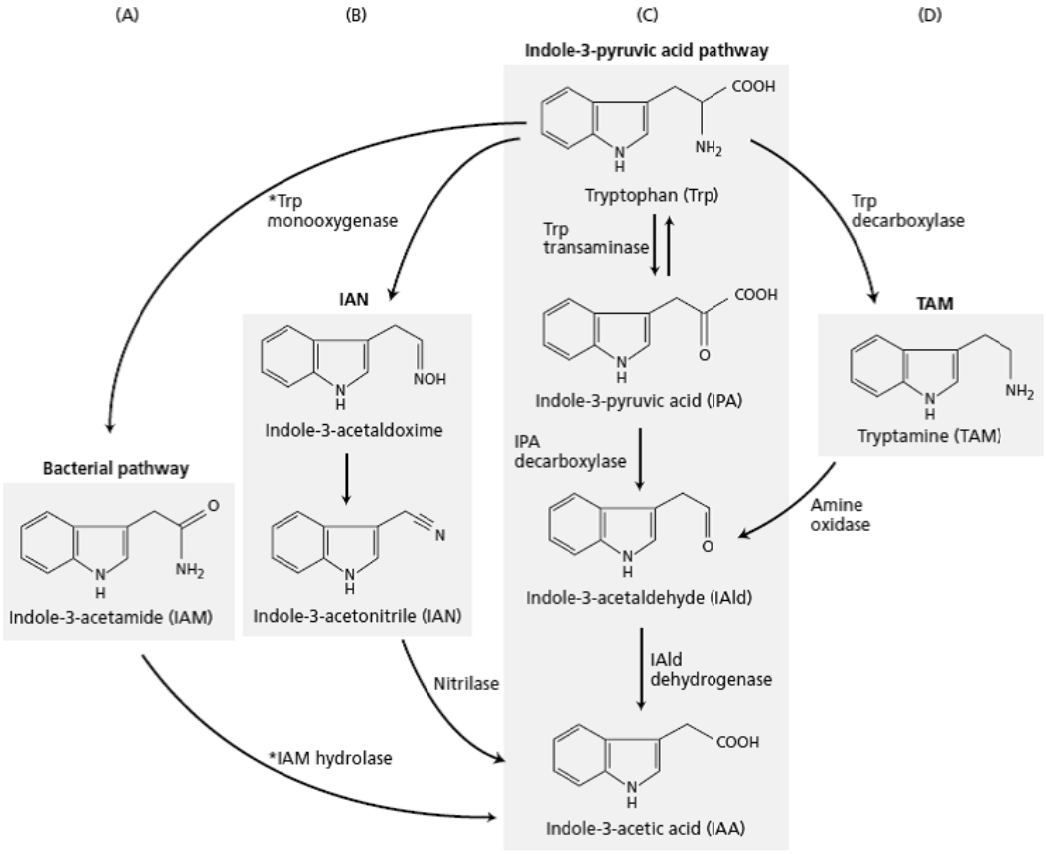


FIGURE 19.6 Tryptophan-dependent pathways of IAA biosynthesis in plants and bacteria. The enzymes that are present only in bacteria are marked with an asterisk. (After Bartel 1997.)

The IAN pathway. In the **indole-3-acetonitrile** (**IAN**) pathway (see Figure 19.6B), tryptophan is first converted to indole-3-acetaldoxime and then to indole-3-acetonitrile. The enzyme that converts IAN to IAA is called *nitrilase*. The IAN pathway may be important in only three plant families: the Brassicaceae (mustard family), Poaceae (grass family), and Musaceae (banana family). Nevertheless, nitrilase- like genes or activities have recently been identified in the Cucurbitaceae (squash family), Solanaceae (tobacco family), Fabaceae (legumes), and Rosaceae (rose family).

Four genes (*NIT1* through *NIT4*) that encode nitrilase enzymes have now been cloned from *Arabidopsis*. When *NIT2* was expressed in transgenic tobacco, the resultant plants acquired the ability to respond to IAN as an auxin by hydrolyzing it to IAA (Schmidt et al. 1996).

Another tryptophan-dependent biosynthetic pathway— one that uses **indole-3-acetamide** (**IAM**) as an intermediate (see Figure19.6A)—is used by various pathogenic bacteria, such as *Pseudomonas savastanoi* and *Agrobacterium tumefaciens*. This pathway involves the two enzymes tryptophan monooxygenase and IAM hydrolase. The auxins produced by these bacteria often elicit morphological changes in their plant hosts.

IAA Is Degraded by Multiple Pathways

Like IAA biosynthesis, the enzymatic breakdown (oxidation) of IAA may involve more than one pathway. For some time it has been thought that peroxidative enzymes are chiefly responsible for IAA oxidation, primarily because these enzymes are ubiquitous in higher plants and their ability to degrade IAA can be demonstrated in vitro (Figure 19.10A). However, the physiological significance of the peroxidase pathway is unclear. For example, no change in the IAA levels of transgenic plants was observed with either a tenfold increase in peroxidase expression or a tenfold repression of peroxidase activity (Normanly et al. 1995).

On the basis of isotopic labeling and metabolite identification, two other oxidative pathways are more likely to be involved in the controlled degradation of IAA. The end product of this pathway is oxindole- 3-acetic acid (OxIAA), a naturally occurring compound in the endosperm and shoot tissues of *Zea mays*. In one pathway, IAA is oxidized without decarboxylation to OxIAA.

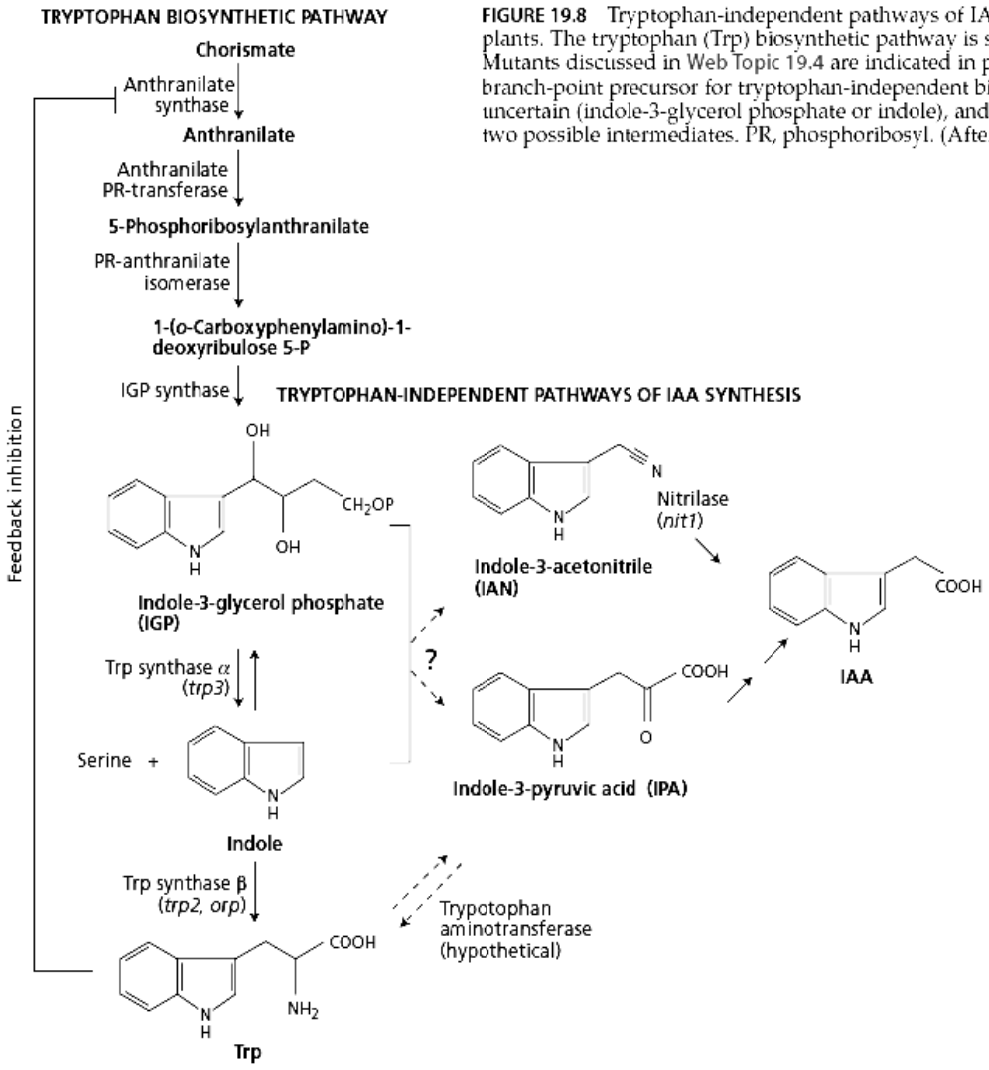


FIGURE 19.8 Tryptophan-independent pathways of IAA biosynthesis in plants. The tryptophan (Trp) biosynthetic pathway is shown on the left. Mutants discussed in Web Topic 19.4 are indicated in parentheses. The branch-point precursor for tryptophan-independent biosynthesis is uncertain (indole-3-glycerol phosphate or indole), and IAN and IPA are two possible intermediates. PR, phosphoribosyl. (After Bartel 1997.)

Physiological Effects of Auxin in Plants:

The following points highlight the eight physiological effects of auxin in plants:

1. Cell Elongation:

The primary physiological effect of auxin in plants is to stimulate the elongation of cells in shoot. A very common example of this can be observed in phototropic curvatures where the unilateral light unequally distributes the auxin in the stem tip (i.e., more auxin on shaded side than on illuminated side).

The higher concentration of auxin on the shaded side causes the cells on that side to elongate more rapidly resulting in bending of the stem tip towards the unilateral light. Many theories have been proposed to explain the mechanism of cell elongation due to auxin.

(i) By increasing the osmotic solutes of the cells,

(ii) By reducing the wall pressure,

(iii) By increasing the permeability of cells to water,

(iv) By an increase in the wall synthesis and

(v) By inducing the synthesis of specific DNA dependent new m-RNA and specific enzymic proteins. The latter bringing about an increase in cell plasticity and extension resulting ultimately in cell enlargement.

2. Apical Dominance:

It has been a common observation in many vascular plants especially the tall and sparsely branched ones that if the terminal bud is intact and growing, the growth of the lateral buds just below it remained suppressed. Removal of the apical bud results in the rapid growth of the lateral buds. This phenomenon in which the apical bud dominates over the lateral buds and does not allow the latter to grow is called as apical dominance.

In recent years, experiment with transgenic plants by plant physiologists and plant molecular biologists have made it quite clear that by removing the apical bud (or decapitating) the shoot), the concentration of auxin in lateral buds situated below was not decreased but there was in-fact a manifold increase in auxin conc., a few hours after decapitation. For instance, Gocal et al (1991) have observed fivefold increase in auxin conc. in the axillary buds of Phaseolus vulgaris 4 hours after decapitation.

It is now generally held that inhibitory effect of auxin from shoot apex on lateral buds is not direct but is indirect possibly through the involvement of other growth hormones such as cytokinins and abscisic acid (ABA). (In many plant species it has been observed that application of cytokinins to lateral buds stimulates bud growth. The auxin in shoot apex makes it a sink for cytokinins which are synthesized in roots. By decapitating the shoot, the supply of cytokinins is diverted to lateral buds, thereby relieving the buds from apical dominance.

Application of auxin to cut apical stump retards accumulation of cytokinins in lateral buds resulting in inhibition of bud growth. The ratio of auxin to cytokinins appears to be one of the key factors involved in apical dominance. Higher auxin to cytokinins ratio suppresses the growth of lateral buds.

3. Root Initiation:

In contrast to the stem, the higher concentration of auxin inhibits the elongation of root but the number of lateral branch roots is considerably increased i.e., the higher conc. of auxin initiates more lateral branch roots. Application of IAA in lanolin paste to the cut end of a young stem results in an early and extensive rooting. This fact is of great practical importance and has been widely utilised to promote root formation in economically useful plants which are propagated by cuttings.

4. Prevention of Abscission:

Natural auxins have controlling influence on the abscission of leaves, fruits etc.

5. Parthenocarpy:

Auxin can induce the formation of parthenocarpic fruits. In nature also, this phenomenon is not uncommon and in such cases the concentration of auxins in the ovaries has been found to be higher

than in the ovaries of plants which produce fruits only after fertilization. In the latter cases, the concentration of the auxin in ovaries increases after pollination and fertilization.

6. Respiration:

It has been established that the auxin stimulates respiration and there is a correlation between auxin induced growth and an increased respiration rate. According to French and Beevers (1953), the auxin may increase the rate of respiration indirectly through increased supply of ADP (Adenosine diphosphate) by rapidly utilizing the ATP in the expanding cells.

7. Callus Formation:

Besides cell elongation, the auxin may also be active in cell division. In fact, in many tissue cultures where the callus growth is quite normal, the continued growth of such callus takes place only after the addition of auxin.

8. Vascular Differentiation:

Auxin induces vascular differentiation in plants. This has also been confirmed in tissue culture experiments and from studies with transgenic plants. Cytokinins are also known to participate in differentiation of vascular tissues and it is believed that vascular differentiation in plants is probably under the control of both auxin and cytokinins.

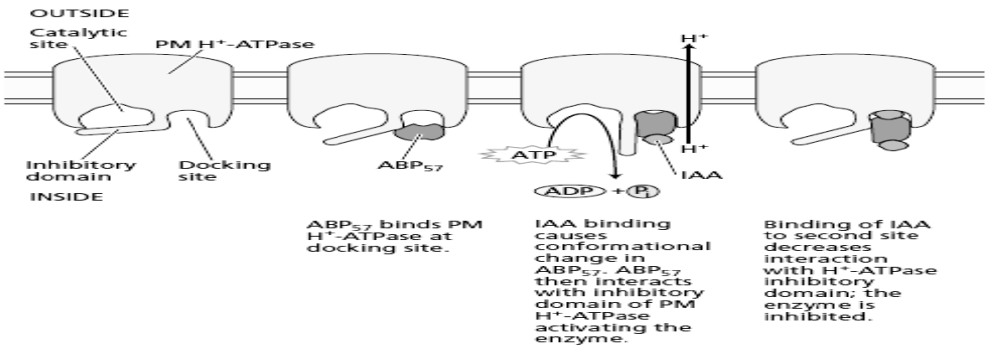
Auxin signal transduction pathways

The ultimate goal of research on the molecular mechanism of hormone action is to reconstruct each step in the signal transduction pathway, from receptor binding to the physiological response. The various signaling pathways that have been implicated in auxin action. Finally we will turn our attention to auxinregulated
gene expression.

ABP1 Functions as an Auxin Receptor

In addition to its possible direct role in plasma membrane H+-ATPase activation (discussed earlier), the auxin-binding protein ABP1 appears to function as an auxin receptor in other signal transduction pathways. ABP1 homologs have been identified in a variety of monocot and dicot species (Venis and Napier 1997). Knockouts of the *ABP1*gene in *Arabidopsis* are lethal, and less severe mutations result in altered development (Chen et al. 2001). Recent studies indicate that, despite being localized primarily on the endoplasmic reticulum (ER), a small amount of ABP1 is secreted to the plasma membrane outer surface where it interacts with auxin to cause protoplast swelling and H+- pumping (Venis et al. 1996; Steffens et al. 2001).

However, it is unlikely that ABP1 mediates all auxin response pathways because expression of a number of auxin-responsive genes is not affected when protoplasts are incubated with anti-ABP1 antibodies. It is also unclear what role the ABP1 in the ER plays in auxin-responsive signal



transduction. Finally, it remains to be determined whether ABP57, the soluble and unrelated ABP from rice that activates the H+-ATPase (see Figure 19.24), is involved in a signal transduction pathway.

Calcium and Intracellular pH Are Possible Signaling Intermediates

Calcium plays an important role in signal transduction in animals and is thought to be involved in the action of certain plant hormones as well. The role of calcium in auxin action seems very complex and, at this point in time, very uncertain. Nevertheless, some experimental evidence shows that auxin increases the level of free calcium in the cell. Changes in cytoplasmic pH can also serve as a second messenger in animals and plants. In plants, auxin induces a decrease in cytosolic pH of about 0.2 units within 4 minutes of application. The cause of this pH drop is not known. Since the cytosolic pH is normally around 7.4, and the pH optimum of the plasma membrane H+-ATPase is 6.5, a decrease in the cytosolic pH of 0.2 units could cause a marked increase in the activity of the plasma membrane H+-ATPase. The decrease in cytosolic pH might also account for the auxin-induced increase in free intracellular calcium, by promoting the dissociation of bound forms. MAP kinases that play a role in signal transduction by phosphorylating proteins in a cascade that ultimately activates transcription factors have also been implicated in auxin responses. When tobacco cells are deprived of auxin, they arrest at the end of either the G1 or the G2 phase and cease dividing; if auxin
is added back into the culture medium, the cell cycle resumes (Koens et al. 1995). Auxin appears to exert its effect on the cell cycle primarily by stimulating the synthesis of the major cyclin-dependent protein kinase (CDK): Cdc2 (*c*ell *d*ivision *c*ycle *2*).

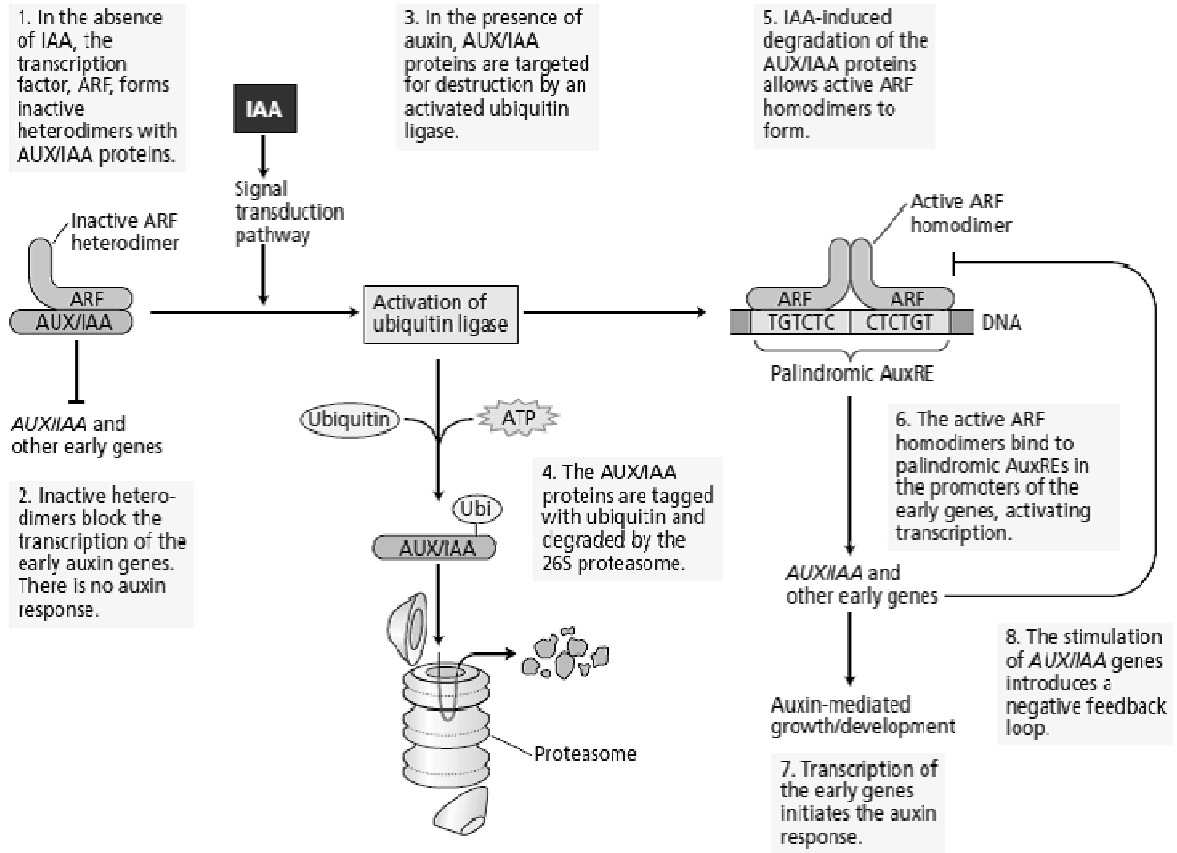


FIGURE 19.41 A model for auxin regulation of transcriptional activation of early response genes by auxin. (After Gray et al. 2001.)

Gibberellins:

In the 1950s the second group of hormones, the gibberellins (GAs), was characterized. The gibberellins are a large group of related compounds (more than 125 are known) that, unlike the auxins, are defined by their chemical structure rather than by their biological activity. Gibberellins are most often associated with the promotion of stem growth, and the application of gibberellin to intact plants can induce large increases in plant height. As we will see, however, gibberellins play important roles in a variety of physiological phenomena.

The biosynthesis of gibberellins is under strict genetic, developmental, and environmental control, and numerous gibberellin-deficient mutants have been isolated. Mendel's tall/dwarf alleles in peas are a famous example. Such mutants have been useful in elucidating the complex pathways of gibberellin biosynthesis.

Biosynthesis and metabolism of gibberellin

Gibberellins Are Synthesized via the Terpenoid Pathway in Three Stages

Gibberellins are tetracyclic diterpenoids made up of four isoprenoid units. Terpenoids are compounds made up of five-carbon (isoprene) building blocks: joined head to tail. Researchers have determined the entire

gibberellin biosynthetic pathway in seed and vegetative tissues of several species by feeding various radioactive precursors and intermediates and examining the production of the other compounds of the pathway (Kobayashi et al. 1996).

The gibberellin biosynthetic pathway can be divided into three stages, each residing in a different cellular compartment (Figure 20.6) (Hedden and Phillips 2000).

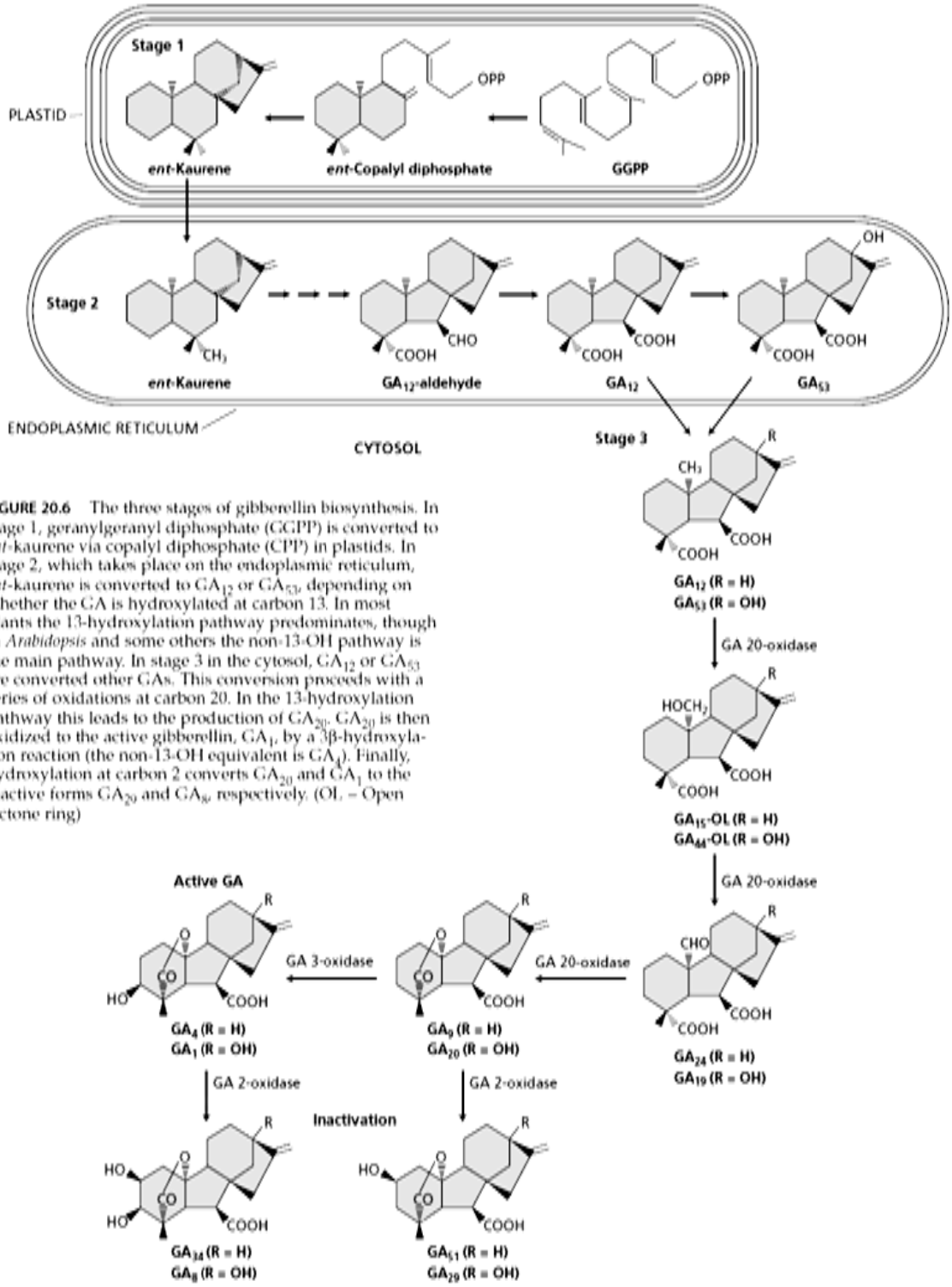


FIGURE 20.6 The three stages of gibberellin biosynthesis. In stage 1, geranylgeranyl diphosphate (GGPP) is converted to *ent*-kaurene via copalyl diphosphate (CPP) in plastids. In stage 2, which takes place on the endoplasmic reticulum, *ent*-kaurene is converted to GA_{12} or GA_{53}, depending on whether the GA is hydroxylated at carbon 13. In most plants the 13-hydroxylation pathway predominates, though in *Arabidopsis* and some others the non-13-OH pathway is the main pathway. In stage 3 in the cytosol, GA_{12} or GA_{53} are converted other GAs. This conversion proceeds with a series of oxidations at carbon 20. In the 13-hydroxylation pathway this leads to the production of GA_{20}. GA_{20} is then oxidized to the active gibberellin, GA_1, by a 3β-hydroxylation reaction (the non-13-OH equivalent is GA_4). Finally, hydroxylation at carbon 2 converts GA_{20} and GA_1 to the inactive forms GA_{29} and GA_8, respectively. (OL = Open lactone ring)

Stage 1: Production of terpenoid precursors and ent-kaurene in plastids.

The basic biological isoprene unit is isopentenyl diphosphate (IPP).2 IPP used in gibberellin biosynthesis in green tissues is synthesized in plastids from glyceraldehyde-3-phosphate and pyruvate (Lichtenthaler et al. 1997). However, in the endosperm of pumpkin seeds, which are very rich in gibberellin, IPP is formed in the cytosol from mevalonic acid, which is itself derived from acetyl-CoA. Thus the IPP used to make gibberellins may arise from different cellular compartments in different tissues. Once synthesized, the IPP isoprene units are added successively to produce intermediates of 10 carbons (geranyl diphosphate), 15 carbons (farnesyl diphosphate), and 20 carbons (geranylgeranyl diphosphate, GGPP). GGPP is a precursor of many terpenoid compounds, including carotenoids and many essential oils, and it is only after GGPP that the pathway becomes specific for gibberellins.

The cyclization reactions that convert GGPP to *ent*-kaurene represent the first step that is specific for the gibberellins (Figure 20.6). The two enzymes that catalyze the reactions are localized in the proplastids of meristematic shoot tissues, and they are not present in mature chloroplasts (Aach et al. 1997). Thus, leaves lose their ability to synthesize gibberellins from IPP once their chloroplasts mature.

Compounds such as AMO-1618, Cycocel, and Phosphon D are specific inhibitors of the first stage of gibberellin biosynthesis, and they are used as growth height reducers.

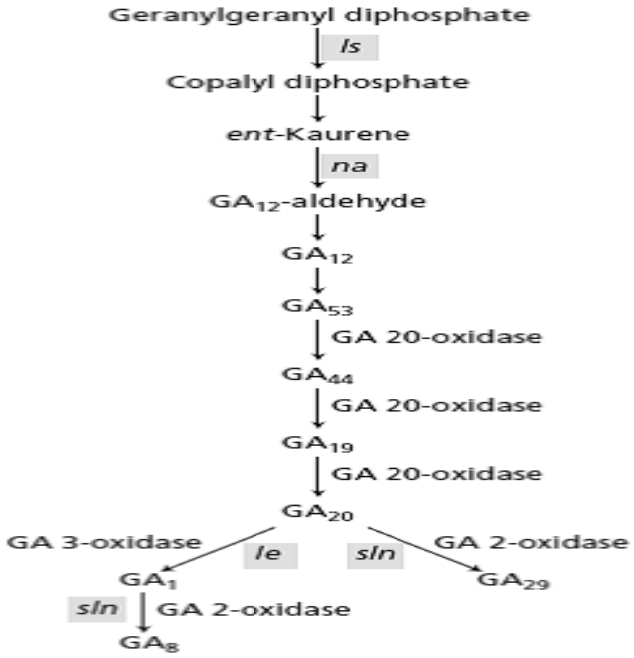


FIGURE 20.7 A portion of the gibberellin biosynthetic pathway showing the abbreviations and location of the mutant genes that block the pathway in pea and the enzymes involved in the metabolic steps after GA_{53}.

Stage 2: Oxidation reactions on the ER form GA12 and GA53.

In the second stage of gibberellin biosynthesis, a methyl group on *ent*-kaurene is oxidized to a carboxylic acid, followed by contraction of the B ring from a six- to a five-carbon ring to give GA12-aldehyde. GA12-aldehyde is then oxidized to **GA12**, the first gibberellin in the pathway in all plants and thus the precursor of all the other gibberellins (see Figure 20.6).

Many gibberellins in plants are also hydroxylated on carbon 13. The hydroxylation of carbon 13 occurs next, forming GA53 from GA12. All the enzymes involved are monooxygenases that utilize cytochrome P450 in their reactions. These P450 monooxygenases are localized on the endoplasmic

reticulum. Kaurene is transported from the plastid to the endoplasmic reticulum, and is oxidized *enroute* to kaurenoic acid by kaurene oxidase, which is associated with the plastid envelope (Helliwell et al. 2001).

Further conversions to GA12 take place on the endoplasmic reticulum. Paclobutrazol and other inhibitors of P450 monooxygenases specifically inhibit this stage of gibberellin biosynthesis before GA12-aldehyde, and they are also growth retardants.

Stage 3: Formation in the cytosol of all other gibberellins from GA12 or GA53.

All subsequent steps in the pathway (see Figure 20.6) are carried out by a group of soluble dioxygenases in the cytosol. These enzymes require 2-oxoglutarate and molecular oxygen as cosubstrates, and they use Fe^{2+} and ascorbate as cofactors.

The specific steps in the modification of GA12 vary from species to species, and between organs of the same species.

Two basic chemical changes occur in most plants:

1. Hydroxylation at carbon 13 (on the endoplasmic reticulum) or carbon 3, or both.
2. A successive oxidation at carbon 20 ($CH_2 \rightarrow CH_2OH \rightarrow CHO$). The final step of this oxidation is the loss of carbon 20 as CO_2 (see Figure 20.6).

When these reactions involve gibberellins initially hydroxylated at C-13, the resulting gibberellin is GA20.

GA20 is then converted to the biologically active form, GA1, by hydroxylation of carbon 3. (Because this is in the beta configuration [drawn as if the bond to the hydroxyl group were toward the viewer], it is referred to as 3β-hydroxylation.)

Finally, GA1 is inactivated by its conversion to GA8 by a hydroxylation on carbon 2. This hydroxylation can also remove GA20 from the biosynthetic pathway by converting it to GA29.

Inhibitors of the third stage of the gibberellin biosynthetic pathway interfere with enzymes that utilize 2-oxoglutarate as cosubstrates. Among these, the compound prohexadione (BX-112), is especially useful because it specifically inhibits GA 3-oxidase, the enzyme that converts inactive GA20 to growth-active GA1.

Gibberellin signal transduction:

Cereal aleurone layers

Genetic analyses of gibberellin-regulated growth, such as the studies described in the previous section, have identified some of the genes and their gene products, but not the biochemical pathways involved in gibberellin signal transduction.

The biochemical and molecular mechanisms, which are probably common to all gibberellin responses, have been studied most extensively in relation to the gibberellin- stimulated synthesis and secretion of α-amylase in cereal aleurone layers (Jacobsen et al. 1995).

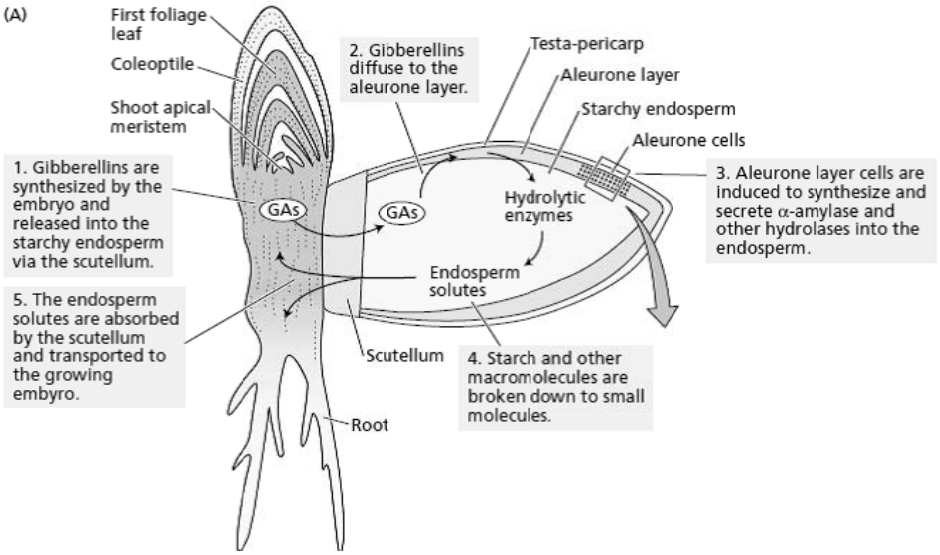
The gibberellin receptor, the transcriptional regulation of the genes for α-amylase and other proteins, and the possible signal transduction pathways involved in the control of α-amylase synthesis and secretion by gibberellin.

Gibberellin from the Embryo Induces α-Amylase

Production by Aleurone Layers

Cereal grains (*caryopses*; singular *caryopsis*) can be divided into three parts: the diploid embryo, the triploid endosperm, and the fused testa–pericarp (seed coat–fruit wall). The embryo part consists of the plant embryo proper, along with its specialized absorptive organ, the *scutellum* (plural *scutella*),

which functions in absorbing the solubilised food reserves from the endosperm and transmitting them to the growing embryo. The endosperm is composed of two tissues: the centrally located starchy endosperm and the aleurone layer (Figure 20.33A).



The starchy endosperm, typically nonliving at maturity, consists of thin-walled cells filled with starch grains. The aleurone layer surrounds the starchy endosperm and is cytologically and biochemically distinct from it. Aleurone cells are enclosed in thick primary cell walls and contain large numbers of protein-storing vacuoles called *protein bodies* (Figures 20.33B–D), enclosed by a single membrane. The protein bodies also contain phytin, a mixed cation salt (mainly Mg2+ and K+) of *myo*-inositolhexaphosphoric acid (phytic acid). During germination and early seedling growth, the stored food reserves of the endosperm—chiefly starch and protein—are broken down by a variety of hydrolytic enzymes, and the solubilized sugars, amino acids, and other products are transported to the growing embryo. The two enzymes responsible for starch degradation are α- and β-amylase. α-Amylase hydrolyzes starch chains internally to produce oligosaccharides consisting of α-1,4-linked glucose residues. β-Amylase degrades these oligosaccharides
from the ends to produce maltose, a disaccharide. Maltase then converts maltose to glucose.
α-Amylase is secreted into the starchy endosperm of cereal seeds by both the scutellum and the aleurone layer (see Figure 20.33A). The sole function of the aleurone layer of the seeds of graminaceous monocots (e.g., barley, wheat, rice, rye, and oats) appears to be the synthesis and release of hydrolytic enzymes. After completing this function, aleurone cells undergo programmed cell death.

Gibberellic Acid Enhances the Transcription of α-Amylase mRNA

Before molecular biological approaches were developed, there was already physiological and biochemical evidence that gibberellic acid might enhance α-amylase production at the level of gene transcription (Jacobsen et al. 1995). The two main lines of evidence were as follows:
1. GA3-stimulated α-amylase production was shown to be blocked by inhibitors of transcription and translation.
2. Heavy-isotope- and radioactive-isotope-labeling studies demonstrated that the stimulation of α-amylase activity by gibberellin involved de novo synthesis of the enzyme from amino acids, rather than activation of preexisting enzyme.

Gibberellin Receptors May Interact with GProteins on the Plasma Membrane

Acell surface localization of the gibberellin receptor is suggested from the fact that gibberellin that has been bound to microbeads that are unable to cross the plasma membrane is still active in inducing

α-amylase production in aleurone protoplasts (Hooley et al. 1991). In addition, microinjection of GA3 into aleurone protoplasts had no effect, but when the protoplasts were immersed in GA3 solution, they produced α-amylase (Gilroy and Jones 1994). These results suggest that gibberellin acts on the outer face of the plasma membrane.

Two gibberellin-binding plasma membrane proteins have been isolated through the use of purified plasma membrane and a radioactively labeled gibberellin that was chemically modified to permanently attach to protein to which it was weakly bound. Because the presence of excess gibberellin reduces binding, and these proteins from a semidwarf, gibberellin-insensitive sweet pea bind gibberellin less strongly, they may represent the gibberellin receptors (Lovegrove et al. 1998).

Cyclic GMP, Ca2+, and Protein Kinases Are Possible Signaling Intermediates

In animal cells, G-proteins can activate the enzyme guanylyl cyclase, the enzyme that synthesizes cGMP from GTP, leading to an increase in cGMP concetration. Cyclic GMP, in turn, can regulate ion channels, Ca2+ levels, protein kinase activity, and gene transcription. Gibberellin has been reported to cause a transient rise in cGMP levels in barley aleurone layers, suggesting a possible role for cGMP in α-amylase production (Pensen et al. 1996).

In conclusion, gibberellin signal transduction in aleurone cells seems to involve G-proteins as well as cyclic

GMP, leading to production of the transcription factor GAMYB, which induces α-amylase gene transcription. α-Amylase secretion has similar initial components but also involves an increase in cytoplasmic calcium and protein phosphorylation. The detailed signaling pathways remain to be worked out.

Physiological Effects of Gibberellins in Plants:

The following points highlight the eight physiological effects of gibberellins: 1. Seed Germination 2. Dormancy of Buds 3. Root Growth 4. Elongation of the Internodes 5. Bolting and Flowering 6. Parthenocarpy 7. Light Inhibited Stem Growth and 8. De novo Synthesis of the Enzyme-α-Amylase.

1. Seed Germination:

Certain light sensitive seeds e.g., lettuce and tobacco show poor germination in dark. Germination starts vigorously if these seeds are exposed to light or red light. This requirement of light is overcome if the seeds are treated with gibberellic acid in dark.

2. Dormancy of Buds:

In temperate regions the buds formed in autumn remain dormant until next spring due to severe colds. This dormancy of buds can be broken by gibberellin treatment. In potatoes also, there is a dormant period after harvest, but the application of gibberellin sprouts the eyes vigorously.

3. Root Growth:

Gibberellins have little or no effect on root growth. At higher concentration in some plants, however, some inhibition of root growth may occur. The initiation of roots is markedly inhibited by gibberellins in isolated cuttings.

4. Elongation of the Internodes:

Most pronounced effect of gibberellins on the plant growth is the elongation of the internodes, so much so that in many plants such as dwarf pea, dwarf maize etc., they overcome the genetic dwarfism. For instance, the light grown dwarf pea plants have short internodes and expanded leaves. But, when treated with gibberellin the internodes elongate markedly and they look like tall plants.

It is considered that in such dwarf plants (i) the gene for producing gibberellin is missing, or (ii) the concentration of the natural inhibitors is higher. When external gibberellin is applied the deficiency of the endogenous gibberellins is made good or the external gibberellin overcomes the effect of natural inhibitors which fall short.

Deepwater rice (*Oryza sativa*) is another notable example of pronounced effect of gibberellins on elongation of internodes so that its foliage may remain above water in the field. Striking growth rates of as much as 25 cms. per day have been observed in rice plants under field conditions.

(Partial submergence of rice plants is believed to reduce partial pressure of O_2 which triggers ethylene biosynthesis in submerged tissues. Ethylene in turn reduces the level of ABA (abscisic acid) which acts as antagonist of GA. Submerged rice tissues thus become more responsive to endogenous GA resulting in marked elongation of internodes).

5. Bolting and Flowering:

In many herbaceous plants the early period of growth shows rosette-habit with short stem and cauline leaves. Under short days the rosette habit is retained while under long days bolting occurs i.e., the stem elongates rapidly and is converted into floral axis bearing flower primordia. This bolting can also be induced in such plants e.g. Rudbeckia speciosa (It is a Long Day Plant) by the application of gibberellin even under non-inductive short days.

In Hyoscyamus niger (also a Long Day Plant) gibberellin treatment causes bolting and flowering under non-inductive short days. While in Long Day Plants the gibberellin treatment usually results in early flowering, its effects are quite variable in Short Day Plants. It may either have no effect, or inhibit, or may activate flowering.

6. Parthenocarpy:

Germination of the pollen grains is stimulated by gibberellins, likewise the growth of the fruit and the formation of parthenocarpic fruits can be induced by gibberellin treatment. In many cases e.g., pome and stone fruits where auxins have failed to induced parthenocarpy the gibberellins have proven to be successful. Seedless and fleshy tomatoes and large sized grapes are produced by gibberellin treatment on commercial scale.

7. Light Inhibited Stem Growth:

It is common observation that the dark grown plants become etiolated and have taller, thinner and pale stems while the light grown plants have shorter, thicker and green stems, and it may be concluded that light has inhibitory effect on stem elongation. Treatment of light grown plants with gibberellin also stimulates the stem growth and they appear to be dark brown. In such cases the protein content of the stem falls while soluble nitrogen content increases probably due to more breakdowns of proteins than their synthesis.

It is considered that the light in some way lowers the level of endogenous gibberellins and inhibits the stem growth.

8. De novo Synthesis of the Enzyme-α-Amylase:

One of the important functions of gibberellins is to cause de novo (i.e., a new) synthesis of the enzyme a- amylase in the aleurone layer surrounding the endosperm of cereal grains during germination. This enzyme brings about hydrolysis of starch to form simple sugars which are then translocated to growing embryo to provide energy source.

Cytokinins:

THE CYTOKININS WERE DISCOVERED in the search for factors that stimulate plant cells to divide (i.e., undergo cytokinesis). Since their discovery, cytokinins have been shown to have effects on many other physiological and developmental processes, including leaf senescence, nutrient mobilization, apical dominance, the formation and activity of shoot apical meristems, floral development, the breaking of bud dormancy, and seed germination. Cytokinins also appear to mediate many aspects of light-regulated development, including chloroplast differentiation, the development of autotrophic metabolism, and leaf and cotyledon expansion.

Although cytokinins regulate many cellular processes, the control of cell division is central in plant growth and development and is considered diagnostic for this class of plant growth regulators. For these reasons

we will preface our discussion of cytokinin function with a brief consideration of the roles of cell division in normal development, wounding, gall formation, and tissue culture.

The regulation of plant cell proliferation by cytokinins. Then turn to cytokinin functions not directly related to cell division: chloroplast differentiation, the prevention of leaf senescence, and nutrient mobilization. Finally, we will consider the molecular mechanisms underlying cytokinin perception and signaling.

Biosynthesis, metabolism of cytokinins:

The side chains of naturally occurring cytokinins are chemically related to rubber, carotenoid pigments, the

plant hormones gibberellin and abscisic acid, and some of the plant defense compounds known as phytoalexins. All of these compounds are constructed, at least in part, from isoprene units.

Isoprene is similar in structure to the side chains of zeatin and iP. These cytokinin side chains are synthesized from an isoprene derivative. Large molecules of rubber and the carotenoids are constructed by the polymerization of many isoprene units; cytokinins contain just one of these units. The precursor(s) for the formation of these isoprene structures are mevalonic acid or pyruvate plus 3-phosphoglycerate, depending on which pathway is involved. These precursors are converted to the biological isoprene unit dimethylallyl diphosphate (DMAPP).

IPT Catalyzes the First Step in Cytokinin Biosynthesis

The first committed step in cytokinin biosynthesis is the transfer of the isopentenyl group of dimethylallyl diphos-phate (DMAPP) to an adenosine moiety. An enzyme that catalyzes such an activity was first identified in the cellular slime mold *Dictyostelium discoideum*, and subsequently the *ipt* gene from *Agrobacterium* was found to encode such an enzyme. In both cases, DMAPP and AMP are converted to isopentenyladenosine-5′-monophosphate (iPMP).

As noted earlier, cytokinins are also present in the tRNAs of most cells, including plant and animal cells. The tRNA cytokinins are synthesized by modification of specific adenine residues within the fully transcribed tRNA. As with the free cytokinins, isopentenyl groups are transferred to the adenine molecules from DMAPP by an enzyme call tRNA-IPT.

The genes for tRNA-IPT have been cloned from many species. The possibility that free cytokinins are derived from tRNA has been explored extensively. Although the tRNAbound cytokinins can act as hormonal signals for plant cells if the tRNA is degraded and fed back to the cells, it is unlikely that any significant amount of the free hormonal cytokinin in plants is derived from the turnover of tRNA.

An enzyme with IPT activity was identified from crude extracts of various plant tissues, but researchers were unable to purify the protein to homogeneity. Recently, plant *IPT* genes were cloned after the *Arabidopsis* genome was analyzed for potential *ipt*-like sequences (Kakimoto 2001; Takei et al. 2001). Nine different *IPT* genes were identified in *Arabidopsis*—many more than are present in

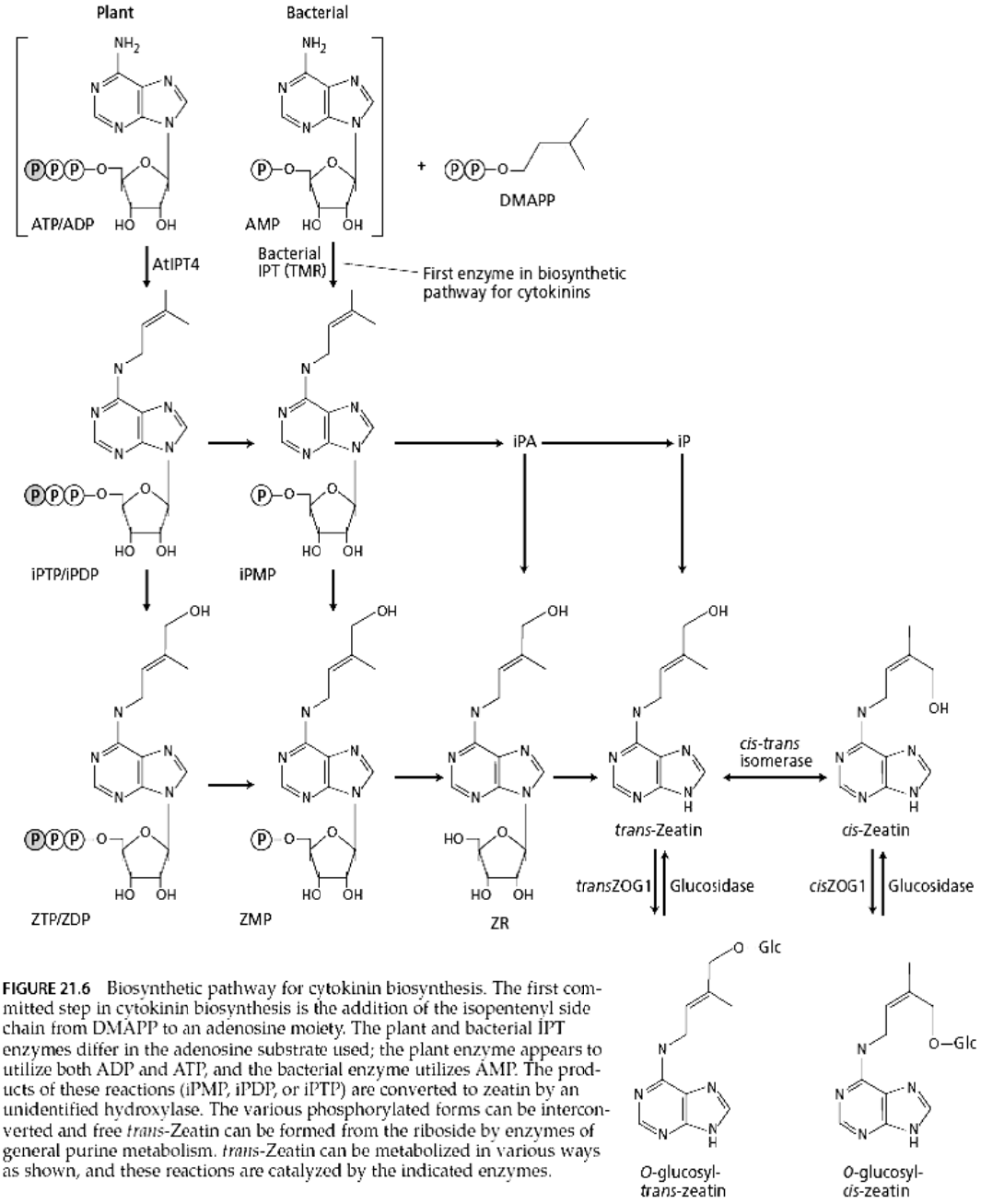


FIGURE 21.6 Biosynthetic pathway for cytokinin biosynthesis. The first committed step in cytokinin biosynthesis is the addition of the isopentenyl side chain from DMAPP to an adenosine moiety. The plant and bacterial IPT enzymes differ in the adenosine substrate used; the plant enzyme appears to utilize both ADP and ATP, and the bacterial enzyme utilizes AMP. The products of these reactions (iPMP, iPDP, or iPTP) are converted to zeatin by an unidentified hydroxylase. The various phosphorylated forms can be interconverted and free *trans*-Zeatin can be formed from the riboside by enzymes of general purine metabolism. *trans*-Zeatin can be metabolized in various ways as shown, and these reactions are catalyzed by the indicated enzymes.

animal genomes, which generally contain only one or two such genes used in tRNA modification. Phylogenetic analysis revealed that one of the *Arabidopsis IPT* genes resembles bacterial tRNA-*ipt*, another resembles eukaryotic tRNA-*IPT*, and the other seven form a distinct group or clade together with other plant sequences. The grouping of the seven *Arabidopsis IPT* genes in this unique plant clade provided a clue that these genes may encode the cytokinin biosynthetic enzyme.

The proteins encoded by these genes were expressed in *E. coli* and analyzed. It was found that, with the exception of the gene most closely related to the animal tRNA-*IPT* genes, these genes encoded

proteins capable of synthesizing free cytokinins. Unlike their bacterial counterparts, however, the *Arabidopsis* enzymes that have been analyzed utilize ATP and ADP preferentially over AMP.

The biological roles of cytokinins

Although discovered as a cell division factor, cytokinins can stimulate or inhibit a variety of physiological, metabolic, biochemical, and developmental processes when they are applied to higher plants, and it is increasingly clear that endogenous cytokinins play an important role in the regulation of these events in the intact plant.

Cytokinins Regulate Cell Division in Shoots and Roots

As discussed earlier, cytokinins are generally required for cell division of plant cells in vitro. Several lines of evidence suggest that cytokinins also play key roles in the regulation of cell division in vivo.

Much of the cell division in an adult plant occurs in the meristems. Localized expression of the *ipt* gene of *Agrobacterium* in somatic sectors of tobacco leaves causes the formation of ectopic (abnormally located) meristems, indicating that elevated levels of cytokinin are sufficient to initiate cell divisions in these leaves (Estruch et al. 1991). Elevation of endogenous cytokinin levels in transgenic *Arabidopsis* results in overexpression of the KNOTTED homeobox transcription factor homologs *KNAT1* and *STM*—genes that are important in the regulation of meristem function. Interestingly, overexpression of *KNAT1* also appears to elevate cytokinin levels in transgenic tobacco, suggesting an interdependent relationship between *KNAT* and the level of cytokinins.

Cytokinins Regulate Specific Components of the Cell Cycle

Cytokinins regulate cell division by affecting the controls that govern the passage of the cell through the cell division cycle. Zeatin levels were found to peak in synchronized culture tobacco cells at the end of S phase, mitosis, and G1 phase.

Cytokinins were discovered in relation to their ability to stimulate cell division in tissues supplied with an optimal level of auxin. Evidence suggests that both auxin and cytokinins participate in regulation of the cell cycle and that they do so by controlling the activity of cyclin-dependent kinases.

cyclin-dependent protein

kinases (*CDKs*), in concert with their regulatory subunits, the *cyclins*, are enzymes that regulate the eukaryotic cell cycle.

The Auxin: Cytokinin Ratio Regulates Morphogenesis in Cultured Tissues

Shortly after the discovery of kinetin, it was observed that the differentiation of cultured callus tissue derived from tobacco pith segments into either roots or shoots depends on the ratio of auxin to cytokinin in the culture medium. Whereas high auxin:cytokinin ratios stimulated the formation of roots, low auxin:cytokinin ratios led to the formation of shoots. At intermediate levels the tissue grew as an undifferentiated callus (Figure 21.13) (Skoog and Miller 1965).

Cytokinins Modify Apical Dominance and Promote Lateral Bud Growth

One of the primary determinants of plant form is the degree of apical dominance. Plants with strong apical dominance, such as maize, have a single growing axis with few lateral branches. In contrast, many lateral buds initiate growth in shrubby plants. Although apical dominance may be determined primarily by auxin, physiological studies indicate that cytokinins play a role in initiating the growth of lateral buds. For example, direct applications of cytokinins to the axillary buds of many species stimulate cell division activity and growth of the buds.

Cytokinins Induce Bud Formation in a Moss

Thus far we have restricted our discussion of plant hormones to the angiosperms. However, many plant hormones are present and developmentally active in representative species throughout the plant kingdom. The moss *Funaria hygrometrica* is a well-studied example. The germination of moss spores gives rise to a filament of cells called a *protonema* (plural *protonemata*). The protonema elongates and undergoes cell divisions at the tip, and it forms branches some distance back from the tip.

Cytokinin Overproduction Has Been Implicated in Genetic Tumors

Many species in the genus *Nicotiana* can be crossed to generate interspecific hybrids. More than 300 such interspecific hybrids have been produced; 90% of these hybrids are normal, exhibiting phenotypic characteristics intermediate between those of both parents. The plant used for cigarette

tobacco, *Nicotiana tabacum*, for example, is an interspecific hybrid. However, about 10% of these interspecific crosses result in progeny that tend to form spontaneous tumors levels in tumor-prone hybrids are five to six times higher
than those found in either parent.

Cytokinins Delay Leaf Senescence

Leaves detached from the plant slowly lose chlorophyll, RNA, lipids, and protein, even if they are kept moist and provided with minerals. This programmed aging process leading to death is termed **senescence**. Leaf senescence is more rapid in the dark than in the light. Treating isolated leaves of many species with
cytokinins will delay their senescence. Although applied cytokinins do not prevent senescence completely, their effects can be dramatic, particularly when the cytokinin is sprayed directly on the intact plant. If only
one leaf is treated, it remains green after other leaves of similar developmental age have yellowed and dropped off the plant. Even a small spot on a leaf will remain green if treated with a cytokinin, after the surrounding tissues on the same leaf begin to senesce.

Cytokinins Promote Movement of Nutrients

Cytokinins influence the movement of nutrients into leaves from other parts of the plant, a phenomenon known as *cytokinin-induced nutrient mobilization*. This process is revealed when nutrients (sugars, amino acids, and so on) radiolabeled with 14C or 3H are fed to plants after one leaf or part of a leaf is treated with a cytokinin. Later the whole plant is subjected to autoradiography to reveal the pattern of movement and the sites at which the labeled nutrients accumulate.

Cytokinins Promote Chloroplast Development

Although seeds can germinate in the dark, the morphology of dark-grown seedlings is very different from that of lightgrown seedlings: Dark-grown seedlings are said to be **etiolated**. The hypocotyl and internodes of
etiolated seedlings are more elongated, cotyledons and leaves do not expand, and chloroplasts do not mature.

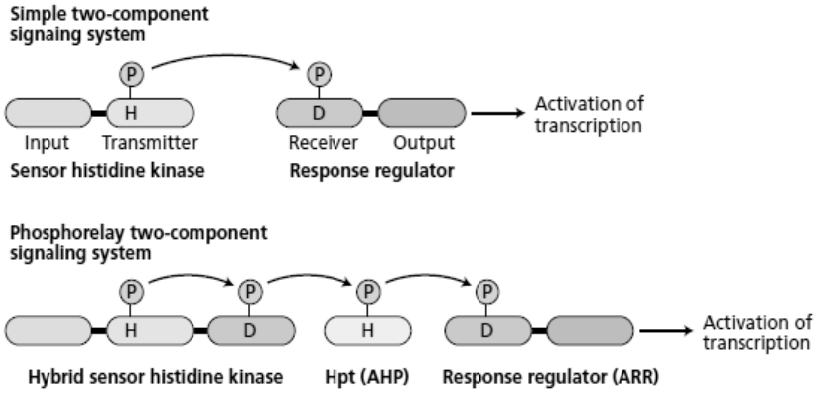


FIGURE 21.22 Simple versus phosphorelay types of two-component signaling systems. (A) In simple two-component systems, the input domain is the site where the signal is sensed. This regulates the activity of the histidine kinase domain, which when activated autophosphorylates on a conserved His residue. The phosphate is then transferred to an Asp residue that resides within the receiver domain of a response regulator. Phosphorylation of this Asp regulates the activity of the output domain of the response regulator, which in many cases is a transcription factor. (B) In the phosphorelay-type two-component signaling system, an extra set of phosphotransfers is mediated by a histidine phosphotransfer protein (Hpt), called AHP in *Arabidopsis*. The *Arabidopsis* response regulators are called ARRs. H = histidine, D = aspartate.

Cytokinins Promote Cell Expansion in Leaves and Cotyledons

The promotion of cell enlargement by cytokinins is most clearly demonstrated in the cotyledons of dicots with leafy cotyledons, such as mustard, cucumber, and sunflower.

The cotyledons of these species expand as a result of cell enlargement during seedling growth. Cytokinin treatment promotes additional cell expansion, with no increase in the dry weight of the treated cotyledons.

Cytokinins Regulate Growth of Stems and Roots

Although endogenous cytokinins are clearly required for normal cell proliferation in the apical meristem, and therefore normal shoot growth (see Figure 21.9), applied cytokinins typically inhibit the process of cell elongation in both stems and roots. For example, exogenous cytokinin inhibits hypocotyl elongation at concentrations that promote leaf and cotyledon expansion in the dark-grown seedlings.

Cellular and molecular modes of cytokinin action

The diversity of the effects of cytokinin on plant growth and development is consistent with the involvement of signal transduction pathways with branches leading to specific responses. Although our knowledge of how cytokinin works at the cellular and molecular levels is still quite fragmentary, significant progress has been achieved.

A Cytokinin Receptor Related to Bacterial Two-Component Receptors Has Been Identified

The first clue to the nature of the cytokinin receptor came from the discovery of the *CKI1* gene. *CKI1* was identified in a screen for genes that, when overexpressed, conferred cytokinin-independent growth on *Arabidopsis* cells in culture.

As discussed already, plant cells generally require cytokinin in order to divide in culture. However, a cell line that overexpresses *CKI1* is capable of growing in culture in the absence of added cytokinin.

Cytokinins Cause a Rapid Increase in the Expression of Response Regulator Genes

One of the primary effects of cytokinin is to alter the expression of various genes. The first set of genes to be upregulated in response to cytokinin are the ***ARR*** (A*rabidopsis r*esponse *r*egulator) genes. These genes are homologous to the receiver domain of bacterial two-component response regulators, the downstream target of sensor histidine kinases.

In *Arabidopsis*, response regulators are encoded by a multigene family. They fall into two basic classes: the **type-A *ARR*** genes, which are made up solely of a receiver domain, and the **type-B *ARR*** genes, which contain a transcription factor domain in addition to the receiver domain (Figure 21.23).

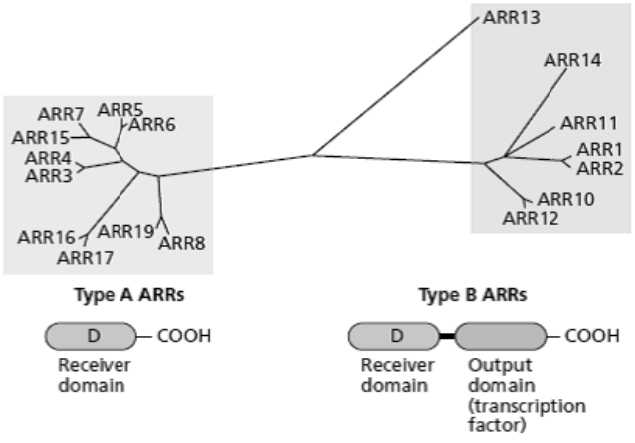


FIGURE 21.23 Phylogenetic tree of *Arabidopsis* response regulators. The top part of the figure shows a phylogenetic tree that represents the degree of relatedness of the receiver domains present in the *Arabidopsis* genome. The closer two proteins are on the tree, the more similar are their amino acid sequences. Note that these proteins fall into two distinct groups, or clades, called the type-A ARRs (blue) and the type-B ARRs (red). These differences in sequence are also reflected in a distinct domain structure, as depicted below the tree. The type-A ARRs consist solely of a receiver domain, but the type-A proteins also contain a fused output domain at the carboxy-terminus.

Histidine Phosphotransferases May Mediate the Cytokinin Signaling Cascade

From the preceding discussions we have seen that cytokinin binds to the CRE1 receptors to initiate a response that culminates in the elevation of transcription of the type-A *ARR*s. The type-A ARR proteins, in turn, may regulate the expression of numerous other genes, as well as the activities of various target proteins that ultimately alter cellular function. How is the signal propagated from CRE1 (which is at the plasma membrane) to the nucleus to alter type-A *ARR* transcription?

Cytokinin-Induced Phosphorylation Activates Transcription Factors

The question now becomes, How do the activated AHPs, once in the nucleus, act to regulate gene transcription? Genetic studies in intact *Arabidopsis* plants and overexpression studies in isolated *Arabidopsis* protoplasts using a cytokinin responsive reporter have provided a likely answer (Hwangand Sheen 2001; Sakai et al. 2001).

The mechanism of action of cytokinin is just beginning to emerge. Acytokinin receptor has been identified in *Arabidopsis*. This transmembrane protein is related to the bacterial two-component sensor histidine kinases. Cytokinins increase the abundance of several specific mRNAs. Some of these are primary response genes that are similar to bacterial two-component response regulators. The signal transduction mechanism from CRE1 to transcriptional activation of the type-A *ARR*s involves other homologs of two-component elements.

Ethylene:

In 1901, Dimitry Neljubov, a graduate student at the Botanical Institute of St. Petersburg in Russia, observed that dark-grown pea seedlings growing in the laboratory exhibited symptoms that were later termed the *triple response*: reduced stem elongation, increased lateral growth (swelling), and abnormal, horizontal growth. When the plants were allowed to grow in fresh air, they regained their normal morphology and rate of growth. Neljubov identified ethylene, which was present in the laboratory air from coal gas, as the molecule causing the response.

The first indication that ethylene is a natural product of plant tissues was published by H. H. Cousins in 1910. Cousins reported that “emanations” from oranges stored in a chamber caused the premature ripening

of bananas when these gases were passed through a chamber containing the fruit. However, given that oranges synthesize relatively little ethylene compared to other fruits, such as apples, it is likely that the oranges used by Cousins were infected with the fungus *Penicillium*, which produces copious amounts of ethylene. In 1934, R. Gane and others identified ethylene chemically as a natural product of plant metabolism, and because of its dramatic effects on the plant it was classified as a hormone.

Structure, biosynthesis, and measurement of ethylene

Ethylene can be produced by almost all parts of higher plants, although the rate of production depends on the type of tissue and the stage of development. In general, meristematic regions and nodal regions are the most active in ethylene biosynthesis. However, ethylene production also increases during leaf abscission and flower senescence, as well as during fruit ripening. Any type of wounding can induce ethylene biosynthesis, as can physiological stresses such as flooding, chilling, disease, and temperature or drought stress.

The amino acid methionine is the precursor of ethylene, and ACC (1-aminocyclopropane-1-carboxylic acid) serves as an intermediate in the conversion of methionine to ethylene. The complete pathway is a cycle, taking its place among the many metabolic cycles that operate in plant cells.

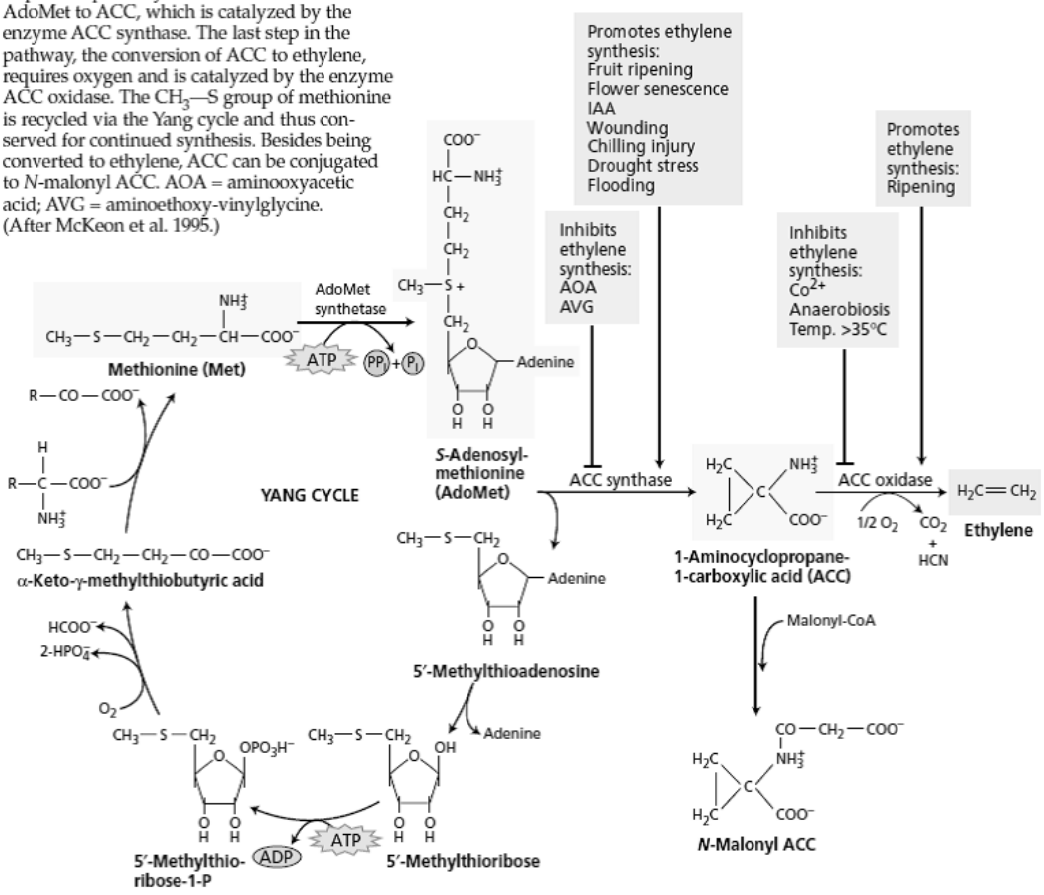


FIGURE 22.1 Ethylene biosynthetic pathway and the Yang cycle. The amino acid methionine is the precursor of ethylene. The rate-limiting step in the pathway is the conversion of AdoMet to ACC, which is catalyzed by the enzyme ACC synthase. The last step in the pathway, the conversion of ACC to ethylene, requires oxygen and is catalyzed by the enzyme ACC oxidase. The CH_3—S group of methionine is recycled via the Yang cycle and thus conserved for continued synthesis. Besides being converted to ethylene, ACC can be conjugated to *N*-malonyl ACC. AOA = aminooxyacetic acid; AVG = aminoethoxy-vinylglycine. (After McKeon et al. 1995.)

Developmental and physiological effects of ethylene:

Ethylene was discovered in connection with its effects on seedling growth and fruit ripening. It has since been shown to regulate a wide range of responses in plants, including seed germination, cell expansion, cell differentiation, flowering, senescence, and abscission.

Ethylene Promotes the Ripening of Some Fruits

In everyday usage, the term *fruit ripening* refers to the changes in fruit that make it ready to eat. Such changes typically include softening due to the enzymatic breakdown of the cell walls, starch hydrolysis, sugar accumulation, and the disappearance of organic acids and phenolic compounds, including tannins. From the perspective of the plant, fruit ripening means that the seeds are ready for dispersal.

For seeds whose dispersal depends on animal ingestion, *ripeness* and *edibility* are synonymous. Brightly colored anthocyanins and carotenoids often accumulate in the epidermis of such fruits, enhancing their visibility. However, for seeds that rely on mechanical or other means for dispersal, *fruit ripening* may mean drying followed by splitting.

Ethylene Induces Lateral Cell Expansion

At concentrations above 0.1 μL L–1, ethylene changes the growth pattern of seedlings by reducing the rate of elongation and increasing lateral expansion, leading to swelling of the region below the hook.

These effects of ethylene are common to growing shoots of most dicots, forming part of the **triple response**. In *Arabidopsis*, the triple response consists of inhibition and swelling of the hypocotyl, inhibition of root elongation, and exaggeration of the apical hook.

Ethylene Breaks Seed and Bud Dormancy in Some Species

Seeds that fail to germinate under normal conditions (water, oxygen, temperature suitable for growth) are said to be dormant. Ethylene has the ability to break dormancy and initiate germination in certain seeds, such as cereals. In addition to its effect on dormancy, ethylene increases the rate of seed germination of several species. In peanuts (*Arachis hypogaea*), ethylene production and seed germination are closely correlated. Ethylene can also break bud dormancy, and ethylene treatment is sometimes used to promote bud sprouting in potato and other tubers.

Ethylene Promotes the Elongation Growth of Submerged Aquatic Species

Although usually thought of as an inhibitor of stem elongation, ethylene is able to promote stem and petiole elongation in various submerged or partially submerged aquatic plants. These include the dicots *Ranunculus sceleratus*, *Nymphoides peltata*, and *Callitriche platycarpa*, and the fern *Regnellidium diphyllum*. Another agriculturally important example is the cereal deepwater rice.

Ethylene Induces the Formation of Roots and Root Hairs

Ethylene is capable of inducing adventitious root formation in leaves, stems, flower stems, and even other roots. Ethylene has also been shown to act as a positive regulator of root hair formation in several species.

This relationship has been best studied in *Arabidopsis*, in which root hairs normally are located in the epidermal cells that overlie a junction between the underlying cortical cells (Dolan et al. 1994).

In ethylene-treated roots, extra hairs form in abnormal locations in the epidermis; that is, cells not overlying a cortical cell junction differentiate into hair cells (Tanimoto et al. 1995). Seedlings grown in the presence of ethylene inhibitors (such as Ag+), as well as ethylene-insensitive mutants, display a reduction in root hair formation in response to ethylene.

These observations suggest that ethylene acts as a positive regulator in the differentiation of root hairs.

Ethylene Induces Flowering in the Pineapple Family

Although ethylene inhibits flowering in many species, it induces flowering in pineapple and its relatives, and it is used commercially in pineapple for synchronization of fruit set. Flowering of other species, such as mango, is also initiated by ethylene. On plants that have separate male and female flowers (monoecious species), ethylene may change the sex of developing flowers. The promotion of female flower formation in cucumber is one example of this effect.

Ethylene Enhances the Rate of Leaf Senescence

As, senescence is a genetically programmed developmental process that affects all tissues of the plant. Several lines of physiological evidence support roles for ethylene and cytokinins in the control of leaf senescence:

The Role of Ethylene in Defense Responses Is Complex

Pathogen infection and disease will occur only if the interactions between host and pathogen are genetically compatible. However, ethylene production generally increases in response to pathogen attack in both compatible (i.e., pathogenic) and noncompatible (nonpathogenic) interactions.

Ethylene Has Important Commercial Uses

Because ethylene regulates so many physiological processes in plant development, it is one of the most

widely used plant hormones in agriculture. Auxins and ACC can trigger the natural biosynthesis of ethylene and in several cases are used in agricultural practice. Because of its high diffusion rate, ethylene is very difficult to apply in the field as a gas, but this limitation can be overcome if an ethylene-releasing compound is used. The most widely used such compound is ethephon, or 2-chloroethylphosphonic acid, which was discovered in the 1960s and is known by various trade names, such as Ethrel.

CELLULAR AND MOLECULAR MODES OF ETHYLENE ACTION

Despite the broad range of ethylene's effects on development, the primary steps in ethylene action are assumed to be similar in all cases: They all involve binding to a receptor, followed by activation of one or more signal transduction pathways leading to the cellular response. Ultimately, ethylene exerts its effects

primarily by altering the pattern of gene expression. In recent years, remarkable progress has been made in our understanding of ethylene perception, as the result of molecular genetic studies of *Arabidopsis thaliana*.

One key to the elucidation of ethylene signaling components has been the use of the triple-response morphology of etiolated *Arabidopsis* seedlings to isolate mutants affected in their response to ethylene

Ethylene Receptors Are Related to Bacterial Two- Component System Histidine Kinases

The first ethylene-insensitive mutant isolated was *etr1*(*et*hylene-*r*esistant *1*). The *etr1* mutant was identified in a screen for mutations that block the response of *Arabidopsis* seedlings to ethylene. The amino acid sequence of the carboxy-terminal half of *ETR1* is similar to bacterial two-component histidine kinases—receptors used by bacteria to perceive various environmental cues, such as chemo-sensory stimuli, phosphate availability, and osmolarity. Bacterial two-component systems consist of a sensor histidine kinase and a response regulator, which often acts as a transcription factor.

High-Affinity Binding of Ethylene to Its Receptor Requires a Copper Cofactor

Even prior to the identification of its receptor, scientists had predicted that ethylene would bind to its receptor via a transition metal cofactor, most likely copper or zinc. This prediction was based on the high affinity of olefins, such as ethylene, for these transition metals. Recent genetic and biochemical studies have borne out these predictions. Analysis of the ETR1 ethylene receptor expressed in yeast demonstrated that a copper ion was coordinated to the protein and that this copper was necessary for highaffinity ethylene binding (Rodriguez et al. 1999). Silver ion could substitute for copper to yield high-affinity binding, which indicates that silver blocks the action of ethylene not by interfering with ethylene binding, but by preventing the changes in the protein that normally occur when ethylene binds to the receptor.

Unbound Ethylene Receptors Are Negative Regulators of the Response Pathway

In *Arabidopsis*, tomato, and probably most other plant species, the ethylene receptors are encoded by multigene families. Targeted disruption (complete inactivation) of the five *Arabidopsis* ethylene receptors (ETR1, ETR2, ERS1, ERS2, and EIN4) has revealed that they are functionally redundant (Hua and Meyerowitz 1998). That is, disruption of any single gene encoding one of these proteins has no effect, but a plant with disruptions in all five receptor genes exhibits a constitutive ethylene response phenotype.

A Serine/Threonine Protein Kinase Is Also Involved in Ethylene Signaling

The recessive *ctr1* (*c*onstitutive *t*riple *r*esponse *1* = triple response in the absence of ethylene) mutation was identified in screens for mutations that constitutively activated ethylene responses. The fact that the mutation

caused an *activation* of the ethylene response suggests that the wild-type protein also acts as a *negative regulator* of the response pathway (Kieber et al. 1993), similar to the ethylene receptors. CTR1 appears to be related to RAF-1, a MAPKKK serine/threonine protein kinase (*m*itogen-*a*ctivated *p*rotein *k*inase *k*inase *k*inase) that is involved in the transduction of various external regulatory signals and developmental signaling pathways in organisms ranging from yeast to humans

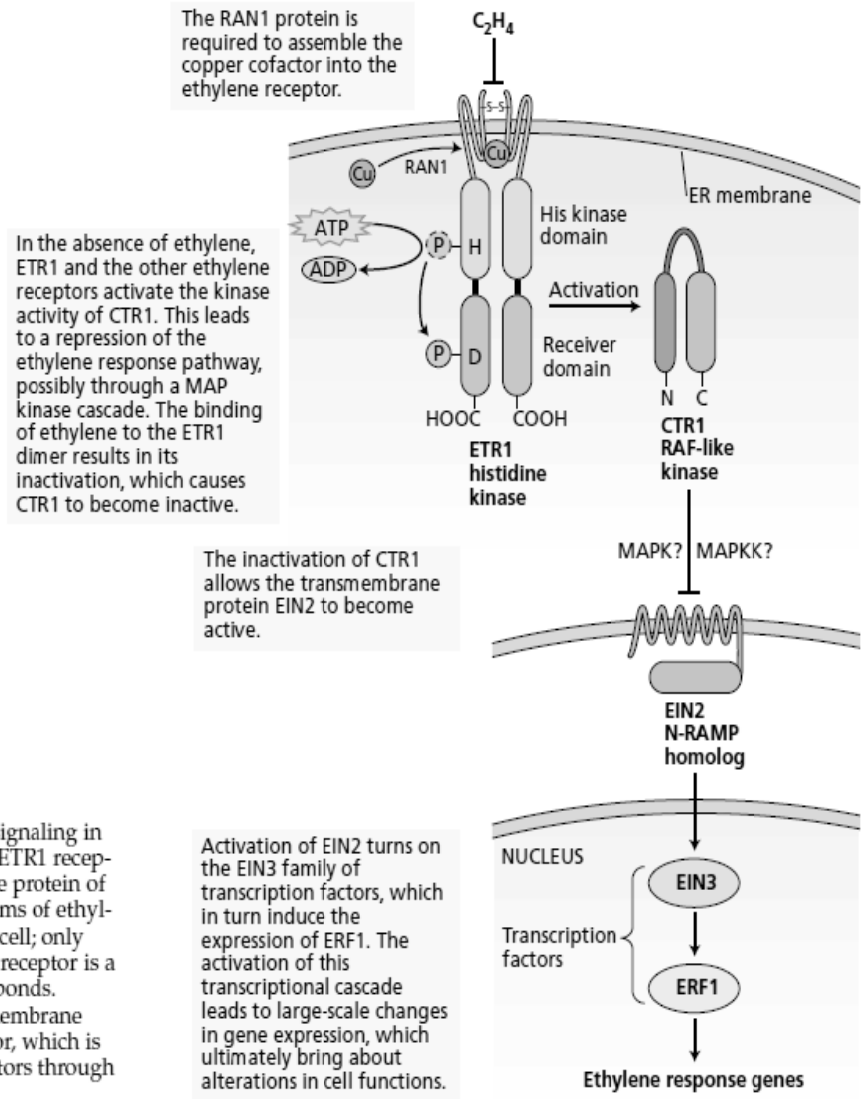


FIGURE 22.16 Model of ethylene signaling in *Arabidopsis*. Ethylene binds to the ETR1 receptor, which is an integral membrane protein of the ER membrane. Multiple isoforms of ethylene receptors may be present in a cell; only ETR1 is shown for simplicity. The receptor is a dimer, held together by disulfide bonds. Ethylene binds within the trans-membrane domain, through a copper co-factor, which is assembled into the ethylene receptors through the RAN1 protein.

Ethylene Regulates Gene Expression

One of the primary effects of ethylene signaling is an alteration in the expression of various target genes. Ethylene affects the mRNA transcript levels of numerous genes, including the genes that encode cellulase, as well as ripening-related genes and ethylene biosynthesis genes. Regulatory sequences called **ethylene response elements**, or **EREs**, have been identified from the ethylene-regulated genes. Key components mediating ethylene's effects on gene expression are the EIN3 family of transcription factors.

Genetic Epistasis Reveals the Order of the Ethylene Signaling Components

The order of action of the genes *ETR1*, *EIN2*, *EIN3*, and *CTR1* has been determined by the analysis of how the mutations interact with each other (i.e., their epistatic order). Two mutants with opposite phenotypes are

crossed, and a line harboring both mutations (the double mutant) is identified in the F2 generation. In the case of the ethylene response mutants, researchers constructed a line doubly mutant for *ctr1*, a constitutive ethylene response mutant, and one of the ethylene-insensitive mutations.

Ethylene regulates fruit ripening and other processes associated with leaf and flower senescence, leaf and fruit abscission, root hair development, seedling growth, and hook opening. Ethylene also regulates the expression of various genes, including ripening-related genes and pathogenesis-related genes.
The ethylene receptor is encoded by a family of genes that encode proteins similar to bacterial two-component histidine kinases. Ethylene binds to these receptors in a transmembrane domain through a copper cofactor. Downstream signal transduction components include CTR1, a member of the RAF family of protein kinases; and EIN2, a channel-like transmembrane protein. The pathway activates a cascade of transcription factors, including the EIN3 and EREBP families, which then modulate gene expression.

Abscisic Acid:

THE EXTENT AND TIMING OF PLANT GROWTH are controlled by the coordinated actions of positive and negative regulators. Some of the most obvious examples of regulated nongrowth are seed and bud dormancy, adaptive features that delay growth until environmental conditions are favorable. For many years, plant physiologists suspected that the phenomena of seed and bud dormancy were caused by inhibitory compounds, and they attempted to extract and isolate such compounds from a variety of plant tissues, especially dormant buds. Early experiments used paper chromatography for the separation of plant extracts, as well as bioassays based on oat coleoptile growth. These early experiments led to the identification of a group of growth-inhibiting compounds, including a substance known as *dormin* purified from sycamore leaves collected in early autumn, when the trees were entering dormancy. Upon discovery that dormin was chemically identical to a substance that promotes the abscission of cotton fruits, *abscisin II*, the compound was renamed **abscisic acid** (**ABA**), to reflect its supposed involvement in the abscission process.
It is now known that ethylene is the hormone that triggers abscission and that ABA-induced abscission of cotton fruits is due to ABA's ability to stimulate ethylene production.

Biosynthesis, metabolism, and transport of ABA

As with the other hormones, the response to ABA depends on its concentration within the tissue and on the sensitivity of the tissue to the hormone. The processes of biosynthesis, catabolism, compartmentation, and transport all contribute to the concentration of active hormone in the tissue at any given stage of development. The complete biosynthetic pathway of ABA was elucidated with the aid of ABA-deficient mutants blocked at specific steps in the pathway.

ABA Is Synthesized from a Carotenoid Intermediate

ABA biosynthesis takes place in chloroplasts and other plastids via the pathway depicted in Figure 23.2. Several ABA-deficient mutants have been identified with lesions at specific steps of the pathway. These mutants exhibit abnormal phenotypes that can be corrected by the application of exogenous ABA. For example, *flacca* (*flc*) and *sitiens* (*sit*) are "wilty mutants" of tomato in which the tendency of the leaves to wilt (due to an inability to close their stomata) can be prevented by the application of exogenous ABA. The *aba* mutants of *Arabidopsis* also exhibit a wilty phenotype. These and other mutants have been useful in
elucidating the details of the pathway (Milborrow 2001). The pathway begins with isopentenyl diphosphate (IPP), the biological isoprene unit, and leads to the synthesis of the C40 xanthophyll (i.e., oxygenated carotenoid) **violaxanthin** (see Figure 23.2). Synthesis of violaxanthin is catalyzed by zeaxanthin epoxidase (ZEP), the enzyme encoded by the *ABA1* locus of *Arabidopsis*. This discovery provided conclusive evidence that ABA synthesis occurs via the "indirect" or carotenoid pathway, rather than as a small molecule.
Maize mutants (*vp*) that are blocked at other steps in the carotenoid pathway also have reduced levels of ABA and exhibit **vivipary**—the precocious germination of seeds in the fruit while still attached to the plant (Figure 23.3). Vivipary is a feature of many ABA-deficient seeds. Violaxanthin is converted to the C40 compound **9′-*cis*neoxanthin**, which is then cleaved to form the C15 com-pound **xanthoxal**, previously called *xanthoxin*, a neutral growth inhibitor that has physiological properties

similar to those of ABA. The cleavage is catalyzed by **9-*cis*-epoxycarotenoid dioxygenase** (**NCED**), so named because it can cleave both 9-*cis*-violaxanthin and 9′-*cis*-neoxanthin.

Developmental and physiological effects of ABA

Abscisic acid plays primary regulatory roles in the initiation and maintenance of seed and bud dormancy and in the plant's response to stress, particularly water stress. In addition, ABA influences many other aspects of plant development by interacting, usually as an antagonist, with auxin, cytokinin, gibberellin, ethylene, and brassinosteroids. In this section we will explore the diverse physiological effects of ABA, beginning with its role in seed development.

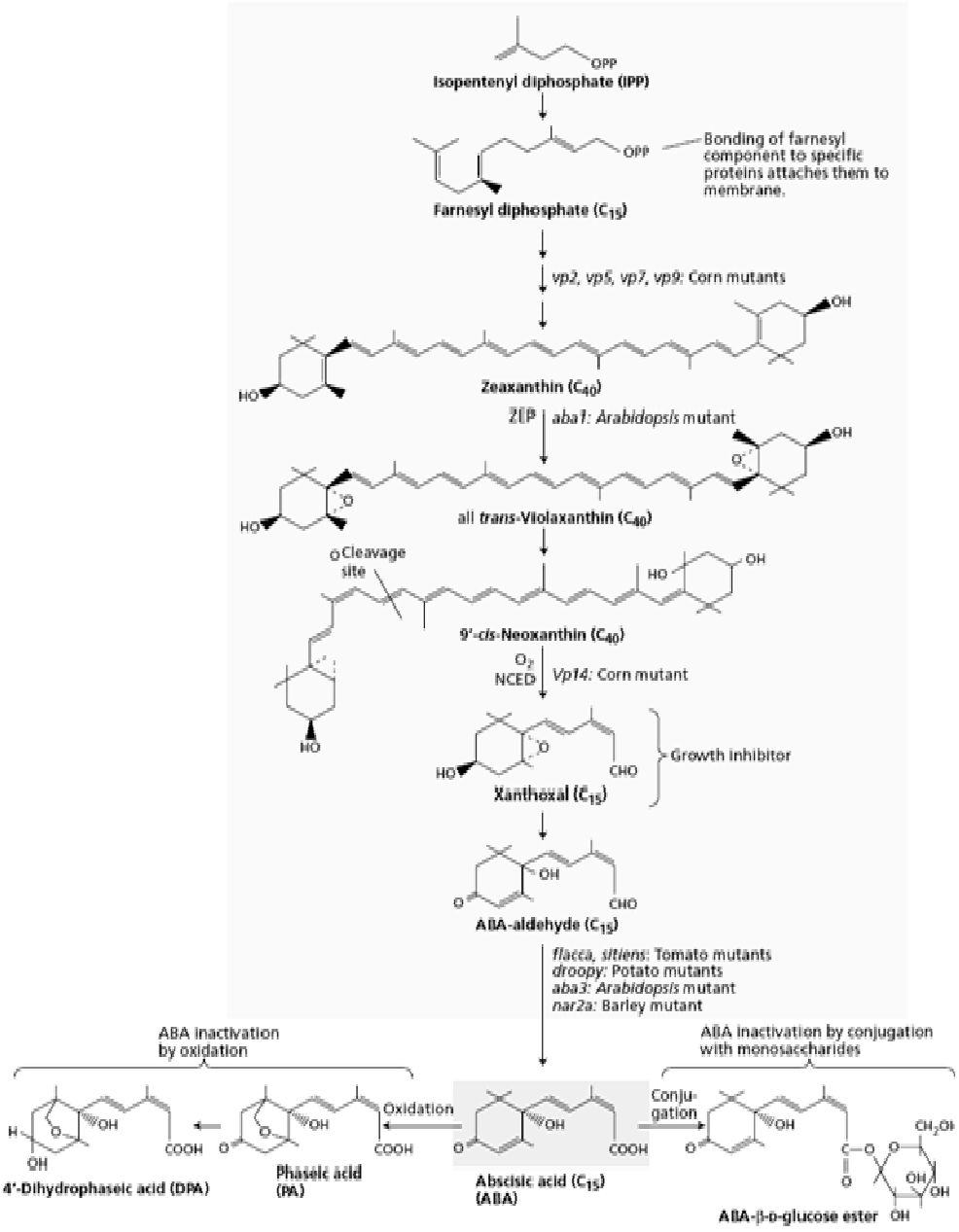


FIGURE 23.2 ABA biosynthesis and metabolism. In higher plants, ABA is synthesized via the terpenoid pathway (see Chapter 13). Some ABA-deficient mutants that have been helpful in elucidating the pathway are shown at the steps at which they are blocked. The pathways for ABA catabolism include conjugation to form ABA-β-D-glucosyl ester or oxidation to form phaseic acid and then dihydrophaseic acid. ZEP = zeaxanthin epoxidase; NCED = 9-cis-epoxycarotenoids dioxygenase.

ABA levels in seeds peak during embryogenesis

Seed development can be divided into three phases of approximately equal duration:

1. During the first phase, which is characterized by cell divisions and tissue differentiation, the zygote undergoes embryogenesis and the endosperm tissue proliferates.
2. During the second phase, cell divisions cease and storage compounds accumulate.
3. In the final phase, the embryo becomes tolerant to desiccation, and the seed dehydrates, losing up to 90% of its water. As a consequence of dehydration, metabolism comes to a halt and the seed enters a **quiescent** ("resting") state. In contrast to dormant seeds, quiescent seeds will germinate upon rehydration.

The latter two phases result in the production of viable seeds with adequate resources to support germination and the capacity to wait weeks to years before resuming growth. Typically, the ABA content of seeds is very low early in embryogenesis, reaches a maximum at about the halfway point, and then gradually falls to low levels as the seed reaches maturity. Thus there is a broad peak of ABA accumulation in the seed corresponding to mid- to late embryogenesis.

The hormonal balance of seeds is complicated by the fact that not all the tissues have the same genotype. The seed coat is derived from maternal tissues the zygote and endosperm are derived from both parents.

Genetic studies with ABA-deficient mutants of *Arabidopsis* have shown that the zygotic genotype controls

ABAsynthesis in the embryo and endosperm and is essential to dormancy induction, whereas the maternal genotype controls the major, early peak of ABA accumulation and helps suppress vivipary in midembryogenesis (Raz et al. 2001).

ABA Promotes Desiccation Tolerance in the Embryo

An important function of ABAin the developing seed is to promote the acquisition of desiccation tolerance. As desiccation can severely damage membranes and other cellular constituents. During the mid- to late stages of seed development, specific mRNAs accumulate in embryos at the time of high levels of endogenous ABA. These mRNAs encode so-called **late-embryogenesis-abundant** (**LEA**) proteins thought to be involved in desiccation tolerance.

Synthesis of many LEA proteins, or related family members, can be induced by ABA treatment of either young embryos or vegetative tissues. Thus the synthesis of most LEA proteins is under ABA control.

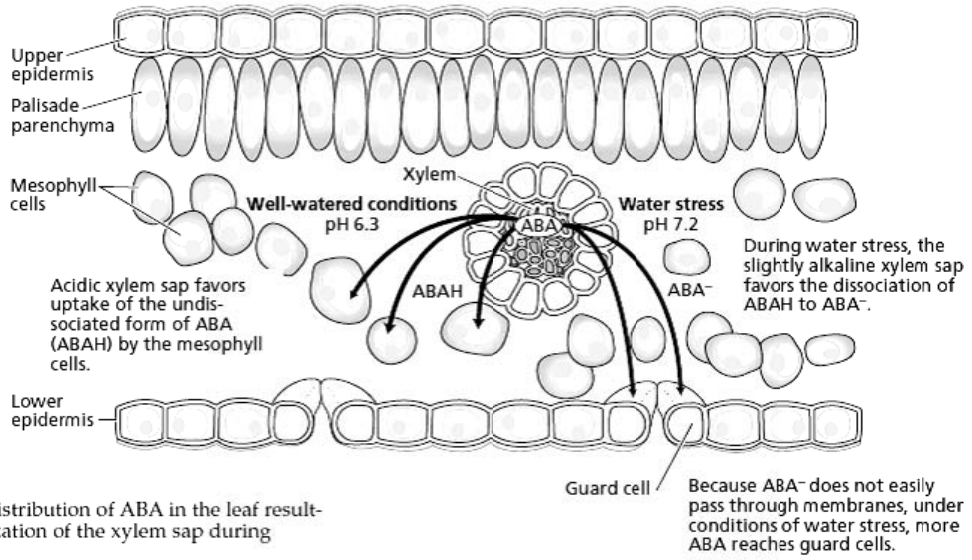


FIGURE 23.4 Redistribution of ABA in the leaf resulting from alkalinization of the xylem sap during water stress.

ABA Promotes the Accumulation of Seed Storage Protein during Embryogenesis

Storage compounds accumulate during mid- to late embryogenesis. Because ABA levels are still high, ABA

could be affecting the translocation of sugars and amino acids, the synthesis of the reserve materials, or both.

Studies in mutants impaired in both ABAsynthesis and response showed no effect of ABA on sugar translocation.

In contrast, ABA has been shown to affect the amounts and composition of storage proteins. For example, exogenous ABA promotes accumulation of storage proteins in cultured embryos of many species, and some ABA-deficient or -insensitive mutants have reduced storage protein accumulation. However, storage protein synthesis is also reduced in other seed developmental mutants with normal ABAlevels and responses, indicating that ABAis only one of several signals controlling the expression of storage protein genes during embryogenesis.

Seed Dormancy May Be Imposed by the Coat or the Embryo

During seed maturation, the embryo enters a quiescent phase in response to desiccation. Seed germination can be defined as the resumption of growth of the embryo of the mature seed; it depends on the same environmental conditions as vegetative growth does. Water and oxygen must be available, the temperature must be suitable, and there must be no inhibitory substances present.

In many cases a viable (living) seed will not germinate even if all the necessary environmental conditions for growth are satisfied. This phenomenon is termed **seed dormancy**. Seed dormancy introduces a temporal delay in the germination process that provides additional time for seed dispersal over greater geographic distances. It also maximizes seedling survival by preventing germination under unfavorable conditions. Two types of seed dormancy have been recognized: coat-imposed dormancy and embryo dormancy.

ABA Inhibits Precocious Germination and Vivipary

When immature embryos are removed from their seeds and placed in culture midway through development before the onset of dormancy, they germinate precociously—that is, without passing through the normal quiescent and/or dormant stage of development. ABA added to the culture medium inhibits precocious germination. This result, in combination with the fact that the level of endogenous ABA is high during mid- to late seed development, suggests that ABA is the natural constraint that keeps developing embryos in their embryogenic state.

ABA Inhibits GA-Induced Enzyme Production

ABA inhibits the synthesis of hydrolytic enzymes that are essential for the breakdown of storage reserves in seeds. For example, GA stimulates the aleurone layer of cereal grains to produce α-amylase and other hydrolytic enzymes that break down stored resources in the endosperm during germination. ABAinhibits this GA-dependent enzyme synthesis by inhibiting the transcription of α-amylase mRNA.

ABA Closes Stomata in Response to Water Stress

Elucidation of the roles of ABA in freezing, salt, and water stress led to the characterization of ABA as a stress hormone. As noted earlier, ABA concentrations in leaves can increase up to 50 times under drought conditions— the most dramatic change in concentration reported for any hormone in response to an environmental signal. Redistribution or biosynthesis of ABA is very effective in causing stomatal closure, and its accumulation in stressed leaves plays an important role in the reduction of water loss by transpiration under water stress conditions.

ABA Promotes Root Growth and Inhibits Shoot Growth at Low Water Potentials

ABAhas different effects on the growth of roots and shoots, and the effects are strongly dependent on the water status of the plant. The growth of shoots and roots of maize seedlings grown under either abundant water conditions (high water potential) or dehydrating conditions (low water potential). Two types of seedlings were used: (1) wild-type seedlings with normal ABA levels and (2) an ABA-deficient, viviparous mutant.

When the water supply is ample (high water potential), shoot growth is greater in the wild-type plant (normal endogenous ABAlevels) than in the ABA-deficient mutant. The reduced shoot growth in the ABA-deficient mutant could be due in part to excessive water loss from the leaves. In maize and tomato, however, the stunted shoot growth of ABA-deficient plants at high water potentials seems to be due to the overproduction of ethylene, which is normally inhibited by endogenous ABA (Sharp et al. 2000). This finding suggests that endogenous ABApromotes shoot growth in well-watered plants by suppressing ethylene production.

ABA Promotes Leaf Senescence Independently of Ethylene

Abscisic acid was originally isolated as an abscission-causing factor. However, it has since become evident that ABA stimulates abscission of organs in only a few species and that the primary hormone causing abscission is ethylene. On the other hand, ABA is clearly involved in leaf senescence, and through its promotion of senescence it might indirectly increase ethylene formation and stimulate abscission.

Cellular and molecular modes of ABA action

ABA is involved in short-term physiological effects (e.g., stomatal closure), as well as long-term developmental processes (e.g., seed maturation). Rapid physiological responses frequently involve alterations in the fluxes of ions across membranes and may involve some gene regulation as well, and long-term processes inevitably involve major changes in the pattern of gene expression.

Genetic studies have shown that many conserved signaling components regulate both short- and long-term responses, indicating that they share common signaling mechanisms.

ABA Is Perceived Both Extracellularly and Intracellularly

Although ABA has been shown to interact directly with phospholipids, it is widely assumed that the ABA receptor is a protein. To date, however, the protein receptor for ABA has not been identified. Experiments have been performed to determine whether the hormone must enter the cell to be effective, or whether it can act externally by binding to a receptor located on the outer surface of the plasma membrane.

The results so far suggest multiple sites of perception. Some experiments point to a receptor on the outer surface of the cell. For example, microinjected ABA fails to alter stomatal opening in the spiderwort *Commelina*, or to inhibit GA-induced α-amylase synthesis in barley aleurone protoplasts (Anderson et al. 1994; Gilroy and Jones 1994). Furthermore, impermeant ABA–protein conjugates have been shown to activate both ion channel activity and gene expression (Schultz and Quatrano 1997; Jeannette et al. 1999).

ABA Increases Cytosolic Ca2+, Raises Cytosolic pH, and Depolarizes the Membrane

As stomatal closure is driven by a reduction in guard cell turgor pressure caused by a massive long-term efflux of K+ and anions from the cell. During the subsequent shrinkage of the cell due to water loss, the surface area of the plasma membrane may contract by as much as 50%. Where does the extra membrane go? The answer seems to be that it is taken up as small vesicles by endocytosis—a process that also involves reorganization of the actin cytoskeleton. However, the first changes detected after exposure of guard cells to ABA are transient membrane depolarization caused by the net influx of positive charge, and transient increases in the cytosolic calcium concentration.

ABAstimulates elevations in the concentration of cytosolic Ca2+ by inducing both influx through plasma membrane channels and release of calcium into the cytosol from internal compartments, such as the central vacuole (Schroeder et al. 2001). Stimulation of influx occurs via a pathway that uses **reactive oxygen species** (**ROS**), such as hydrogen peroxide (H_2O_2) or superoxide (O_2), as secondary messengers leading
to plasma membrane channel activation (Pei et al. 2000).

ABA Activation of Slow Anion Channels Causes Long-Term Membrane Depolarization

The rapid, transient depolarizations induced by ABA are insufficient to open the K+ efflux channels, which require long-term membrane depolarization in order to open. However, long-term depolarizations in response to ABA have been demonstrated. According to a widely accepted model,

long-term membrane depolarization is triggered by two factors: (1) an ABA-induced transient depolarization of the plasma membrane, coupled with (2) an increase in cytosolic calcium.

ABA Stimulates Phospholipid Metabolism

As discussed previously, much evidence supports a role for calcium both in the promotion of stomatal closing and in the inhibition of stomatal opening. According to the classic calcium-dependent signal transduction pathway of animal cells, IP3 is released, along with diacylglycerol (DAG), when phospholipase C is activated by a G-protein in the plasma membrane. Does ABAuse the same pathway when it induces stomatal closure?

1. ABA binds to its receptors.

2. ABA-binding induces the formation of reactive oxygen species, which activate plasma membrane Ca^{2+} channels.

3. ABA increases the levels of cyclic ADP-ribose and IP_3, which activate additional calcium channels on the tonoplast.

4. The influx of calcium initiates intracellular calcium oscillations and promotes the further release of calcium from vacuoles.

5. The rise in intracellular calcium blocks K^+_{in} channels.

6. The rise in intracellular calcium promotes the opening if Cl^-_{out} (anion) channels on the plasma membrane, causing membrane depolarization.

7. The plasma membrane proton pump is inhibited by the ABA-induced increase in cytostolic calcium and a rise in intracellular pH, further depolarizing the membrane.

8. Membrane depolarization activates K^+_{out} channels.

9. K^+ and anions to be released across the plasma membrane are first released from vacuoles into the cytosol.

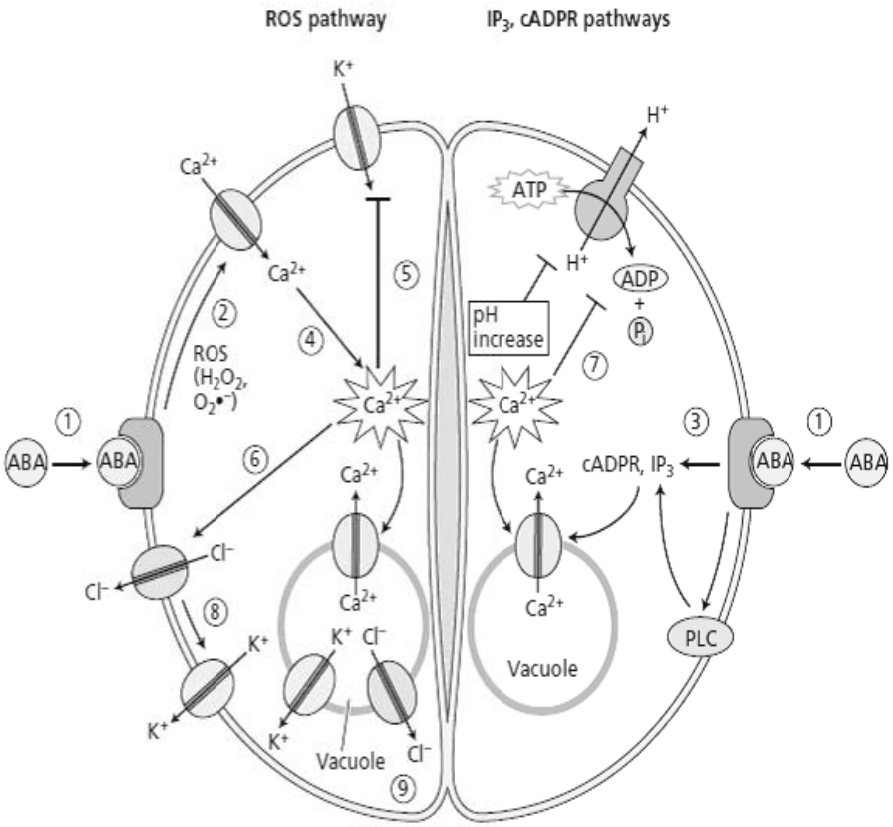


FIGURE 23.12 Simplified model for ABA signaling in stomatal guard cells. The net effect is the loss of potassium and its anion (Cl^- or $malate^{2-}$) from the cell. (R = receptor; ROS = reactive oxygen species; cADPR = cyclic ADP-ribose; G-protein = GTP-binding protein; PLC = phospholipiase C.)

Protein Kinases and Phosphatases Participate in ABA Action

Nearly all biological signaling systems involve protein phosphorylation and dephosphorylation reactions at some step in the pathway. Thus we can expect that signal transduction in guard cells, with their multiple sensory inputs, involves protein kinases and phosphatases. Artificially raising the ATP concentration inside guard cells by allowing the cytoplasm to equilibrate with the solution inside a patch pipette strongly activates the slow anion channels.

This activation of the slow anion channels by ATP is abolished by the inclusion of protein kinase inhibitors in the patch pipette solution (Schmidt et al. 1995). Protein kinase inhibitors also block ABA-induced stomatal closing.

ABA Regulation of Gene Expression Is Mediated by Transcription Factors

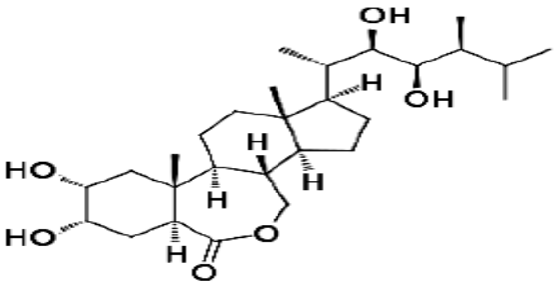
Downstream of the early ABA signal transduction processes already discussed, ABA causes changes in gene

expression. ABAhas been shown to regulate the expression of numerous genes during seed maturation and under certain stress conditions, such as heat shock, adaptation to low temperatures, and salt tolerance (Rock 2000). The ABAand stress-induced genes are presumed to contribute to adaptive aspects of induced tolerance. They include genes encoding proteases, chaperonins, proteins similar to LEA proteins, enzymes of sugar or other compatible solute metabolism, ion and water channel proteins, enzymes that detoxify active oxygen species, and regulatory proteins such as transcription factors and protein kinases.

In general, the ABAresponse appears to be regulated by more than one signal transduction pathway, even within a single cell type. This redundancy is consistent with the ability of plant cells to respond to multiple sensory inputs. There is genetic evidence for cross-talk between ABA signalling and the signaling of all other major classes of phytohormones, as well as sugars.

Brassinosteroids:

Brassinosteroids (BRs) are a class of polyhydroxysteroids that have been recognized as a sixth class of plant hormones and may have utility as an anticancer drug for endocrine-responsive cancers to induce apoptosis and inhibit growth. These brassinosteroids were first explored during the 70s, when Mitchell et al. reported promotion in stem elongation and cell division by the treatment of organic extracts of rapeseed (*Brassica napus*) pollen. Brassinolide was the first isolated brassinosteroid in 1979, when pollen from *Brassica napus* was shown to promote stem elongation and cell divisions, and the biologically active molecule was isolated. The yield of brassinosteroids from 230 kg of *Brassica napus* pollen was only 10 mg. Since their discovery, over 70 BR compounds have been isolated from plants.



Brassinolide, the first brassinosteroid isolated and shown to have biological activity

Biosynthesis

The BR is biosynthesised from campesterol. The biosynthetic pathway was elucidated by Japanese researchers and later shown to be correct through the analysis of BR biosynthesis mutants in *Arabidopsis thaliana*, tomatoes, and peas. The sites for BR synthesis in plants have not been experimentally demonstrated. One well-supported hypothesis is that all tissues produce BRs, since BR biosynthetic and signal transduction genes are expressed in a wide range of plant organs, and short distance activity of the hormones also supports this. Experiments have shown that long distance transport is possible and that the flow is from the base to the tips (acropetal), but it is not known if this movement is biologically relevant.

Hormonal activity

BRs have been shown to be involved in numerous plant processes: Promotion of cell expansion and cell elongation; works with auxin to do so. It has an unclear role in cell division and cell wall regeneration.

Promotion of vascular differentiation; BR signal transduction has been studied during vascular differentiation. Is necessary for pollen elongation for pollen tube formation. Acceleration of senescence in dying tissue cultured cells; delayed senescence in BR mutants supports that this action may be biologically relevant.

Can provide some protection to plants during chilling and drought stress. Extract from the plant *Lychnis viscaria* contains a relatively high amount of Brassinosteroids. Lychnis viscaria increases the disease resistance of surrounding plants. 24-Epibrassinolide (EBL), a brassinosteroid isolated from *Aegle marmelos* Correa (Rutaceae), was further evaluated for the antigenotoxicity against maleic hydrazide (MH)-induced genotoxicity in *Allium cepa* chromosomal aberration assay. It was shown that the percentage of chromosomal aberrations induced by maleic hydrazide (0.01%) declined significantly with 24-epibrassinolide treatment. BRs have been reported to counteract both abiotic and biotic stress in plants. Application of brassinosteroids to cucumbers was demonstrated to increase the metabolism and removal of pesticides, which could be beneficial for reducing the human ingestion of residual pesticides from non-organically grown vegetables. In all Type of brassinosteroids 28-homoBL is the most effective type of brassinosteroids. Brassinosteroids increased tolerance to high temperature in Brassica juncea L. The ability of 28-homobrassinolide to confer resistance to stress in *Brassica juncea* L. has also established. Application of 24-epiBL have any protective role on shoot, root length, soluble protein, proline content and peroxidases along with proline content PPO and IAA in seedlings of *B. juncea* L. under seasonal stress BRs have also been reported to have a variety of effects when applied to rice seeds (*Oryza sativa* L.). Seeds treated with BRs were shown to reduce the growth inhibitory effect of salt stress. When the developed plants fresh weight was analyzed the treated seeds outperformed plants grown on saline and non-saline medium however when the dry weight was analyzed BR treated seeds only outperformed untreated plants that were grown on saline medium. When dealing with tomatoes (*Lycopersicon esculentum*) under salt stress the concentration of cholophyll a and cholophyll b were decreased and thus pigmentation was decreased as well. BR treated rice seeds considerably restored the pigment level in plants that were grown on saline medium when compared to non-treated plants under the same conditions.

Signalling mechanism

BRs are perceived at the cell membrane by a co-receptor complex, comprising BRASSINOSTEROID INSENSITIVE 1(BRI1) and BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1). BRI1 acts as a kinase, but in the absence of BR its action is inhibited by another protein, BRI1 KINASE INHIBITOR 1 (BKI1). When BR binds to the BRI1:BAK1 complex, BKI1 is released, and a phosphorylation cascade is triggered which results in the de-activation of another kinase, BRASSINOSTEROID INSENSITIVE 2 (BIN2). BIN2 and its close homologues inhibit several transcription factors.

The inhibition of BIN2 by BR releases these transcription factors to bind to DNA and to enact certain developmental pathways.

Agricultural uses

BR might reveal to have a prominent interest in the role of horticultural crops. Based on extensive research BR has the ability to improve the quantity and quality of horticultural crops and protect plants against many stresses that can be present in the local environment. With the many advances in technology dealing with the synthesis of more stable synthetic analogues and the genetic manipulation of cellular BR activity, using BR in the production of horticultural crops has become a more practical and hopeful strategy for improving crop yields and success. BR could also help bridge the gap of the consumers' health concerns and the producers need for growth. A major benefit of using BR is that it does not interfere with the environment because they act in natural doses in a natural way. Since it is a “plant strengthening substance” and it is natural, BR application would be more favorable than pesticides and does not contribute to the co-evolution of pests.

In Germany, extract from the plant is allowed for use as a "plant strengthening substance."

Polyamines:

A **polyamine** is an organic compound having more than two amino groups. Alkyl polyamines occur naturally but are also synthetic. Alkylpolyamines are colorless, hygroscopic, and water soluble. Near neutral pH, they exist as the ammonium derivatives. Most aromatic polyamines are crystalline solids at room temperature.

Natural polyamines

Low-molecular-weight linear polyamines are found in all forms of life. The principal examples are the triamine and tetraamine spermidine and spermine. They are structurally and biosynthetically related to the diamines putrescine and cadaverine. Polyamine metabolism is regulated by the activity of the enzyme ornithine decarboxylase (ODC). Polyamines are found in high concentrations in the mammalian brain.

Synthetic polyamines

Several synthetic polyamines are used in chemical industry and the research laboratory. They are mainly of interest as additives to motor oil and as co-reactants (hardeners) with epoxy resins. Many synthetic polyamines feature NCH2CH2N linkages:

Diethylenetriamine, abbreviated dien or DETA, The related permethylated derivative pentamethyldiethylenetriamine is used as a chelating agent in organolithium chemistry. Triethylenetetramine tetraethylenepentamine, pentaethylenehexamine.

Biological function

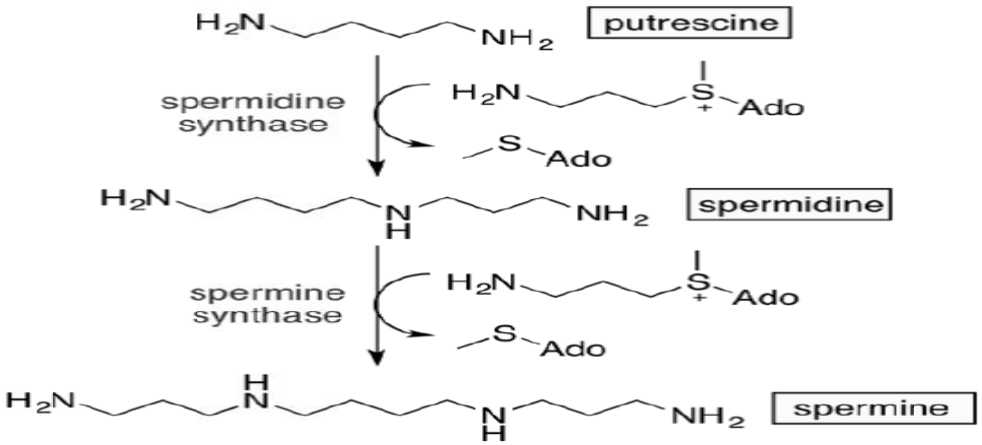
Although it is known that the biosynthesis of polyamines is highly regulated, the biological function of polyamines is only partly elucidated. In their cationic ammonium form, they bind to DNA, and, in structure, they represent compounds with cations that are found at *regularly spaced intervals* (unlike, say, Mg^{2+} or Ca^{2+} which are point charges). They have also been found to act as promoters of programmed ribosomal frameshifting during translation.

Inhibition of polyamine biosynthesis, retards or stops cell growth. The provision of exogenous polyamines restores the growth of these cells. Most eukaryotic cells have a polyamine transporter system on their cell membrane that facilitates the internalization of exogenous polyamines. This system is highly active in rapidly proliferating cells and is the target of some chemotherapeutics currently under development.

Polyamines are also modulators of a variety of ion channels, including NMDA receptors and AMPA receptors. They block inward-rectifier potassium channels so that the currents of the channels are inwardly rectified, thereby the cellular energy, i.e. K+ ion gradient across the cell membrane, is conserved. In addition, polyamine participate in initiating the expression of SOS response of Colicin E7 operon and down-regulate proteins that are essential for colicin E7 uptake, thus conferring a survival advantage on colicin-producing *E. coli* under stress conditions. Polyamines can enhance the permeability of the blood–brain barrier. They are involved in modulating senescence of organs in plants and are therefore considered as a plant hormone. In addition, they are directly involved in regulation of programmed cell death.

Biosynthesis of spermidine, spermine, thermospermine

Spermidine is synthesized from putrescine, using an aminopropyl group from decarboxylated *S*-adenosyl-L-methionine (SAM).



Biosynthesis of spermidine and spermine from putrescine. Ado = 5'-adenosyl.

The reaction is catalyzed by spermidine synthase. Spermine is synthesized from the reaction of spermidine with SAM in the presence of the enzyme spermine synthase. Thermospermine (NH_2-$(CH2)_3$-NH-$(CH_2)_3$-NH-$(CH_2)_4$-NH_2) is a structural isomer of spermine and a novel type of plant growth regulator. It is produced from spermidine by the action of thermospermine synthase, which is encoded by a gene named ACAULIS5 (ACL5).

Polyamine analogues

The critical role of polyamines in cell growth has led to the development of a number of agents that interfere with polyamine metabolism. These agents are used in cancer therapy. Polyamine analogues upregulate p53 in a cell leading to restriction of proliferation and apoptosis. It also decreases the expression of estrogen receptor alpha in ER positive breast cancer.

Jasmonates:

Jasmonate (**JA**) and its derivatives are lipid-based plant hormones that regulate a wide range of processes in plants, ranging from growth and photosynthesis to reproductive development. In particular, JAs are critical

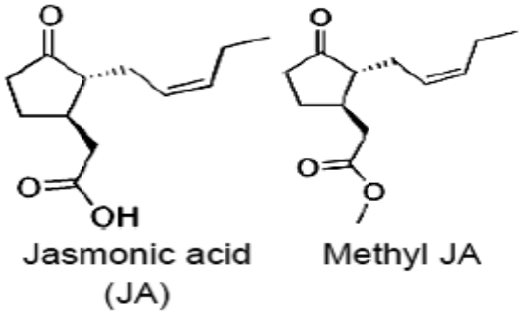
for plant defense against herbivory and plant responses to poor environmental conditions and other kinds of abiotic and biotic challenges. Some JAs can also be released as volatile organic compounds (VOCs) to permit communication between plants in anticipation of mutual dangers.

The isolation of methyl jasmonate from jasmine oil derived from *Jasminum grandiflorum* led to the discovery of the molecular structure of jasmonates and their name.

Chemical structure

Jasmonates (JA) are an oxylipin, i.e. a derivative of oxygenated fatty acid. It is biosynthesized from linolenic acid in chloroplast membranes. Synthesis is initiated with the conversion of linolenic acid to 12-oxo-phytodienoic acid (OPDA), which then undergoes a reduction and three rounds of oxidation to form (+)-7-iso-JA, jasmonic acid. Only the conversion of linolenic acid to OPDA occurs in the chloroplast; all subsequent reactions occur in the peroxisome. JA itself can be further metabolized into active or inactive derivatives. Methyl JA (MeJA) is a volatile compound that is potentially responsible for interplant communication. JA conjugated with amino acid isoleucine (Ile) results in JA-Ile, which is currently the only known JA derivative needed for JA signaling. JA undergoes decarboxylation to give cis-jasmone.

Mechanism of signalling



Structures of active jasmonate derivatives

In general, the steps in jasmonate (JA) signaling mirror that of auxin signaling: the first step comprises E3 ubiquitin ligase complexes, which tag substrates with ubiquitin to mark them for degradation by proteasomes. The second step utilizes transcription factors to effect physiological changes. One of the key molecules in this pathway is JAZ, which serves as the on-off switch for JA signaling. In the absence of JA, JAZ proteins bind to downstream transcription factors and limit their activity. However, in the presence of JA or its bioactive derivatives, JAZ proteins are degraded, freeing transcription factors for expression of genes needed in stress responses. Because JAZ did not disappear in null *coi1* mutant plant backgrounds, protein COI1 was shown to mediate JAZ degradation. COI1 belongs to the family of highly conserved

F-box proteins, and it recruits substrates for the E3 ubiquitin ligase SCFCOI1. The complexes that ultimately form are known as SCF complexes. These complexes bind JAZ and target it for proteasomal degradation. However, given the large spectrum of JA molecules, not all JA derivatives activate this pathway for signaling, and the range of those participating in this pathway is unknown. Thus far, only JA-Ile has been shown to be necessary for COI1-mediated degradation of JAZ11. JA-Ile and structurally related derivatives can bind to COI1-JAZ complexes and promote ubiquitination and thus degradation of the latter.

This mechanistic model raises the possibility that COI1 serves as an intracellular receptor for JA signals. Recent research has confirmed this hypothesis by demonstrating that the COI1-JAZ complex acts as a co-receptor for JA perception.

Specifically, JA-Ile binds both to a ligand-binding pocket in COI1 and to a 20 aminoacid stretch of the conserved Jas motif in JAZ. This JAZ residue acts as a plug for the pocket in COI1, keeping JA-Ile bound in the pocket. Additionally, co-purification and subsequent removal of inositol pentakisphosphate (InsP5) from COI1 suggest InsP5 is a necessary component of the co-receptor and plays a role in potentiating the co-receptor complex. Once freed from JAZ, transcription factors can activate genes needed for a specific JA response. The best-studied transcription factors acting in this pathway belong to the MYC family of transcription factors, which are characterized by a basic helix-loop-helix (bHLH) DNA binding motif. These factors (of which there are three, MYC2, 3, and 4) tend to act additively. For example, a plant that has only lost one myc becomes more susceptible to insect herbivory than a normal plant. A plant that has lost all three will be as susceptible to damage as coi1 mutants, which are completely unresponsive to JA and cannot mount a defense against herbivory. However, while all these MYC molecules share functions, they vary greatly in expression patterns and transcription functions. For instance, MYC2 has a greater effect

on root growth compared to MYC3 or MYC4.

Additionally, MYC2 will loop back and regulate JAZ expression levels, leading to a negative feedback loop. These transcription factors all have different impacts on JAZ levels after JA signaling. JAZ levels in turn affect transcription factor and gene expression levels. In other words, on top of activating different response genes, the transcription factors can vary JAZ levels to achieve specificity in response to JA signals.

Function

Although jasmonate (JA) regulates many different processes in the plant, its role in wound response is best understood. Following mechanical wounding or herbivory, JA biosynthesis is rapidly activated, leading to expression of the appropriate response genes. For example, in the tomato, wounding produces defense molecules that inhibit leaf digestion in the insect's gut. Another indirect result of JA signaling is the volatile emission of JA-derived compounds. MeJA on leaves can travel airborne to nearby plants and elevate levels of transcripts related to wound response. In general, this emission can further upregulate JA synthesis and signaling and induce nearby plants to prime their defenses in case of herbivory.

Following its role in defense, JAs have also been implicated in cell death and leaf senescence. JA can interact with many kinases and transcription factors associated with senescence. JA can also induce mitochondrial death by inducing the accumulation of reactive oxygen species (ROSs). These compounds disrupt mitochondria membranes and compromise the cell by causing apoptosis, or programmed cell death. JAs' roles in these processes are suggestive of methods by which the plant defends itself against biotic challenges and limits the spread of infections. JA and its derivatives have also been implicated in plant development, symbiosis, and a host of other processes included in the list below.

By studying mutants overexpressing JA, one of the earliest discoveries made was that JA inhibits root growth. The mechanism behind this event is still not understood, but mutants in the COI1-dependent signaling pathway tend to show reduced inhibition, demonstrating that the COI1 pathway is somehow necessary for inhibiting root growth. JA plays many roles in flower development. Mutants in JA synthesis or in JA signaling in Arabidopsis present with male sterility, typically due to delayed development. The same genes promoting male fertility in Arabidopsis promote female fertility in tomatoes. Overexpression of 12-OH-JA can also delay flowering. JA and MeJA inhibit the germination of nondormant seeds and stimulate the germination of dormant seeds. High levels of JA encourage the accumulation of storage proteins; genes encoding vegetative storage proteins are JA responsive. Specifically, tuberonic acid, a JA derivative, induces the formation of tubers. JAs also play a role in symbiosis between plants and microorganisms; however, its precise role is still unclear. JA currently appears to regulate signal exchange and nodulation regulation between legumes and rhizobium. On the other hand, elevated JA levels appear to regulate carbohydrate partitioning and stress tolerance in mycorrhizal plants.

Role in pathogenesis

Pseudomonas syringae causes bacterial speck disease in tomatoes by hijacking the plant's jasmonate (JA) signalling pathway. This bacteria utilizes a type III secretion system to inject a cocktail of viral effector proteins into host cells.

One of the molecules included in this mixture is the phytotoxin coronatine (COR). JA-insensitive plants are highly resistant to *P. syringae* and unresponsive to COR; additionally, applying MeJA was sufficient to rescue virulence in COR mutant bacteria. Infected plants also expressed downstream JA and wound response genes but repressed levels of pathogenesis-related (PR) genes. All these data suggest COR acts through the JA pathway to invade host plants.

Activation of a wound response is hypothesized to come at the expense of pathogen defense. By activating the JA wound response pathway, *P. syringae* could divert resources from its host's immune system and infect more effectively. Plants produce N-acylamides that confer resistance to necrotrophic pathogens by activating JA biosynthesis and signalling. Arachidonic acid (AA), the counterpart of the JA precursor α-LeA occurring in metazoan species but not in plants, is perceived by plants and acts through an increase in JA levels concomitantly with resistance to necrotrophic pathogens. AA is an evolutionarily conserved signalling molecule that acts in plants in response to stress similar to that in animal systems.

Cross talk with other defense pathways

While the jasmonate (JA) pathway is critical for wound response, it is not the only signaling pathway mediating defense in plants. To build an optimal yet efficient defense, the different defense pathways must be capable of cross talk to fine-tune and specify responses to abiotic and biotic challenges.

One of the best studied examples of JA cross talk occurs with salicylic acid (SA). SA, a hormone, mediates defense against pathogens by inducing both the expression of pathogenesis-related genes and systemic acquired resistance (SAR), in which the whole plant gains resistance to a pathogen after localized exposure to it. Wound and pathogen response appear to be interact negatively. For example, silencing phenylalanine ammonia lyase (PAL), an enzyme synthesizing precursors to SA, reduces SAR but enhances herbivory resistance against insects.

Similarly, overexpression of PAL enhances SAR but reduces wound response after insect herbivory. Generally, it has been found that pathogens living in live plant cells are more sensitive to SA-induced defenses, while herbivorous insects and pathogens that derive benefit from cell death are more susceptible to JA defenses. Thus, this trade-off in pathways optimizes defense and saves plant resources.

Cross talk also occurs between JA and other plant hormone pathways, such as those of abscisic acid (ABA) and ethylene (ET). These interactions similarly optimize defense against pathogens and

herbivores of different lifestyles. For example, MYC2 activity can be stimulated by both JA and ABA pathways, allowing it to integrate signals from both pathways. Other transcription factors such as ERF1 arise as a result of JA and ET signaling. All these molecules can act in combination to activate specific wound response genes.
Finally, cross talk is not restricted for defense: JA and ET interactions are critical in development as well, and a balance between the two compounds is necessary for proper apical hook development in *Arabidopsis* seedlings. Still, further research is needed to elucidate the molecules regulating such cross talk.

9. Growth and developmental physiology: Embryogenesis and differentiation of plant organs Seed germination and seedling growth.

The vegetative phase of development begins with embryogenesis, but development continues throughout the life of a plant. Plant developmental biologists are concerned with questions such as, How does a zygote give rise to an embryo, an embryo to a seedling? How do new plant structures arise from preexisting structures? Organs are generated by cell division and expansion, but they are also composed of tissues in which groups of cells have acquired specialized functions, and these tissues are arranged in specific patterns. How do these tissues form in a particular pattern, and how do cells differentiate? What are the basic principles that govern the size increase (growth) that occurs throughout plant development?

Understanding how growth, cell differentiation, and pattern formation are regulated at the cellular, biochemical, and molecular levels is the ultimate goal of developmental biologists.

Embryogenesis

The developmental process known as **embryogenesis** initiates plant development. Although embryogenesis usually begins with the union of a sperm with an egg, forming a single-celled *zygote*, somatic cells also may undergo embryogenesis under special circumstances. Fertilization also initiates three other developmental programs: endosperm, seed, and fruit development. Here we will focus on embryogenesis because it provides the key to understanding plant development.
Embryogenesis transforms a single-celled zygote into a multicellular, microscopic, embryonic plant. A completed **embryo** has the basic body plan of the mature plant and many of the tissue types of the adult, although these are present in a rudimentary form.
In plants, as in all other eukaryotes, the union of one sperm with the egg forms a single-celled zygote. In angiosperms, however, this event is accompanied by a second fertilization event, in which another sperm unites with two polar nuclei to form the triploid endosperm nucleus, from which the ***endosperm*** (the tissue that supplies food for the growing embryo) will develop.
Embryogenesis occurs within the **embryo sac** of the ovule while the ovule and associated structures develop into the **seed**. Embryogenesis and endosperm development typically occur in parallel with seed development, and the embryo is part of the seed. Endosperm may also be part of the mature seed, but in some species the endosperm disappears before seed development is completed. Embryogenesis and seed development are highly ordered, integrated processes, both of which are initiated by double fertilization.
When completed, both the seed and the embryo within it become dormant and are able to survive long periods unfavorable for growth. The ability to form seeds is one of the keys to the evolutionary success of angiosperms as well as gymnosperms. the zygote is genetically programmed to develop in a particular way, and that cell division, cell expansion, and cell differentiation are tightly controlled during embryogenesis.
If these processes were to occur at random in the embryo, the result would be a clump of disorganized cells with no definable form or function. In this section we will examine these changes in greater detail. We will focus on molecular genetic studies that have been conducted with the model plant *Arabidopsis* that have provided insights into plant development.

Embryogenesis Establishes the Essential Features of the Mature Plant

Plants differ from most animals in that embryogenesis does not directly generate the tissues and organs of the adult. For example, angiosperm embryogenesis forms a rudimentary plant body, typically consisting of an embryonic axis and two cotyledons (if it is a dicot). Nevertheless, embryogenesis establishes the two basic developmental patterns that persist and can easily be seen in the adult plant:

1. The apical–basal axial developmental pattern.
2. The radial pattern of tissues found in stems and roots.

Embryogenesis also establishes the **primary meristems**. Most of the structures that make up the adult plant are generated after embryogenesis through the activity of merisstems. Although these primary meristems are established during embryogenesis, only upon germination will they become active and begin to generate the organs and tissues of the adult.

Axial patterning. Almost all plants exhibit an *axial polarity* in which the tissues and organs are arrayed in a precise order along a linear, or polarized, axis. The shoot apical meristem is at one end of the axis, the root apical meristem at the other. In the embryo and seedling, one or two cotyledons are attached just below the shoot apical meristem. Next in this linear array is the hypocotyl, followed by the root, the root apical meristem, and the root cap. This axial pattern is established during embryogenesis.

For example, whereas adventitious roots develop from the basal ends of stem cuttings, buds develop from the apical ends, even if they are inverted.

Radial patterning. Different tissues are organized in a precise pattern within plant organs. In stems and roots the tissues are arranged in a radial pattern extending from the outside of a stem or a root into its center. If we examine a root in cross section, for example, we see three concentric rings of tissues arrayed along a radial axis: An outermost layer of epidermal cells (the epidermis) covers a cylinder of cortical tissue (the cortex), which in turn overlies the vascular cylinder (the endodermis, pericycle, phloem, and xylem).

The **protoderm** is the meristem that gives rise to the epidermis, the **ground meristem** produces the future cortex and endodermis, and the **procambium** is the meristem that gives rise to the primary vascular tissue and vascular cambium.

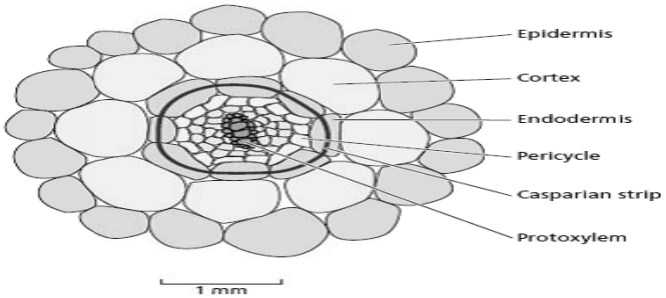


FIGURE 16.2 The radial pattern of tissues found in plant organs can be observed in a crosssection of the root. This crosssection of an *Arabidopsis* root was taken approximately 1 mm back from the root tip, a region in which the different tissues have formed.

***Arabidopsis* Embryos Pass through Four Distinct Stages of Development**

The *Arabidopsis* pattern of embryogenesis has been studied extensively and is the one we will present here, but keep in mind that angiosperms exhibit many different patterns of embryonic development, and this is only one type.

The most important stages of embryogenesis in *Arabidopsis*, and many other angiosperms, are these:

1. *The **globular stage** embryo*. After the first zygotic division, the apical cell undergoes a series of highly ordered divisions, generating an eight-cell (**octant**) globular embryo by 30 hours after fertilization. Additional precise cell divisions increase the number of cells in the sphere.

2. *The **heart stage** embryo*. This stage forms through rapid cell divisions in two regions on either side of
the future shoot apex. These two regions produce outgrowths that later will give rise to the cotyledons and give the embryo bilateral symmetry
3. *The **torpedo stage** embryo*. This stage forms as a result of cell elongation throughout the embryo axis and
further development of the cotyledons.
4. *The **maturation stage** embryo*. Toward the end of embryogenesis, the embryo and seed lose water and
become metabolically quiescent as they enter dormancy.

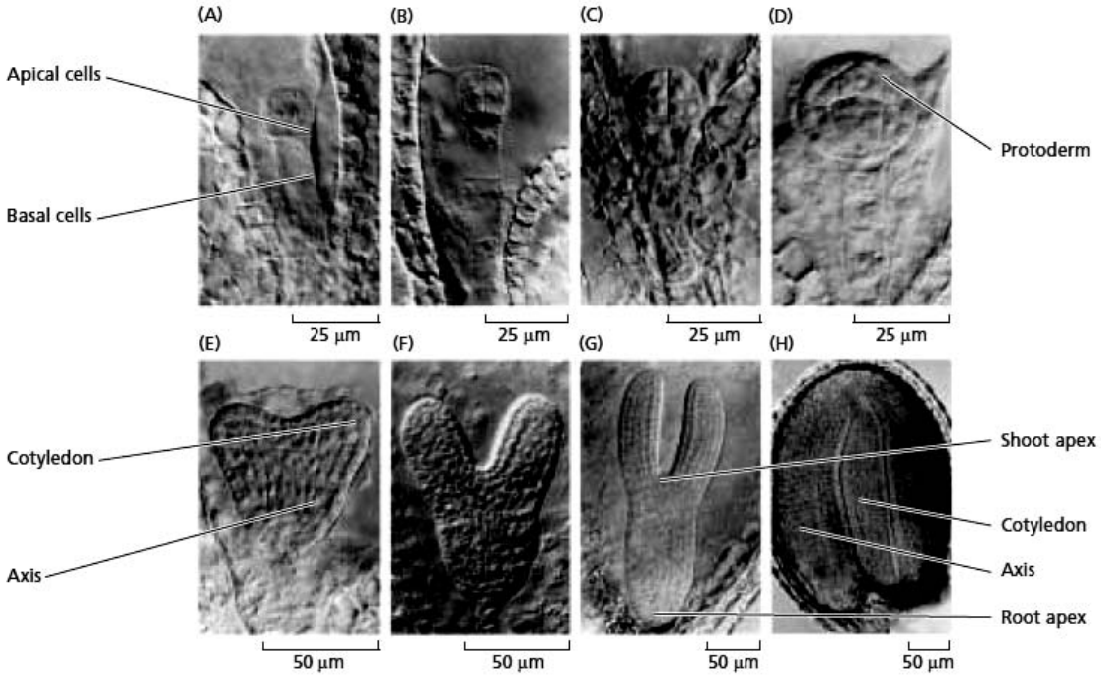


FIGURE 16.3 *Arabidopsis* embryogenesis is characterized by a precise pattern of cell division. Successive stages of embryogenesis are depicted here. (A) One-cell embryo after the first division of the zygote, which forms the apical and basal cells; (B) two-cell embryo; (C) eight-cell embryo; (D) early globular stage, which has developed a distinct protoderm (surface layer); (E) early heart stage; (F) late heart stage; (G) torpedo stage; (H) mature embryo. (From West and Harada 1993 photographs taken by K. Matsudaira Yee; courtesy of John Harada, © American Society of Plant Biologists, reprinted with permission.)

Cotyledons are food storage organs for many species, and during the cotyledon growth phase, proteins, starch, and lipids are synthesized and deposited in the cotyledons to be utilized by the seedling during the heterotrophic (nonphotosynthetic) growth that occurs after germination.
Although food reserves are stored in the *Arabidopsis* cotyledons, the growth of the cotyledons is not as extensive in this species as it is in many other dicots. In monocots, the food reserves are stored mainly in the endosperm. In *Arabidopsis* and many other dicots, the endosperm develops rapidly early in embryogenesis but then is reabsorbed, and the mature seed lacks endosperm tissue.

Embryogenesis Requires Specific Gene Expression

Analysis of *Arabidopsis* mutants that either fail to establish axial polarity or develop abnormally during embryogenesis has led to the identification of genes whose expression participates in tissue patterning during embryogenesis.

The* GNOM *gene: Axial patterning. Seedlings homozygous for mutations in the *GNOM* gene lack both roots and cotyledons. Defects in *gnom* embryos first appear during the initial division of the zygote, and they persist throughout embryogenesis. In the most extreme mutants, *gnom* embryos are

spherical and lack axial polarity entirely. We can conclude that *GNOM* gene expression is required for the establishment of axial polarity.1

The* MONOPTEROS *gene: Primary root and vascular tissue.

Mutations in the *MONOPTEROS* (*MP*) gene result in seedlings that lack both a hypocotyl and a root, although they do produce an apical region. The apical structures in the *mp* mutant embryos are not structurally normal, however, and the tissues of the cotyledons are disorganized (Berleth and Jürgens 1993). Embryos of *mp* mutants first show abnormalities at the octant stage, and they do not form a procambium in the lower part of the globular embryo, the part that should give rise to the hypocotyl and root. Although the *mp* mutant embryos lack a primary root when they germinate, they will form adventitious roots as the seedlings grow into adult plants.

The* SHORT ROOT *and* SCARECROW *genes: Ground tissue development.

Genes have been identified that function in the establishment of the radial tissue pattern in the root and hypocotyl during embryogenesis. These genes also are required for maintenance of the radial pattern during

postembryonic development. To identify these genes, investigators isolated *Arabidopsis* mutants that caused roots to grow slowly. Analysis of these mutants identified several that have defects in the radial tissue pattern. Two of the affected genes, *SHORT ROOT* (*SHR*) and *SCARECROW* (*SCR*), are necessary for tissue differentiation and cell differentiation not only in the embryo, but also in both primary and secondary roots and in the hypocotyl. Mutants of *SHR* and *SCR* both produce roots with a single- celled layer of ground tissue. Cells making up the single-celled layer of ground tissue have a mixed identity and show characteristics of both endodermal and cortical cells in plants with the *scr* mutation. These *scr* mutants also lack the cell layer called the **starch sheath**, a structure that is involved in the growth response to gravity.

The* HOBBIT *gene: The root meristem.

The primary root and shoot meristems are established during embryogenesis. Because in most cases they do not become active at this time, the term *promeristem* may be more appropriate to describe these structures. A **promeristem** may be defined as an embryonic structure that will become a meristem upon germination.

A molecular marker for the root promeristem has not yet been identified, but it appears to be determined early in embryogenesis. Root cap stem cells (the cells that divide to produce the root cap) are formed from the hypophysis at the heart stage of embryogenesis, indicating that the root promeristem is established at least by this stage of embryogenesis. The expression of the *HOBBIT* gene may be an early marker of root meristem identity (Willemsen et al. 1998).

The SHOOTMERISTEMLESS gene: The shoot promeristem.

The shoot promeristem can be recognized morphologically by the torpedo stage of embryogenesis in *Arabidopsis*. Oriented cell divisions of some of the cells between the cotyledons result in a layered appearance of this region that is characteristic of the shoot apical meristem (as described later in the chapter). However, the progenitors of these cells probably acquired the molecular identity of the shoot apical meristem cells much earlier, during the globular stage.

Embryo Maturation Requires Specific Gene Expression

The *Arabidopsis* embryo enters dormancy after it has generated about 20,000 cells. Dormancy is brought about by the loss of water and a general shutting down of gene transcription and protein

synthesis, not only in the embryo, but also throughout the seed. To adapt the cell to the special conditions of dormancy, specific gene expression is required.

For example, the *ABSCISIC ACID INSENSITIVE3* (*ABI3*) and *FUSCA3* genes are necessary for the initiation of dormancy and are sensitive to the hormone abscisic acid, which is the signaling molecule that initiates seed and embryo dormancy. *ABI3* also controls the expression of genes encoding the storage proteins that are deposited in the cotyledons during the maturation phase of embryogenesis.

The *LEAFY COTYLEDON1* (*LEC1*) gene also is active in late embryogenesis. Because *lec1* mutants cannot survive desiccation and do not enter dormancy, the embryos die unless they are rescued through isolation before desiccation occurs. The normal appearance and development of the mature *lec1* mutants indicates that the *LEC1* gene is required only during embryogenesis. Although the most obvious defects of the *lec1* mutants are seen only in the maturation phase embryo, mRNA from *LEC1* gene expression can be detected throughout embryogenesis.

Meristems in plant development

Meristems are populations of small, isodiametric (having equal dimensions on all sides) cells with embryonic characteristics. Vegetative meristems are self-perpetuating. Not only do they produce the tissues that will form the body of the root or stem, but they also continuously regenerate themselves. Ameristem can retain its embryonic character indefinitely, possibly even for thousands of years in the case of trees. The reason for this ability is that some meristematic cells do not become committed to a differentiation pathway, and they retain the capacity for cell division, as long as the meristem remains vegetative. Undifferentiated cells that retain the capacity for cell division indefinitely are said to be **stem cells**. Although historically called *initial cells* in plants, in function they are very similar, if not identical, to animal stem cells (Weigel and Jürgens 2002).

When stem cells divide, on average one of the daughter cells retains the identity of the stem cell, while the other is committed to a particular developmental pathway (Figure 16.12). Stem cells usually divide slowly. Their committed daughters, however, may enter a period of rapid cell division before they stop dividing and can be recognized as specific cell types. Stem cells represent the ultimate source of all the cells in the meristem and the entire rest of the plant— both roots, leaves, and other organs, as well as stems.

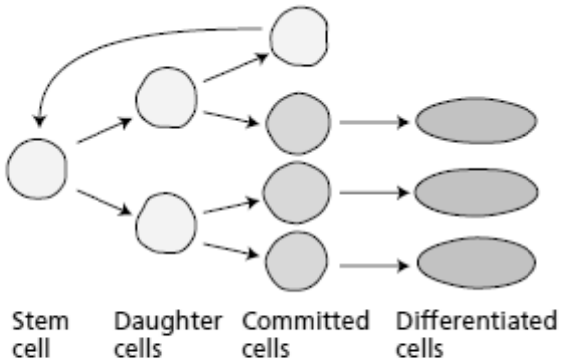


FIGURE 16.12 Stem cells generate daughter cells, some of which remain uncommitted and retain the property of stem cells, while others become committed to differentiate.

The Shoot Apical Meristem Contains Different Functional Zones and Layers

The shoot apical meristem consists of different functional regions that can be distinguished by the orientation of the cell division planes and by cell size and activity. The angiosperm vegetative shoot apical meristem usually has a highly stratified appearance, typically with *three distinct layers of cells*. These layers are designated **L1**, **L2**, and **L3**, where L1 is the outermost layer (Figure 16.13). Cell

divisions are anticlinal in the L1 and L2 layers; that is, the new cell wall separating the daughter cells is oriented at right angles to the meristem surface. Cell divisions tend to be less regularly oriented in the L3 layer. Each layer has its
own stem cells, and all three layers contribute to the formation of the stem and lateral organs.
Active apical meristems also have an organizational pattern called **cytohistological zonation**. Each zone is composed of cells that may be distinguished not only on the basis of their division planes, but also by differences in size and by degrees of vacuolation (see Figure 16.13B). These zones exhibit different patterns of gene expression, reflecting the different functions of each zone (Nishimura et al. 1999; Fletcher and Meyerowitz 2000).

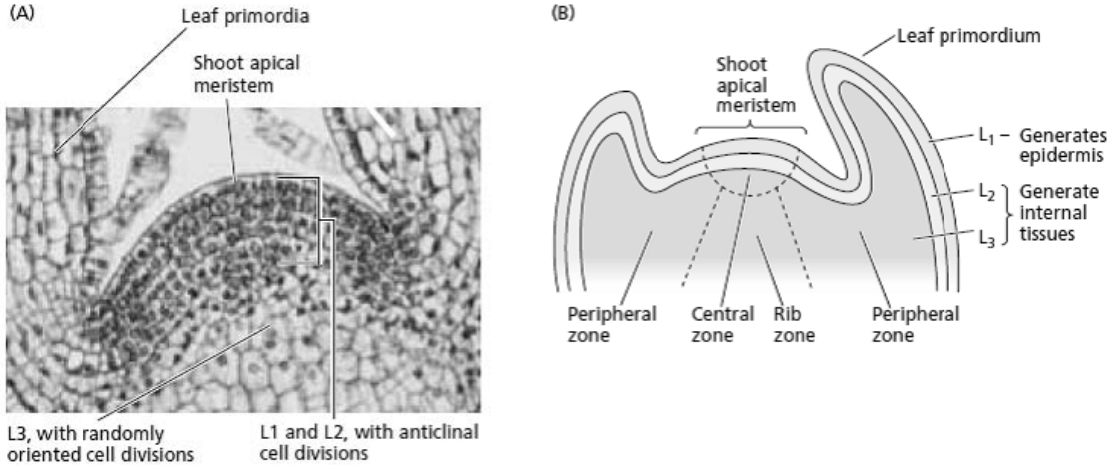


FIGURE 16.13 The shoot apical meristem generates the aerial organs of the plant. (A) This longitudinal section through the center of the shoot apex of *Coleus blumei* shows the layered appearance of the shoot apical meristem. Most cell divisions are anticlinal in the outer L1 and L2 layers, while the planes of cell divisions are more randomly oriented in the L3 layer. The outermost (L1) layer generates the shoot epidermis; the L2 and L3 layers generate internal tissues. (B) The shoot apical meristem also has cytohistological zones, which represent regions with different identities and functions. The central zone contains the stem cells, which divide slowly but are the ultimate source of the tissues that make up the plant body. The peripheral zone, in which cells divide rapidly, surrounds the central zone and produces the leaf primordia. A rib zone lies below the central zone and generates the central tissues of the stem. (A ©J. N. A. Lott/Biological Photo Service.)

The center of an active meristem contains a cluster of relatively large, highly vacuolate cells called the **central zone**. The central zone is somewhat comparable to the quiescent center of root meristems (which will be discussed later in the chapter). Adoughnut-shaped region of smaller cells, called the **peripheral zone**, flanks the central zone. A **rib zone** lies underneath the central cell zone and gives rise to the internal tissues of the stem.
These different zones most likely represent different developmental domains. The peripheral zone is the region in which the first cell divisions leading to the formation of leaf primordia will occur. The rib zone contributes cells that become the stem. The central zone contains the pool of stem cells, some fraction of which remains uncommitted, while others replenish the rib and peripheral zone populations (Bowman and Eshed 2000).

Leaf development

The leaves of most plants are the organs of photosynthesis. This is where light energy is captured and used to drive the chemical reactions that are vital to the life of the plant.

Although highly variable in size and shape from species to species, in general leaves are thin, flat structures with dorsiventral polarity. This pattern contrasts with that of the shoot apical meristem and stem, both of which have radial symmetry. Another important difference is that leaf primordial exhibit determinate growth, while the vegetative shoot apical meristem is indeterminate. As described in the

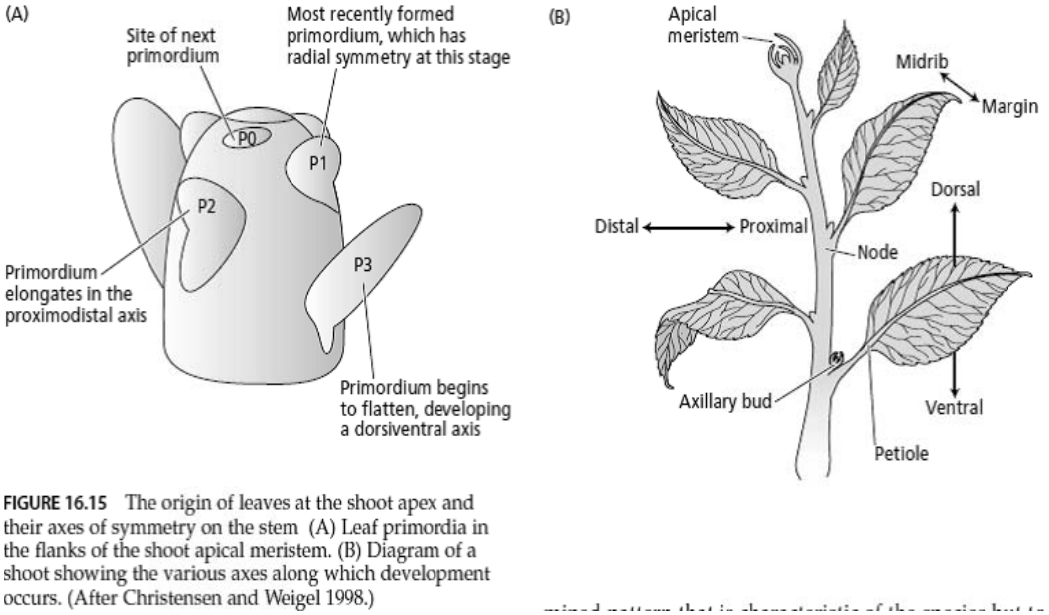


FIGURE 16.15 The origin of leaves at the shoot apex and their axes of symmetry on the stem (A) Leaf primordia in the flanks of the shoot apical meristem. (B) Diagram of a shoot showing the various axes along which development occurs. (After Christensen and Weigel 1998.)

sections that follow, several distinct stages can be recognized in leaf development (Sinha 1999).

Stage 1: Organogenesis. Asmall number of cells in the L1 and L2 layers in the flanks of the apical dome of the shoot apical meristem acquire the **leaf founder cell** identity. These cells divide more rapidly than surrounding cells and produce the outgrowth that represents the **leaf primordium** (plural *primordia*) (Figure 16.15A). These primordial subsequently grow and develop into leaves.

Stage 2: Development of suborgan domains. Different regions of the primordium acquire identity as specific parts of the leaf. This differentiation occurs along three axes: **dorsiventral** (abaxial–adaxial), **proximodistal** (apical–basal), and **lateral** (margin–blade–midrib) (Figure 16.15B). The upper (adaxial) side of the leaf is specialized for light absorption; the lower (abaxial) surface is specialized for gas exchange. Leaf structure and maturation rates also vary along the proximodistal and lateral axes.

Stage 3: Cell and tissue differentiation. As the developing leaf grows, tissues and cells differentiate. Cells derived from the L1 layer differentiate as epidermis (epidermal cells, trichomes, and guard cells), derivatives of the L2 layer differentiate as the photosynthetic mesophyll cells, and vascular elements and bundle sheath cells are derived from the L3 layer. These cells differentiate in a genetically determined pattern that is characteristic of the species but to some degree modified in response to the environment.

The Arrangement of Leaf Primordia Is Genetically Programmed

The timing and pattern with which the primordia form is genetically determined and usually is a characteristic of the species. The number and order in which leaf primordial form is reflected in the subsequent arrangement of leaves around the stem, known as **phyllotaxy** (Figure 16.16).

Root development

Roots are adapted for growing through soil and absorbing the water and mineral nutrients in the capillary spaces between soil particles. These functions have placed constraints on the evolution of root structure. For example, lateral appendages would interfere with their penetration through the soil. As a result, roots have a streamlined axis, and no lateral organs are produced by the apical meristem.

Branch roots arise internally and form only in mature, nongrowing regions. Absorption of water and minerals is enhanced by fragile root hairs, which also form behind the growth zone. These long, threadlike cells greatly increase the root's absorptive surface area.

The Root Tip Has Four Developmental Zones

Roots grow and develop from their distal ends. Although the boundaries are not sharp, four developmental zones can be distinguished in a root tip: the root cap, the meristematic zone, the elongation zone, and the maturation zone (Figure 16.17).

These four developmental zones occupy only a little more than a millimeter of the tip of the *Arabidopsis* root. The developing region is larger in other species, but growth is still confined to the tip. With the exception of the root cap, the boundaries of these zones overlap considerably:

The **root cap** protects the apical meristem from mechanical injury as the root pushes its way through the soil. Root cap cells form by specialized root cap stem cells. As the root cap stem cells produce new cells, older cells are progressively displaced toward the tip, where they are eventually sloughed

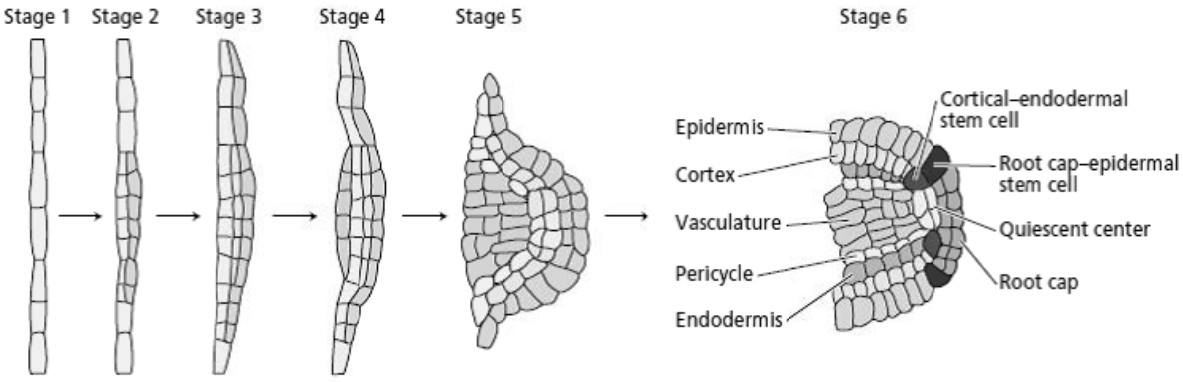


FIGURE 16.18 Model for lateral root formation in *Arabidopsis*. Six major stages are shown in the development of the primordium. The different tissue types are designated by colors. By stage 6, all tissues found in the primary root are present in the typical radial pattern of the branch root. (From Malamy and Benfey 1997.)

off. As root cap cells differentiate, they acquire the ability to perceive gravitational stimuli and secrete mucopolysaccharides (slime) that help the root penetrate the soil.

• The **meristematic zone** lies just under the root cap, and in *Arabidopsis* it is about a quarter of a millimeter

long. The root meristem generates only one organ, the primary root. It produces no lateral appendages.

• The **elongation zone**, as its name implies, is the site of rapid and extensive cell elongation. Although some cells may continue to divide while they elongate within this zone, the rate of division decreases progressively to zero with increasing distance from the meristem.

• The **maturation zone** is the region in which cells acquire their differentiated characteristics. Cells enter

the maturation zone after division and elongation have ceased. Differentiation may begin much earlier, but cells do not achieve the mature state until they reach this zone. The radial pattern of differentiated tissues becomes obvious in the maturation zone. Later in the chapter we will examine the differentiation and maturation of one of these cell types, the tracheary element.

As discussed earlier, lateral or branch roots arise from the pericycle in mature regions of the root. Cell divisions in the pericycle establish secondary meristems that grow out through the cortex and epidermis, establishing a new growth axis (Figure 16.18). The primary and the secondary root meristems behave similarly in that divisions of the cells in the meristem give rise to progenitors of all the cells of the root.

Cell differentiation

Differentiation is the process by which a cell acquires metabolic, structural, and functional properties that are distinct from those of its progenitor cell. In plants, unlike animals, cell differentiation is frequently reversible, particularly when differentiated cells are removed from the plant and placed in tissue culture. Under these conditions, cells dedifferentiate (i.e., lose their differentiated characteristics), reinitiate cell division, and in some cases, when provided with the appropriate nutrients and hormones, even regenerate whole plants.

This ability to dedifferentiate demonstrates that differentiated plant cells retain all the genetic information

required for the development of a complete plant, a property termed **totipotency**. The only exceptions to this rule are cells that lose their nuclei, such as sieve tube elements of phloem, and cells that are dead at maturity, such as vessel elements and tracheids (collectively referred to as tracheary elements) in xylem.

Initiation and regulation of Developmental pathways

Rapid progress has been made in identifying genes that play critical roles in regulating growth, cell differentiation, and pattern formation. This progress is largely a consequence of an intensive, international effort focused on *Arabidopsis*— first to sequence its genome, and subsequently to understand the function of all of its genes. However, many important discoveries have been made as a result of studies with other species, including *Antirrhinum*, maize, petunia, tomato, and tobacco.

However, many of these developmentally important genes have been found to encode either transcription factors (proteins with the ability to bind to specific DNA sequences and thus control the expression of other genes) or components of signaling pathways. The nature of these genes suggests some possible ways that development might be regulated.

Where these molecular genetic studies have been coupled with clonal analysis, cell biological, physiological,

and/or biochemical studies, it has been possible to identify important principles of plant development. Although we are far from a complete understanding, these insights include the following:

• The expression of genes that encode transcription factors determines cell, tissue, and organ identity.
• The fate of a cell is determined by its position and not its clonal history.
• Developmental pathways are controlled by networks of interacting genes.
• Development is regulated by cell-to-cell signaling.

Transcription Factor Genes Control Development

Transcription factors are proteins that have an affinity for DNA. They are able to turn the expression of genes on or off by binding to specific DNA sequences.

These 1500 transcription factor genes belong to numerous families. However, many members of two of

these families—the MADS box and homeobox genes**—** have been found to be particularly important in plant development.

MADS box genes are key regulators of important biological functions in plants, animals, and fungi.2 There are about 30 MADS box genes in the *Arabidopsis* genome, many of which control aspects of development. Specific MADS box genes are important for developmental events in the root, leaf, flower, ovule, and fruit (Riechmann and Meyerowitz 1997).

Homeobox genes encode homeodomain proteins that act as transcription factors. **Homeodomain proteins** play a major role in regulating developmental pathways in all eukaryotes. As with the MADS box genes, each homeobox gene participates in regulating a unique developmental event by controlling the expression of a unique set of target genes.

Many Plant Signaling Pathways Utilize Protein Kinases

Protein kinases are ATP-dependent enzymes that add phosphate groups to proteins. Protein phosphorylation is a key regulatory mechanism that is utilized extensively to regulate the activity of enzymes and transcription factors. Although widely utilized by all eukaryotes, plant genomes are especially rich in genes that encode these enzymes. The *Arabidopsis* genome contains over 1200

genes that encode protein kinases. Of these, more than 600 encode *receptor protein kinases* (Shiu and Bleecker 2001).

The functions of most of these receptor protein kinases are unknown, but recently some have been shown to play important signaling roles in plant development. *Arabidopsis* has two such genes: *BRI1*, which encodes a receptor kinase that functions in brassinosteroid signaling and *CLAVATA1* (*CLV1*), which encodes a

receptor kinase that participates in regulating the size of the uncommitted cell population in shoot apical meristem.

A Cell's Fate Is Determined by Its Position

In both the root and shoot meristem, a small number of stem cells are the ultimate source of any particular tissue, and most of the cells in a given tissue are clonal, having arisen. Inappropriate expression of the *KN1* gene during leaf development causes severe abnormalities around the leaf veins. The gain-of-function mutation *kn1*causes cell proliferation after normal cell division ceases; in addition, the division planes are abnormal, causing gross distortion of the blade surface.

However, most evidence supports the view that *cell fate does not depend on cell lineage, but instead is determined by positional information* (Scheres 2001). In the vast majority of cases, shoot epidermal cells are derived from a small number of stem cells in the L1 layer. However, the derivatives of the L1 layer are committed to become epidermal cells because they occupy the outermost layer and lie on top of the cortical cell layer, not because they were clonally derived from the stem cells in the L1 layer.

The plane in which a cell divides will determine the position of its daughter cells within a tissue, and this positioning in turn plays the most significant role in determining the fate of the daughter cells. The strongest evidence for the importance of position in determining a cell's ultimate fate comes from an examination of the fate of cells that are displaced from their usual position, such that they come to occupy a different layer.

Developmental Pathways Are Controlled by Networks of Interacting Genes

We have a great deal more to learn about the regulatory networks that control developmental pathways. However, several discoveries point to a model in which local and long-distance signaling events control the expression of genes that encode transcription factors. These transcription factors in turn determine the character or activities of a given tissue or cell. Often these mechanisms involve feedback loops in which two or more genes interact to regulate each other's expression. These interactions are seen most clearly in the case of the shoot apical meristem.

Expression of the *KNOX* gene *STM* (*SHOOTMERISTEMLESS*) is essential for the formation of the shoot apical meristem in the *Arabidopsis* embryo and for meristem function in the growing plant. *STM* is expressed throughout the apical dome of the vegetative meristem, except in the developing leaf primordia. Similarly, *STM* is expressed in the dome of the floral meristem, but it is silenced as floral organs appear. Two additional *KNOX* genes—*KNAT1* and *KNAT2*—also are expressed in the apical meristem of *Arabidopsis* and participate in maintaining the meristem cells in an undifferentiated state.

Development Is Regulated by Cell-to-Cell Signaling

How do cells know where they are? If a cell's fate is determined by its position and not by clonal lineage, then cells must be able to sense their position relative to other cells, tissues, and organs. Neighboring cells and distant tissues and organs provide positional information. Cells in multicellular plants usually are in close contact with others around them, and the behavior of each cell is carefully

coordinated with that of its neighbors throughout the life of the plant. Furthermore, each cell occupies a specific position within the tissue and organ to which it belongs.

Coordination of cellular activity requires cell–cell communication. That is, some developmentally important genes act *nonautonomously*. They do not have to be expressed in a given cell to affect the fate of that cell. Agiven gene or set of genes can exert an effect on development in neighboring cells or even cells in distant tissues through cell–cell communication, via at least three different mechanisms:

1. Ligand-induced signaling
2. Hormonal signaling
3. Signaling via trafficking of regulatory proteins and/or mRNAs

Other signaling mechanisms remain to be discovered.

The mechanism by which cells communicate has not been established in other cases, although it is clear that positional information is exchanged between cells in different tissues.

As presented earlier, the *SHR* and *SCR* genes are important for the establishment of the radial tissue patterns in roots. They encode rather similar transcription factors, but these two genes are expressed and function in different tissues. *SCR* is required for the asymmetric cell division that forms the epidermis and cortex, and it also determines the endodermis cell fate. *SCR* is expressed in the stem cell that will give rise to the ground tissue before it divides asymmetrically to form the precursors of endodermis and cortex.

The analysis of plant growth

How do plants grow? This deceptively simple question has challenged plant scientists for more than 150 years. New cells form continually in the apical meristems. Cells enlarge slowly in the apical meristem and more rapidly in the subapical regions. The resulting increase in cell volume can range from severalfold to 100-fold, depending on the species and environmental conditions. Classically, plant growth has been analyzed in terms of cell number or overall size (or mass).

However, these measures tell only part of the story. Tissue growth is neither uniform nor random. The derivatives of the apical meristems expand in predictable and site-specific ways, and the expansion patterns in these subapical regions largely determine the size and shape of the primary plant body. The total growth of the plant can be thought of as the sum of the local patterns of cell expansion.

The analysis of the motions of cells or “tissue elements” (and the related problem of cell expansion) is called *kinematics*. In this section we will discuss both the classical definitions of growth and the more modern, kinematic approach. As we will see, the advantage of the kinematic approach is that it allows one to describe the growth patterns of organs mathematically in terms of the expansion patterns of their component cells.

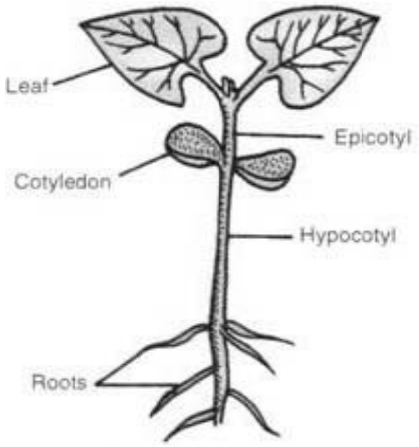


Fig. 3.3(*b*). Epicotyl and Hypocotyl.

Seed Germination:

Germination is a process by which the embryo in the seed becomes activated and begins to grow into a new seedling.

Types of Germination:

There are two types of germination:

1. Epigeal Germination:

In this type of germination, the hypocotyl elongates rapidly and arches upwards pulling the cotyledons which move above the soil. Bean, cotton, papaya, gourd, castor and onion have germination of this kind.

2. Hypogeal Germination:

In this type of germination, the epicotyl elongates and the cotyledons remain below the soil. Pea, mango, maize, rice, gram and groundnut have germination of this kind.

Epicotyl → The upper portion of the axis of the seedling above the cotyledons.

Hypocotyl → The portion of seedling between the cotyledons and the radicle.

3. Vivipary (Viviparous Germination):

Vivipary is the phenomenon of giving birth to young ones in advanced stage of development. It occurs in mammals (among animals) and mangrove plants. In mangrove plants (e.g., Rhizophora, Sonneratia, Heritiera) the seeds cannot germinate on the ground because of the excessive salt content and lack of oxygen in marshy habitat. In such plants seed dormancy is absent.

The embryo of the seed (present inside the fruit) continues growth while the latter is attached to the parent plant. Hypocotyl elongates and pushes the radicle out of the seed and the fruit. Growth continues till the hypocotyl and radicle become several centimetres long (more than 70 cm in Rhizophora). The seedling becomes heavy.

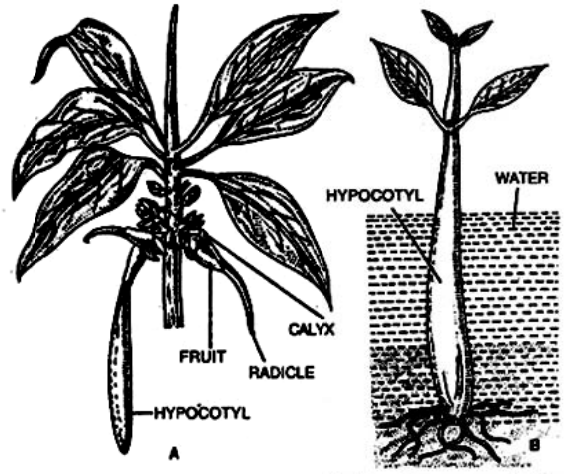


Fig. 4.9. Vivipary. A. twig of *Rhizophora* showing viviparous germination, **B. A seedling has become established on tidal soil.**

10. Senescence and programmed cell death: Types of senescence, metabolic changes associated with senescence and its regulation, influence of hormones.

Senescence is the process of aging in plants. Plants have both stress-induced and age-related developmental aging. Chlorophyll degradation during leaf senescence reveals the carotenoids, and is the cause of autumn leaf color in deciduous trees. Leaf senescence has the important function of recycling nutrients, mostly nitrogen, to growing and storage organs of the plant. Unlike animals, plants continually form new organs and older organs undergo a highly regulated senescence program to maximize nutrient export.

The plants or their organs like all other living organisms have a certain span of life during which they develop, grow, attain maturity and after some time at the end die. But prior to death, distinctive but natural deteriorative processes occur in them in growth and synthetic activities.

These deteriorative processes that naturally terminate their functional life are collectively called as senescence and the plants or plant organs at this stage are called as senescent. These deteriorative processes may terminate in death either gradually or abruptly depending upon the plant. Senescence is a normal energy dependent developmental process which is controlled by plants own genetic programme and the death of the plant or plant part consequent to senescence is called as programmed cell death (PCD).

Senescence is not confined only to whole plant. It may be limited to a particular plant organ such as leaf and flowers or cells such as phloem and xylem or cell-organelles such as chloroplasts and mitochondria etc. Senescence is closely associated with the phenomenon of aging and both are sometimes considered as the same by many workers. But according to Medawar (1957), the term senescence should be used to refer to natural changes towards termination of life while ‘aging’ to refer to changes in time without reference to the natural development of death.

Types of senescence:

Leopold (1961) has recognised 4 types of senescence patterns in whole plant (Fig17.41) which are as follows:

1. Overall Senescence:

This type of senescence occurs in annuals where whole of the plant is affected and dies.

2. Top Senescence:

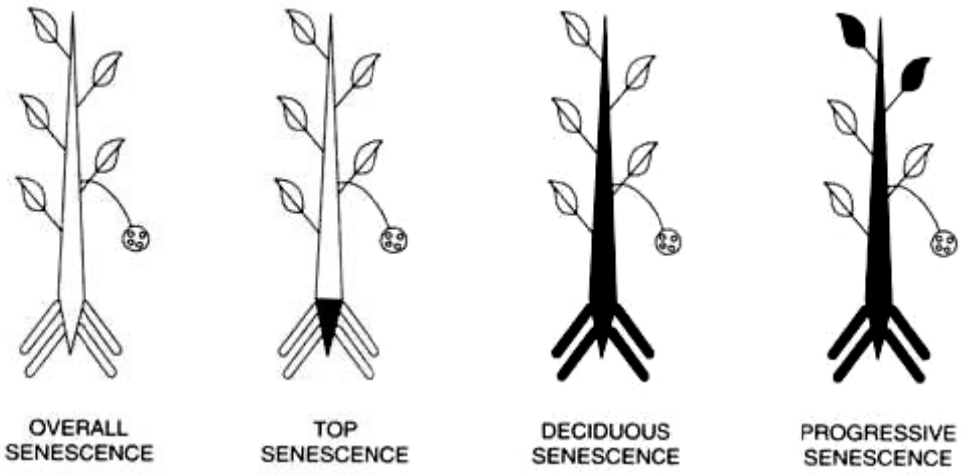
This is represented by perennial herbs where senescence occurs only in the above ground parts, the root system and underground system remaining viable.

3. Deciduous Senescence:

This type of senescence is less drastic and takes place in woody deciduous plants. Here senescence occurs in all the leaves simultaneously but the bulk of the stem and root system remains alive.

4. Progressive Senescence:

This is characterized by gradual progression of senescence and death of leaves from the base upwards as the plant grows. (The senescence of the entire plant after a single reproductive cycle is also known as monocarpic senescence) Senescence can best be studied in leaves or similar other organs of plants e.g., cotyledons, sepals, petals etc. or cell organelles like isolated chloroplast.



Some of the metabolic changes recognized so far are given briefly here:

i. Senescing cells and tissues are metabolically very active and an ordered series of cytological and biochemical events occur during senescence.

ii. Senescence is characterized by increased respiration, declining photosynthesis and an orderly disintegration of macromolecules.

iii. At the cellular level, chloroplasts are the first organelles to be disintegrated. Nuclei remain structurally and functionally intact until the last stage of senescence. Meanwhile, other cell organelles and bio-membranes also gradually deteriorate.

iv. Expression of senescence down-regulated genes (SDGs) decreases. Such genes encode proteins in photosynthesis and other biosynthetic processes. Concentration of growth promoting hormones especially cytokinins decline.

v. Expression of senescence associated genes (SAGs) increases. Such genes encode hydrolytic enzymes such as proteases, ribonucleases and lipases as well as enzymes involved in biosynthesis of deteriorative hormones such as abscisic acid (ABA) and ethylene.

vi. Some of the SAGs have secondary functions in senescence that are useful to plant. These genes encode enzymes that are involved in conversion and remobilization of nutrients and substrates from senescing tissues and their reallocation to other parts of the plant that survive (i.e., not senescing).

vii. Brilliant Colors are developed in leaves of many plants during senescence.

viii. This is due to degradation of chlorophylls, resulting in unmasking of more stable carotenoid pigments.

ix. Towards the end of senescence, the cells and tissues also lose respiratory control.
x. It is marked by general decline in ATP synthesis and chloroplast activities.
xi. Decrease in DNA and RNA
xii. Semi permeability of cytoplasmic membranes in the cells starts declining.
xiii. Impairment of the process of formation of new cells to replace the old and dead cells.
xiv. Due to anomalies in protein synthesis caused by senescence chromosomal aberrations and gene mutations may occur in the body cells which may be fatal.

Several environmental factors especially those which suppress normal plant growth also tend to enhance the rate of senescence. These are deficiency of soil nutrients, high temperatures, water deficit, darkness etc. In many plants on the other hand, removal of flowers, fruits and vegetative growing points can markedly delay the senescence of leaves. The role of externally supplied cytokinins in delaying senescence especially in detached plant parts is also well established.

Regulation of Senescence Process in Plants:

Senesce processes are tightly controlled processes in which the sequence of events is usually highly ordered until the terminal stages (death) are underway. Various factors which control the processes of senescence in different organs during life cycle of a flowering plant are given below.

It is evident from these details that at all stages a wide range of growth substances appears to be involved:

(a) Senescence of root: which also includes senescence of vascular tissues, root cap and hairs, is regulated by auxins and cytokinins.
(b) Senescence of cotyledons: is regulated by axis, shoot, light fruit, root and all hormones.
(c) Senescence of leaf: which also includes senescence of abscission zone, is regulated by fruit, root, light, all hormones and ethylene.
(d) Senescence of stem: which also includes senescence of vascular tissues, is regulated by auxins, cytokinins and sugars.
(e) Senescence of fruit: which also includes senescence of seeds is controlled by all hormones and ethylene.
(f) Senescence of flower: is regulated by pollination, ABA (Abscisic acid), cytokinins and ethylene.
(g) Senescence of apex: is regulated by fruits, day-length and gibberellins.
(h) Senescence of whole plant (monocarpism): is regulated by fruits, roots, day-length, auxins, ABA (Abscisic acid) and cytokinins.

Hormonal influence of senescence:

Programmed senescence seems to be heavily influenced by plant hormones. The hormones abscisic acid, ethylene, jasmonic acid and salicylic acid are accepted by most scientists as promoters of senescence, but at least one source lists gibberellins, brassinosteroids and strigolactone as also being involved. Cytokinins help to maintain the plant cell and expression of cytokinin biosynthesis genes late in development prevents leaf senescence.A withdrawal of or inability of the cell to perceive cytokinin may cause it to undergo apoptosis or senescence. In addition, mutants that cannot perceive ethylene show delayed senescence. Genome-wide comparison of mRNAs expressed during dark-induced senescence versus those expressed during age-related developmental senescence demonstrate that jasmonic acid and ethylene are more important for dark-induced (stress-related) senescence while salicylic acid is more important for developmental senescence.

Annual versus perennial benefits

Some plants have evolved into annuals which die off at the end of each season and leave seeds for the next, whereas closely related plants in the same family have evolved to live as perennials. This may be a programmed "strategy for the plants.

The benefit of an annual strategy may be genetic diversity, as one set of genes does continue year after year, but a new mix is produced each year. Secondly, being annual may allow the plants a better survival strategy, since the plant can put most of its accumulated energy and resources into seed production rather than saving some for the plant to overwinter, which would limit seed production. Conversely, the perennial strategy may sometimes be the more effective survival strategy, because the plant has a head start every spring with growing points, roots, and stored energy that have survived through the winter. In trees for example, the structure can be built on year after year so that the tree and root structure can become larger, stronger, and capable of producing more fruit and seed than the year before, out-competing other plants for light, water, nutrients, and space. This strategy will fail when environmental conditions change rapidly. If a certain bug quickly takes advantage and kills all of the nearly identical perennials, then there will be a far lesser chance that a random mutation will slow the bug compared to more diverse annuals.

Plant self-pruning

There is a speculative hypothesis on how and why a plant induces part of itself to die off. The theory holds that leaves and roots are routinely pruned off during the growing season whether they are annual or perennial. This is done mainly to mature leaves and roots and is for one of two reasons; either both the leaves and roots that are pruned are no longer efficient enough nutrient acquisition-wise or that energy and resources are needed in another part of the plant because that part of the plant is faltering in its resource acquisition.

•Poor productivity reasons for plant self pruning – the plant rarely prunes young dividing meristematic cells, but if a fully grown mature cell is no longer acquiring nutrients that it should acquire, then it is pruned.

oShoot efficiency self pruning reasons – for instance, presumably a mature shoot cell must on average produce enough sugar, and acquire enough oxygen and carbon dioxide to support both it and a similar sized root cell. Actually, since plants are obviously interested in growing it is arguable, that the "directive" of the average shoot cell, is to "show a profit" and produce or acquire more than enough sugar and gases than is necessary to support both it and a similar sized root cell. If this "profit" isn't shown, the shoot cell is killed off and resources are redistributed to "promising" other young shoots or leaves in the hope that they will be more productive.

oRoot efficiency self pruning reasons – similarly a mature root cell must acquire on average, more than enough minerals and water needed to support both it and a similar sized shoot cell that does not acquire water and minerals. If this does not happen, the root is killed off and resources sent to new young root candidates.

•Shortage/need-based reason for plant self pruning – this is the other side of efficiency problems.

oShoot shortages – if a shoot is not getting enough root derived minerals and water, the idea is that it will kill part of itself off, and send the resources to the root to make more roots.

oRoot shortages – the idea here is that if the root is not getting enough shoot derived sugar and gases it will kill part of itself off and send resources to the shoot, to allow more shoot growth.

This is an oversimplification, in that it is arguable that some shoot and root cells serve other functions than to acquire nutrients. In these cases, whether they are pruned or not would be "calculated" by the plant using some other criteria. It is also arguable that, for example, mature nutrient-acquiring shoot cells would have to acquire more than enough shoot nutrients to support both it and its share of both shoot and root cells that do not acquire sugar and gases whether they are of a structural, reproductive, immature, or just plain, root nature.

The idea that a plant does not impose efficiency demands on immature cells is that most immature cells are part of so-called dormant buds in plants. These are kept small and non-dividing until the plant needs them. They are found in buds, for instance in the base of every lateral stem.

Theory of hormonal induction of senescence

There is little theory on how plants induce themselves to senesce, although it is reasonably widely accepted that some of it is done hormonally. Plant scientists generally concentrate on ethylene and abscisic acid as culprits in senescence, but neglect gibberellin and brassinosteroid which inhibits root

growth if not causing actual root pruning. This is perhaps because roots are below the ground and thus harder to study.

1. Shoot pruning – it is now known that ethylene induces the shedding of leaves much more than abscisic acid. ABA originally received its name because it was discovered to have a role in leaf abscission. Its role is now seen to be minor and only occurring in special cases.

oHormonal shoot pruning theory – a new simple theory says that even though ethylene may be responsible for the final act of leaf shedding, it is ABA and strigolactones that induces senescence in leaves due to a run away positive feedback mechanism. What supposedly happens is that ABA and strigolactones are released by mostly mature leaves under water and or mineral shortages. The ABA and strigolactones act in mature leaf cells however, by pushing out minerals, water, sugar, gases and even the growth hormones auxin and cytokinin (and possibly jasmonic and salicylic acid in addition). This causes even more ABA and strigolactones to be made until the leaf is drained of all nutrients. When conditions get particularly bad in the emptying mature leaf cell, it will experience sugar and oxygen deficiencies and so lead to gibberellin and finally ethylene emanation. When the leaf senses ethylene it knows its time to excise.

2. **Root pruning** – the concept that plants prune the roots in the same kind of way as they abscise leaves, is not a well discussed topic among plant scientists, although the phenomena undoubtedly exists. If gibberellin, brassinosteroid and ethylene are known to inhibit root growth it takes just a little imagination to assume they perform the same role as ethylene does in the shoot,that is to prune the roots too.

o**Hormonal root pruning theory** – in the new theory just like ethylene, GA, BA and Eth are seen both to be induced by sugar (GA/BA) and oxygen (ETH) shortages (as well as maybe excess levels of carbon dioxide for Eth) in the roots, and to push sugar and oxygen, as well as minerals, water and the growth hormones out of the root cell causing a positive feedback loop resulting the emptying and death of the root cell. The final death knell for a root might be strigolactone or most probably ABA as these are indicators of substances that should be abundant in the root and if they cannot even support themselves with these nutrients then they should be senesced.

3**. Parallels to cell division** – the theory, perhaps even more controversially, asserts that just as both auxin and cytokinin seem to be needed before a plant cell divides, in the same way perhaps ethylene and GA/BA (and ABA and strigolactones) are needed before a cell would senesce.

Senescence is the process of aging in plants. Plants have both stress-induced and age-related developmental aging. Chlorophyll degradation during leaf senescence reveals the carotenoids, and is the cause of autumn leaf color in deciduous trees. Leaf senescence has the important function of recycling nutrients, mostly nitrogen, to growing and storage organs of the plant. Unlike animals, plants continually form new organs and older organs undergo a highly regulated senescence program to maximize nutrient export.

The plants or their organs like all other living organisms have a certain span of life during which they develop, grow, attain maturity and after some time at the end die. But prior to death, distinctive but natural deteriorative processes occur in them in growth and synthetic activities.

These deteriorative processes that naturally terminate their functional life are collec¬tively called as senescence and the plants or plant organs at this stage are called as senes¬cent. These deteriorative processes may terminate in death either gradually or abruptly depending upon the plant. Senescence is a normal energy dependent developmental process which is controlled by plants own genetic programme and the death of the plant or plant part con¬sequent to senescence is called as programmed cell death (PCD).

Senescence is not confined only to whole plant. It may be limited to a particular plant organ such as leaf and flowers or cells such as phloem and xylem or cell-organelles such as chloroplasts and mitochondria etc. Senescence is closely associated with the phenomenon of aging and both are sometimes considered as the same by many workers. But according to Medawar (1957), the term senes¬cence should be used to refer to natural changes towards termination of life while ‘aging’ to refer to changes in time without reference to the natural development of death**.**

11. **Control of flowering: Floral meristem and floral organ development, floral evocation.**

Growth and development of plants are influenced by several environmental factors including light. However, light causes several responses other than photosynthesis. These responses greatly influence the course of plant development and the final plant appearance. They are photomorphogenetic responses.

For example, the seeds of many plants do not germinate unless they are exposed to light. Germination of seeds in light shows that the seedlings require light to grow. Phototropic responses of seedlings and of leaves of mature plants are also beneficial photomorphogenetic processes.

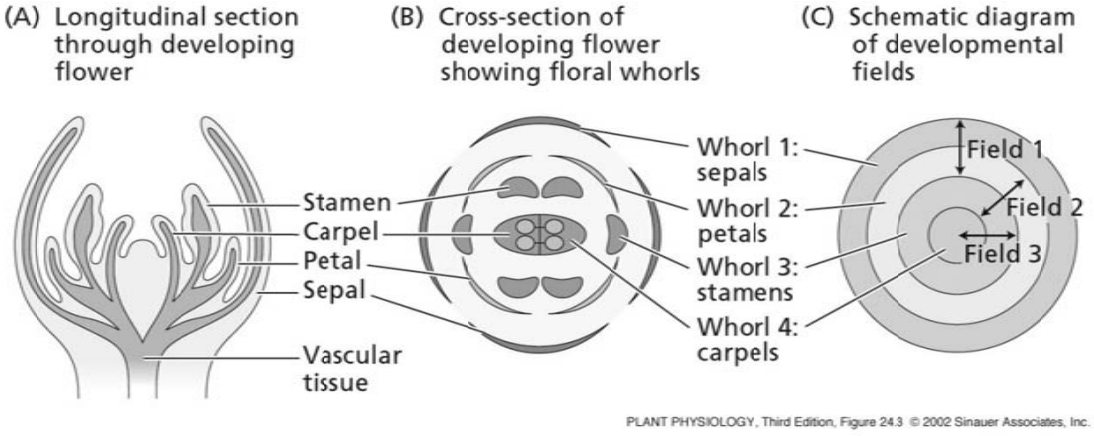
Photomorphogenetic responses are also important to older plants. Many such responses respond to the relative lengths of day and night by forming reproductive structures or by forming dormant buds that can resist a cold winter (i.e., phenomenon of photoperiodism and vernalization.)

Flower formation is a transitional phase in the life cycle of a plant. It is of immense importance for perpetuation and origin of variability in the next generations. Flower initiation takes place by the transformation of vegetative apex into a reproductive structure.It signifies a transition from vegetative to the floral state. The shoot meristem is reduced and is also induced to develop sepals, petals, stamens and carpels in place of leaves. The pattern and timing of flower initiation vary from species to species. Thus, the flowers may be terminal, axillary, single or grouped into an inflorescence. A plant must attain a specific state of 'ripeness to respond' before it flowers.Once this stage is reached, then it can be induced to flower. Thus by and large, two phases are recognized at this point. These are flower induction and flower differentiation. Both the processes are subjected to variable and diverse controls. The induction of flowering implies whereby all the cells of the shoot meristem instead of giving rise to leaves are turned towards the formation of floral organs.Once such a switch over has been accomplished then the differentiation steps leading to flower formation follow. Of the two phases, the former has attracted the attention of several plant physiologists for a long time. One simple reason could be that once the induction of flowering has been accomplished, the differentiation would follow automatically.With experience it is now widely accepted that during flower induction a plant must be subjected, for specific period of time, to an external condition e.g., light, temperature or even some chemicals, etc.This time interval is generally referred to as induction period and the external conditions as inductive-conditions. Similarly, the external conditions under which a given plant continues to grow vegetatively is called non-inductive conditions.The two main inductive conditions (i.e., light and temperature), which induce flowering. The flowering response to day length is called photoperiodism while low temperature treatment is called vernalization.A large number of plant species undergo a state of vigorous vegetative growth and during this period, they cannot be induced to flower. This is called a juvenile phase. It is as if such species must attain 'ripeness-to-flower' even though suitable conditions are provided. On the contrary, several grass species like Loliumremotum, L. temulentum can be made to flower at any stage of growth.The duration for ripeness-to-flower varies considerably. It may be a few days (*Xanthium*), a few weeks (*Lunaria*) or even several years (Mains, *Citrus*). In such instances, the plant must develop specific number of nodes and also minimal number of leaves before flowering is induced. It is this difference in the number of leaves which is critical for different species to flower at variable times.In a longitudinal section of the vegetative shoot apex, a central dome or corpus is seen along with one or several layered tunica. Corpus is differentiated into central mother cell zone, flank meristem and rib meristem. During the transition of vegetative apex into reproductive primordium, several histological and biochemical changes occur.One of these is an increase in the mitotic activity between rib meristem and central mother zone. Cells derived from this zone become central core of floral primordium. The reproductive shoot is an enlarged structure with several cells. Such a transformation also induces synthesis of nucleic acids.Through the usage of antimetabolites i.e., inhibitors of nucleic acids synthesis, the

suppression of such transformation is clearly made out. In Chenopodium there is a rapid increase in RNA content following photoperiodic induction. Similarly, with radio-active precursors of RNA an enormous amount of incorporation was observed in Lolium following inductive long-day conditions.Murret (1977) has reviewed on the environmental interaction and the genetics of flowering. In brief, it may be said that before the initiation of flowering, there is reprogramming of genetic activity in the shoot apices. It is generally assumed that stimulus induces some of the passive genes blocks.The latter are activated and these undertake synthesis of new messenger RNA; such genes are activated floral genes. The RNA thus synthesized controls flowering through the synthesis of new enzyme systems.It is also believed that the flowering stimulus is a transmissible molecule which has the potentiality to combine with and inactivate the repressor i.e., floral gene repressors. Moreover, DNA may also be exerting a significant regulatory role through nucleotides. Genes may influence flowering through enzyme synthesis, membrane and transport phenomenon, energy supply, switching and regulator)' mechanism, etc.In tomato, flower differentiation is solely determined by genotype of the plant. In several plant species in addition to genetic make-up, day length, intensity and quality of light, temperature and mineral nutrition also influence flower formation enormously. In fact, the response of plants to the factors mentioned above is an adaptive property for their perpetuation during unfavourable conditions.

Floral meristem and floral organ development

The four different types of floral organs are initiated sequentially in separate, concentric whorls.



Two major types of genes regulate floral development

1. **Meristem identity genes :** Positive regulators of floral organ identity in the developing floral meristem. Encode transcription factors that are necessary for initial induction of organ identity genes.

- **FLORICAULA (FLO) :**
- Identified from snapdragon (*Antirrhinum*)
- *flo* mutants develop an inflorescence that does not produce flowers i.e. develop additional inflorescence meristems in the bract axils.
- The wild type FLO gene controls the determining step in which floral meristem identity is established.
- **LEAFY (LFY)** and **SUPRESSOR OF CONSTANS 1 (SOC1):**
- LFY is *Arabidopsis* version of FLO gene.
- Both LFY and SOC1 serve as master regulators for the initiation of floral development.
- **APETALA1 (AP1) :** another floral meristem identity gene identified from *Arabidopsis*

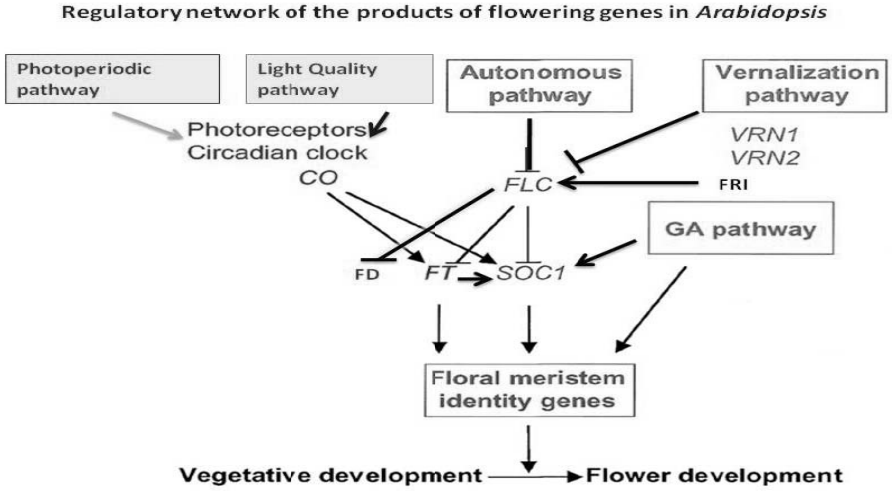
2. **Floral organ identity genes :** directly control floral identity. The proteins encoded by these genes are transcription factors that likely control the expression of other genes whose products are involved in the formation and/ or function of floral organs.

- The genes that determine floral organ identity are known as **floral homeotic genes**.
- Most of plant homeotic genes belong to a class of relate sequences known as **MADS box genes**.
- The **MADS box genes** have a charateristic conserved nucleotide sequence known as **MADS box**, which encodes a protein known as **MADS domain**.
- The MADS domain enables these transcription factors to bind DNA at specific site.
- Some floral homeotic genes: **DEFICIENS** gene of snapdragon and the **AGAMOUS (AG)**, **PISTILLATA (PI)** and **APETALA3 (AP3)** genes of *Arabidopsis*.

Mutations in homeotic floral identity genes alter the types of organs produced in each of whorls.

Control of Flower Primordium Specification by LEAFY and APETALAI/CAULI FLOWER:

The



knowledge regarding the mechanism by which flowers are specified on the flanks of the shoot apex is well understood though same cannot be said regarding mechanisms involved in the production of floral stimulus in the leaves.

The available information is:

i. Replacement of flowers with indeterminate shoots in lfy and apcal double mutants indicates that LFY and API/CAL are crucial for flower primordia specifications.

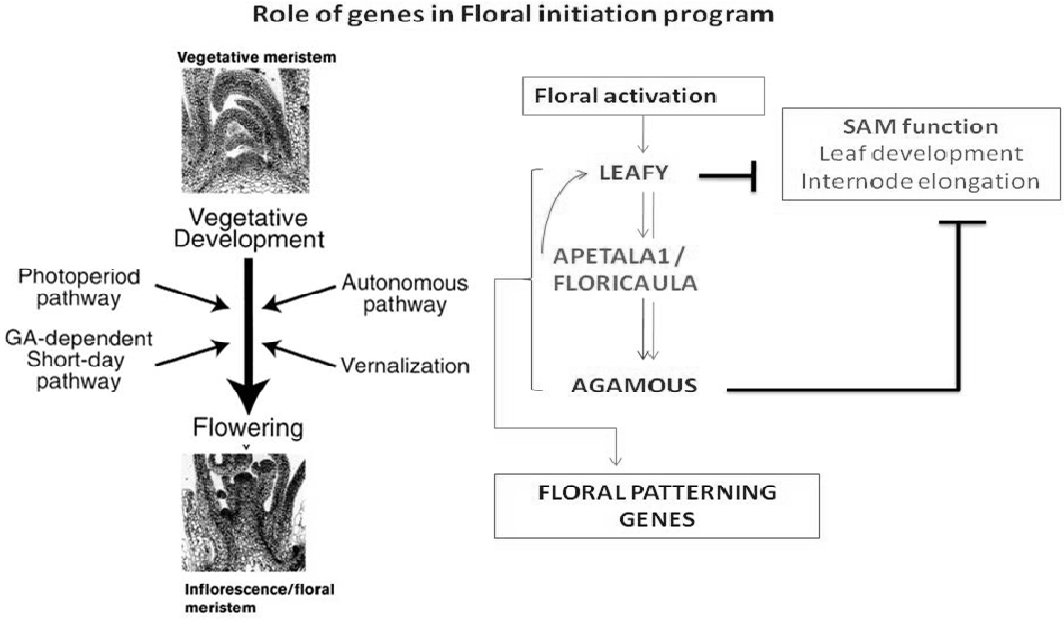
ii. In ecotypically expressed LFY and API these genes are sufficient to specify flowers when expressed in shoot primordia.

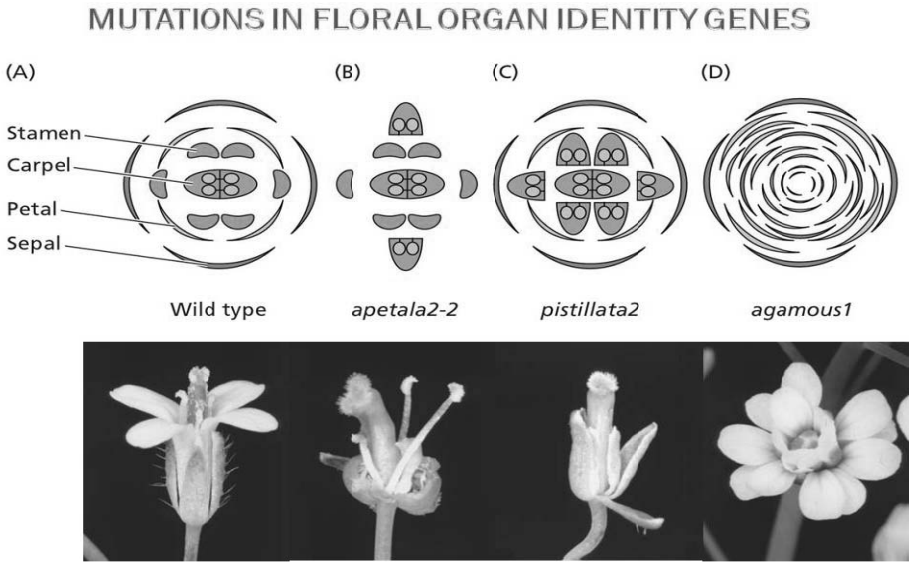
iii. Many other genes may be involved in the specifications of flowers along with LFY and API/CAL.

Hempel (2000) have discussed molecular interactions between LFY and MADS-BOX genes, API/CAL and AGAMOUS (AG). In wild type LFY is expressed throughout flower primordia early in their ontogeny. API and CAL expression also takes place throughout flower primordia, though the expression of these two genes occurs in primordia only after they have become distinct from the meristem.

The upregulation of API during floral induction treatments does not occur until several hours after LFY has been upregulated. In lfy mutants, API expression is weak and delayed whereas ecotypic expression of LFY induces the ecotypic expression of LFY induces the ecotypic expression of API in leaf primordia and in axillary flower primordia.

These data show that LFY is a formal regulator of API. The recent data also show that the API promoter is a direct target of LFY, and it has a LFY-responsive enhancer that is needed for its activity. Clearly LFY has direct and distinct roles in the specification of flowers and in the patterning of floral organs.





Interactions between LFY, API and TERMINAL FLOWERI (TFLI) during the Transition to Flowering: It is the interactions between these genes (LFY and API/CAL) and TFLI that inflorescence

is regulated. TFLI prevents the expression of floral meristem identity genes in the shoot meristem and promotes indeterminate growth.

ABC model of flowering:

The ABC model of flower development in angiosperm demonstrates the presence of three classes of genes that regulate the development of floral organs. The genes are referred to as class A genes, class B genes and class C gene. These genes and the interaction between them induce the development of floral organs.Many literatures on molecular genetics and Internet Websites provide articles on ABC model. In the following essay the basic concept of ABC model will be discussed in brief. The analysis of ABC model is based on the use of molecular genetics and formulated on the observation that mutants induce right floral organs to develop in wrong whorls.

In the flower of angiosperms there are usually four concentric whorls of organs, i.e. sepal, petal, stamen and carpel that are formed in whorl 1, whorl 2, whorl 3 and whorl 4 respectively, the whorl 1 being on the peripheral side. In the whorl 1 class A genes when expressed induce the development of sepals. The interaction between class A and class B genes induce the development of petals in the whorl 2. Stamens are formed in the whorl 3 as a result of interaction between class B and class C genes. In the whorl 4 class C gene induces the formation of carpel. So the summary of ABC model is: class A genes together and class C gene alone are responsible for the development of sepals and carpel respectively. The class B genes and class A genes function cooperatively to determine the development of petals. The class B genes and class A genes function cooperatively to determine the development of stamens.

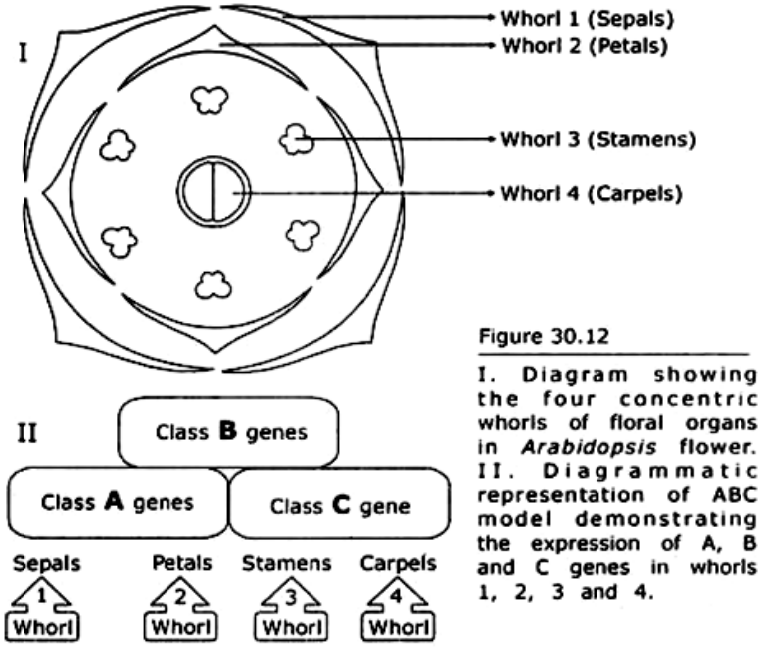


Figure 30.12

I. Diagram showing the four concentric whorls of floral organs in *Arabidopsis* flower. II. Diagrammatic representation of ABC model demonstrating the expression of A, B and C genes in whorls 1, 2, 3 and 4.

Coen et al. (1991) formulated the ABC model. While analyzing the mutations affecting flower structure Coen et al. identified the class ABC genes that direct flower development. They also formulated the molecular models of how floral meristem and organ identity may be specified. They

have shown that the distantly related angiosperm plants use homologous mechanisms in pattern formation of floral organs. Ex. Arabidopsis thaliana and *Antirrhinum majus*.

The following two have led to formulate ABC model:

(1) The discovery of homeotic mutants (homeotic genes identify specific floral organs and help the organ to develop in respective whorl. The homeotic mutant has inappropriate expression—that is, it induces right organ to develop in wrong whorl. As for example - petals emerge in the whorl where normally stamens develop).

(2) The observation that each of the genes that induce the formation of an organ in a flower has an effect on two groups of floral organs, i.e. sepal and petals or petals and stamens.

Class A, B and C genes are homeotic genes. They determine the identity of different floral organs and induce the organs to develop in their respective whorls. The homeotic mutants have defects in floral organ development and induce the right organs to develop in wrong whorls/place, i.e. one floral organ develops in the whorl, which is the normal position of another floral organs. Petals, for example, develop in the whorl where stamens are normally to be formed.In each whorl of a flower there is one or more homeotic genes and their cooperative functions determine the organ to be formed in that whorl. For example, the activity of class A genes is restricted to whorls 1 and 2. The class B genes have function in whorls 2 and 3. The class C gene functions in whorls 3 and 4.Another way of describing the function of class A, B and C genes is that —in whorl 1, the class A gene-function alone determines the formation of sepals; in whorl 2, class A and B gene-functions both determine the formation of petals; in whorl 3, class B and C gene-functions both determine the emergence of stamens and in whorl 4, class C gene-function alone determines the carpel formation.

In Arabidopsis there are two genes in class A, two genes in class B and one gene in class C. The most characteristic feature of these homeotic genes is in the identification of floral organs and in the determinacy of position / whorl of their emergence in a floral meristem. The two genes of class A and the two genes of class B act cooperatively.

The function of class A genes is confined to whorls 1 and 2. Similarly the function of class C gene is restricted in whorls 3 and 4. This can be interpreted in another way. In the whorls 1 and 2 the function of class A genes prevents class C gene from functioning in the same whorls. Similarly the function of class C gene prevents class A genes from functioning in the whorls 3 and 4.

Any mutation in class A genes with defects in floral organ development will invite class C gene to express in whorls 1 and 2. The class C gene, in class A mutants, will express in whorls 1 and 2 in addition to the normal whorls 3 and 4.

Similarly any mutation in class C gene with defects in floral organ development will lead to the encroachment of the function of class A genes. The class A genes will express in the whorls 3 and 4 in addition to the normal whorls 1 and 2.

Class	*Arabidopsis*
Class A	*APETALA 1 (AP1), APETALA 2 (AP2)*
Class B	*APETALA 3 (AP3), PISTILLATA (P1)*
Class C	*AGAMOUS (AG)*

Table 30.1
Illustrating Homeotic genes in *Arabidopsis*.

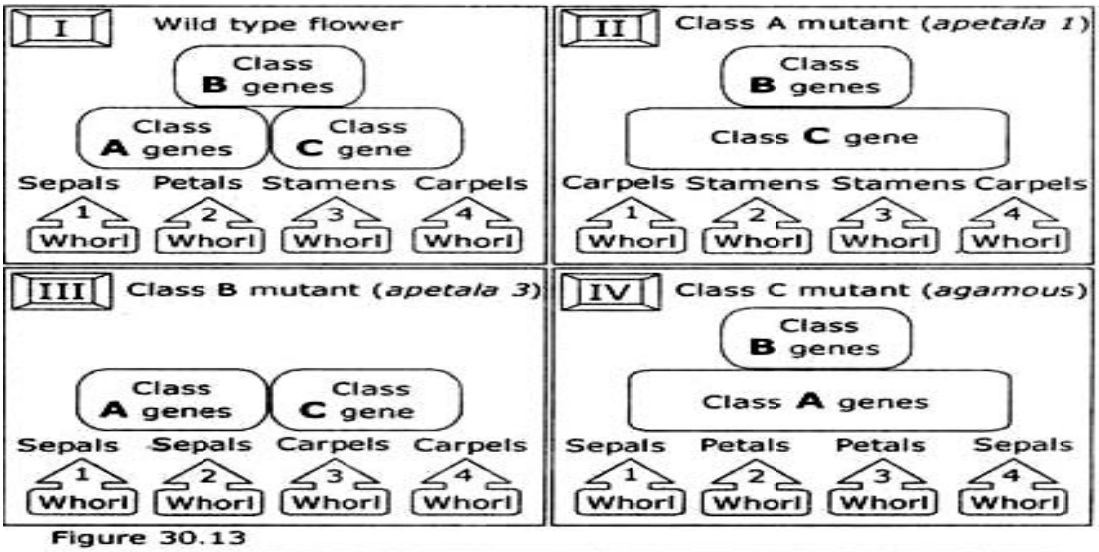


Figure 30.13

Diagrammatic illustrations of the floral organs formed in wild type and homeotic mutants of *Arabidopsis* flower. I. Wild type flower. The individual and cooperative expressions of class A, B and C genes are in the formation of sepals, petals, stamens and carpels in whorls of 1, 2, 3 and 4 respectively. II. Class A mutant, *apetala 1*. This homeotic mutant contains loss-of-function A genes. Class C gene expresses alone in whorl 1 in addition to whorl 4. Class C gene and class B genes together express in whorls 2 and 3. As a result stamens are formed in both whorls 2 and 3. III. Class B mutant, *apetala 3*. This homeotic mutant contains loss-of-function B genes. Class A genes express in whorls 1 and 2. As a result sepals are formed in both whorls 1 and 2. Class C gene expresses in whorls 3 and 4. So carpels are formed in both whorls 3 and 4. IV. Class C mutant, *agamous*. This homeotic mutant contains loss-of-function C gene. Class A genes express in whorls 3 and 4 in addition to whorls 1 and 2. As a result petals and sepals are formed in whorls 3 and 4 respectively. In whorl 3 class A and class B genes together form petals. In whorls 1 and 4 the sole group of class A genes expresses and so sepals are formed.

The following three examples of homeotic mutant genes will illustrate the above discussion:

(1) The flower of Arabidopsis with class A mutants, such as apetala 1(ap 1) shows the following pattern of floral organs (Fig. 3.13.II): whorl 1 shows bract-like structure with carpelloid characteristics; whorl 2 shows stamens; whorl 3 shows stamens and whorl 4 shows carpel.

The pattern of floral organ formation in whorls 1 and 2 is changed. In ap 1 mutants the activity of two genes of class A is lost. So the class C gene expressed in whorls 1 and 2 in addition to whorls 3 and 4. As a result carpelloid organ developed in whorl 1 and stamens formed in whorl 2. In the whorls 3 and 4 stamens and carpel respectively are formed similar to wild type (Fig. 30.13.I).

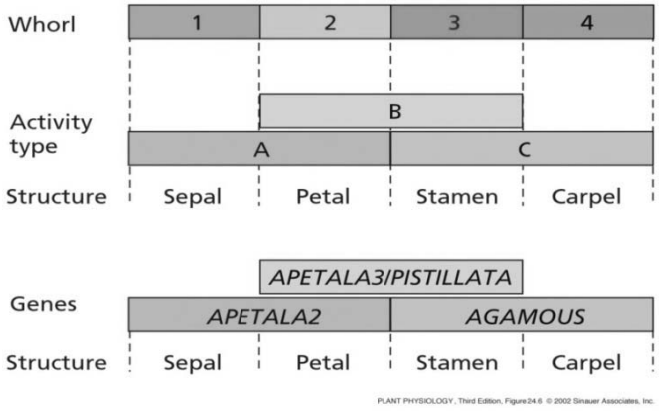
(2) Example: Flower of Arabidopsis with class B mutant, such as apetala 3 (ap 3): The flower shows sepals only both in whorls 1 and 2, while the whorls 3 and 4 show carpel only. Class B mutant

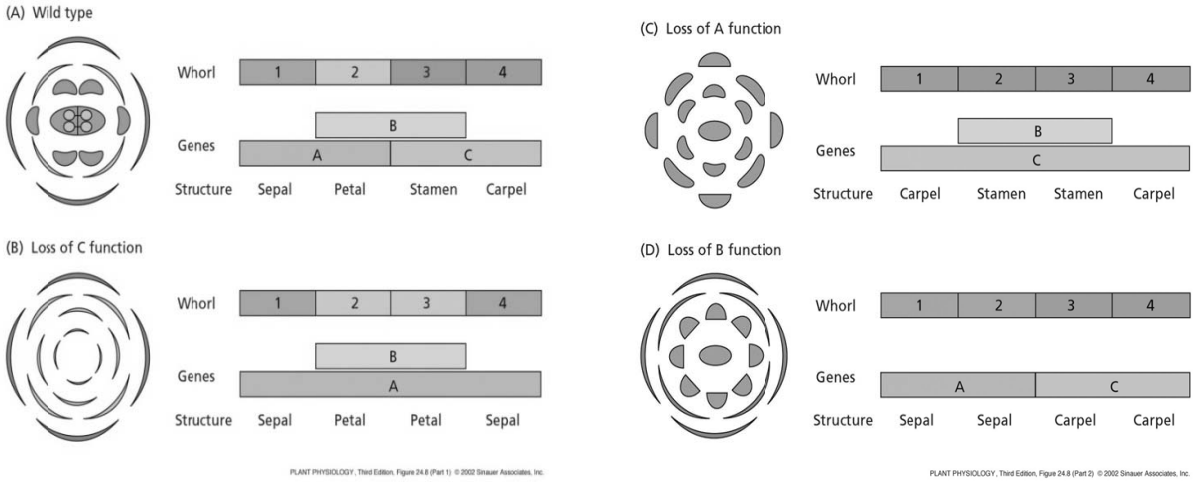
contains loss-of-function genes and as a result class A genes express in whorls 1 and 2; and class C gene alone expresses in whorls 3 and 4. In ap 3 mutants in whorl 2, sepals are formed instead of petals and in whorl 3, carpel is formed instead of stamens.

(3) In Arabidopsis the class C gene contains the sole gene agamous (ag). Arabidopsis flower with agamous (ag) mutant consists of many sepals and petals. The reproductive organs – stamens and carpel are not formed in the whorls 3 and 4. Class C gene with ag mutant contains loss-of-function gene. As a result class A genes express in whorls 3 and 4 in addition to 1 and 2. In ag mutant sepals and petals are formed in whorls 3 and 4 instead of stamens and carpel. The literature of Howell provides the scan electron micrograph of flower phenotypes of the floral homeotic mutants of class A, B and C genes.

In Arabidopsis it was observed that in all the mutants one homeotic gene remains functional in each whorl. The flower with class ABC triple mutant shows sepals in each whorl. In ABC triple mutant, the genes required for floral organ formation become nonfunctional. As a result sepals or leaves are formed in each whorl, as homeotic mutants specify no floral organs. This observation led Botanists to regard ‘flowers as modified leaves’ on the basis of molecular genetics.

The important feature of ABC model is that it can predict the type of floral organ to be induced to develop in any whorl. Krizek et al. (1996) was successful to induce any one of the four different floral parts in whorl 1 of Arabidopsis flower. This became possible by genetic manipulations of right combination of homeotic selector genes.The ABC model appears to be simple, but a completely different picture is obtained when it is analyzed on the basis of molecular genetics and in molecular terms.The analysis includes the structure of different classes of homeotic genes, the homeotic mutants, the co-operative function between homeotic genes, mutual exclusion in the expression of class A and C genes in the same whorl, the identification of floral homeotic genes and their isolation by cloning, the production of MADS box protein by homeotic mutants, the study of genes that mediate the interaction between floral meristem and floral organ development, presence or absence of different classes of transcription factors etc., the details of which can be obtained in the literatures on molecular genetics.





Arabidopsis thaliana belongs to the family Brassicaceae and has become the model organism for understanding the genetics and molecular biology of flowering plants like mice and Drosophila in animal researches due to following reasons:

(i) It has five chromosomes (n = 5) and so this small-size-genome is advantageous in gene mapping and sequencing.
(ii) The size of plant is small and so can be cultivated in a small space and requires modest indoor facilities.
(iii) It has rapid life cycle and takes about six weeks from germination to mature seeds.
(iv) An individual plant produces several thousand seeds.
(v) 'The Arabidopsis genome is among the smallest in higher plants, with a haploid size of about 100 megabases (mb) of DNA. With a small genome size it was expected that there would be fewer problems with gene duplication'— Howell.
(vi) It is easily transformable with T-DNA mediated transformation.

In 2004 ABCE model has been formulated. The characterization of sepallata 1, 2, 3 triple mutants in Arabidopsis has led to the above formulation. It is regarded that the class E genes have important role in the development of floral organs.

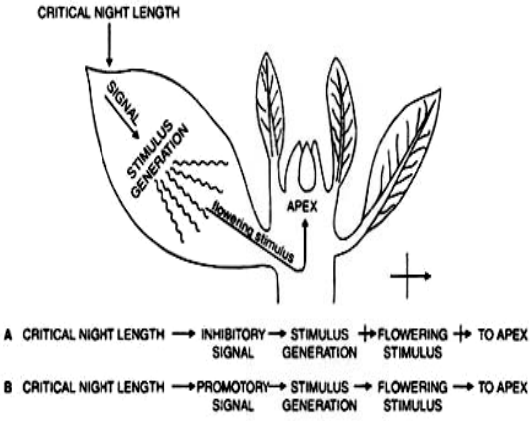


Fig. 22-12. Regulation of flowering in long day (A) and short day plants (B).

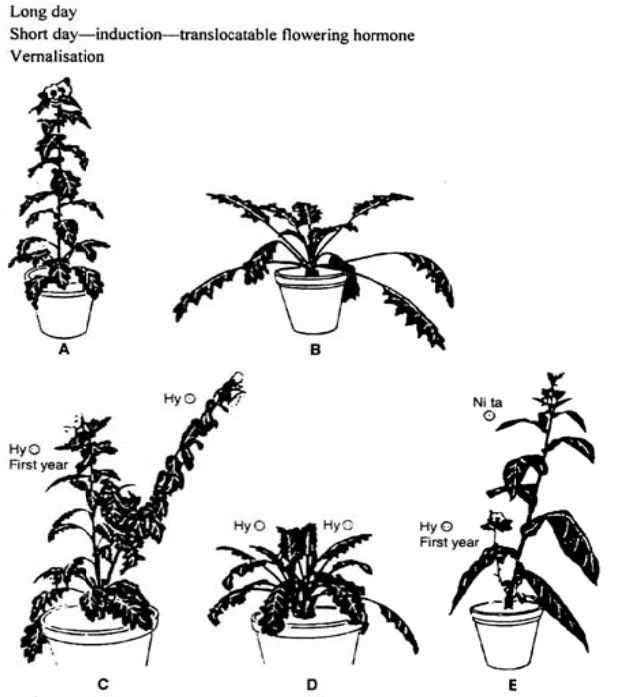


Fig. 22-12 A. Grafting experiments on *Hyoscyamus*. (A) Annual, (B) biennial in the first year, (C) annual (right) grafted on biennial in the first year, (D) control biennial graft on biennial in first year ; (E) *Nicotiana tabacum* graft on biennial in the first year (from Kuhn, 1965)

Floral Induction:
Floral induction is not a rapid process and the transformation of vegetative apex to flowering depends upon the intensity of stimulus. Different plant species require different number of inductive photoperiods for complete flowering. The inductive stimulus has to be of specific intensity and the induction can be reversed.

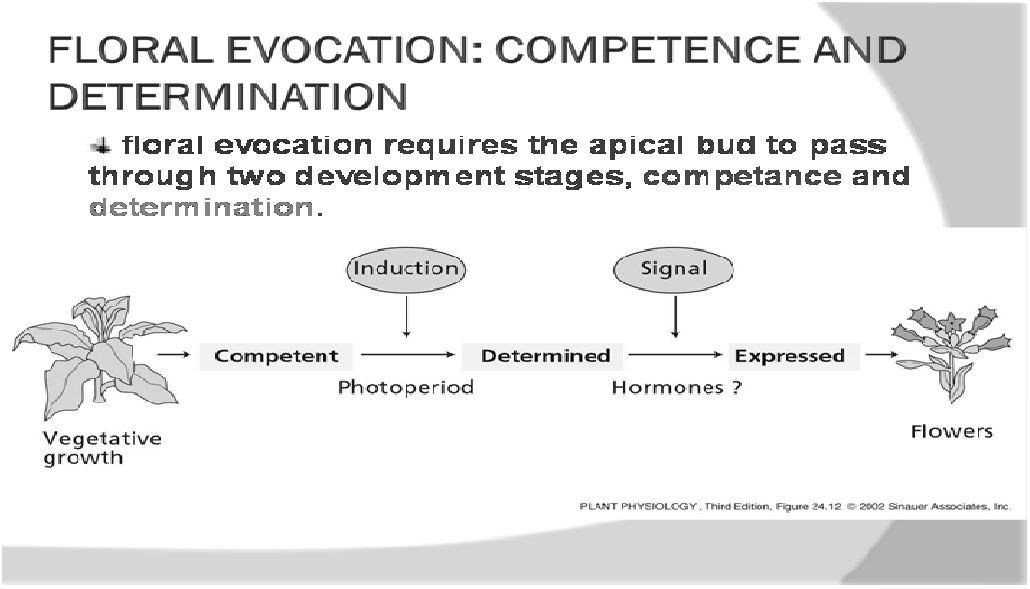
We have already shown that leaf is the real site of perception of the light signals that induce or stop flowering. Obviously something must be translocated from the leaf to the stem tip. According to the Russian plant physiologist C. Kh. Chailakhyan, flowering response needs atleast four steps and these are perception of stumulus; the transformation of the perceiving organ; the translocation of stimulus; the translocation of a stimulator and finally a specific response in the growing tip and thus the formation of flower.
It has come to be realized that perception is through phytochrome. The stimulus takes several hours to move out of the leaf and then initiate flowering. L.T. Evans (1971) has also shown that rate of translocation of the flowering stimulus varies in the LD and SD plants. He also showed that the role of stimulus was different from photosynthate. Flowering inducing stimulus are possibly translocated through phloem. .
Thus differences in LD and SD plants could be due to the concentrations of flowering hormone or else secondary reactions may be induced in the two types as follows:
In general it is believed that florigens are the floral stimulating substances. Florigens are in fact a concept. Some experiments have indicated the occurrence of flowering inhibitors. This inhibitor effect is opposite to that of florigen.

Floral evocation : the events occurring in the shoot apex that specifically commit the apical meristem to produce flowers.

Internal (autonomous) and external (environment-sensing) control systems enable plant to precisely regulate and time flowering for reproductive success.

Floral development can be response to:

- PHOTOPERIODISM: changes in day length.
- VERNALISATION: prolonged cold temperature.



Circadian Rhythms:

Like all other living organisms, the plants are and have always been exposed to strong and rhythmic environmental changes caused by planetary movements. It is but natural, that these environmental rhythmicities or periodicities find their counterparts in biological rhythms which control many behavioural and physiological activities of living organisms including plants. When the period of biological rhythmicities matches with those of the cycles of day and night, such rhythms are called as circadian rhythms (Circa = about; diem = a day).

Rhythmic behaviour in plants under natural conditions in known to scientist for over two thousand years. The so-called sleep movements (or up and down movements) of leaves in certain plants such as bean (Phaseolus multiflorus) is one such example and amongst the earliest observations on circadian phenomena.

In 1729, the French astronomer De Mairan carried out a very important experiment in which he transferred the plants to the continuous darkness of a cellar (a room under a house) and observed that the leaves showed rhythmic up and down movements for a number of days. He concluded that these were not dependent on the daily cycle of light and darkness even though they were normally synchronized with the latter. According to him these movements were controlled by an internal mechanism and were, therefore endogenous.

All circadian rhythms oscillate with a period length close to, but seldom equal to 24 hrs. when organisms are kept under constant conditions of light, temperature and other possible geophysical factors. The pattern of phase response curves may vary from organism to organism.

The persistence of rhythm varies with quality of light and also duration of exposure. In many animals, circadian rhythms in locomotory activity persist in continuous light (LL) as well as in constant darkness (DD) but with their 't' altered.

Circadian rhythms are endogenous. They are both an organismal and a cellular phenomenon. Light/dark cycle works as one of the strongest entrainers of these rhythms, although other periodic cycles can also entrain circadian rhythms. They free-run in the absence of a light/dark cycle.

This suggests that there is a pacemaker(s) which generates circadian rhythms in various physiological, biochemical and behavioural variables. Thus a basic circadian system has necessarily three important components such as photoreceptors, pacemaker(s), and observable rhythmic outputs.

The entraining pathways transduce information between the photo receptive elements and the pacemaker(s). The coupling pathways are also necessary as a link (the efferent pathways) between the pacemaker(s) and the multiple effector systems. The effector outputs are the overt rhythms that allow us to study the properties of the central clockwork (pacemaker) (Figs. 11.1 and 11.2).

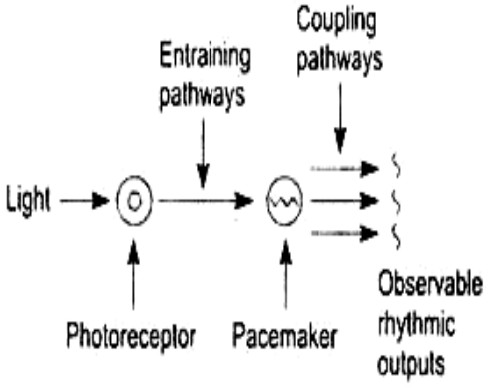


Fig. 11.1: A model showing a simple unidirectional pathway consisting of basic components of circadian systems.

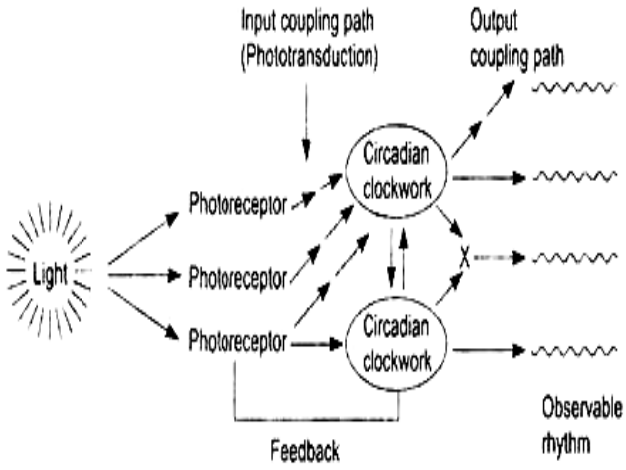


Fig. 11.2: A hypothetical model for the organisation of the major components of circadian systems

Occurrence of Circadian Rhythms in Plants:

Circadian rhythms have been found in some members of almost all major groups of plants except Bryophyta and Gymnosperms. Some of the examples of circadian rhythms in plants are given in Table 22.1.

Table 22.1. Some Examples of Circadian Rhythms in Plants.

Plant Group	Name of Organisms	Rhythmic Process
Algae	*Gonyaulax polyedra* (Unicellular dinoflagellate)	Bioluminescence, Photosynthetic capacity, Growth.
	Euglena gracilis (Unicellular)	Phototaxis
	Oedogonium cardiacum	Sporulation
	Acetabularia major (Unicellular)	Photosynthetic capacity
Fungi	*Pilobolus sphaerosporous*	Spore discharge
	Neurospora crassa	Mycelial growth
Pteridophyta	*Selaginella serpens*	Plastid Shape
Angiosperms	*Phaseolus multiflorus*	Leaf movements.
	Avena sativa	Growth rate of coleoptile
	Bryophyllum fedtschenkoi	Dark CO_2 fixation
	Kalanchoe blossfeidiana	Petal movements

Biological Clock:

The plants showing endogenous circadian rhythms have time measuring system or 'biological clock' inside their cells which measures the passage of time in much the same way as a pendulum. The nature and functioning of biological clock is not yet clearly understood.

There are, however, several evidences for the possible participation of:

(i) Membrane physiology, cytoplasmic organelles or

(ii) Nucleus and protein synthesis or

(iii) Higher frequency biochemical oscillations in the cells.

Accordingly, different models have been given by scientists to explain the working of circadian oscillator. For example, in Chronon model, the 24 hrs. period is ascribed to the time taken for the transcription of a hypothetical linear sequence of DNA, cistron by cistron. According to a membrane model, this period is attributed to the slower processes involved in the lateral fusion of proteins with the lipid bilayer. According to another model, the circadian period is believed to be the result of interactions between higher frequency biochemical oscillations within the cell.

The location of the 'biological clock' too is not clear in the cells. Because circadian rhythms are not observed in cell-free extracts or isolated cell-organelles, it is likely that the biological clock does not lie within the cell but the whole cell itself probably acts as the biological clock.

Photoperiodism:

The plants in order to flower require a certain day length i.e., the relative length of day and night which is called as photoperiod. The response of plants to the photoperiod expressed in the form of flowering is called as photoperiodism.

The phenomenon of photoperiodism was first discovered by Garner and Allard (1920, 22) who observed that the Biloxi variety of Soybeans (*Glycine max*) and 'Maryland Mammoth' variety of tobacco (*Nicotiana tabacum*) could be made to flower only when the daily exposure to the light was reduced below a certain critical duration and after many complex experiments concluded that 'the relative length of the day is a factor of the first importance in the growth and development of plants'. Depending upon the duration of the photoperiod, they classified plants into three categories.

(1) Short Day Plants (SDP):

These plants require a relatively short day light period (usually 8-10 hours) and a continuous dark period of about 14-16 hours for subsequent flowering (Fig. 18.1A). Some examples of these plants which are also known as long-night-plants are Maryland Mammoth variety of tobacco (*Nicotiana tabacum*) Biloxi variety of Soybeans (*Glycine max*), Cocklebur (*Xanthium pennsylvanicum*).

i. In short day plants the dark period is critical and must be continuous. If this dark period is interrupted even with a brief exposure of red light (660-665 mμ wavelength), the short day plant will not flower.

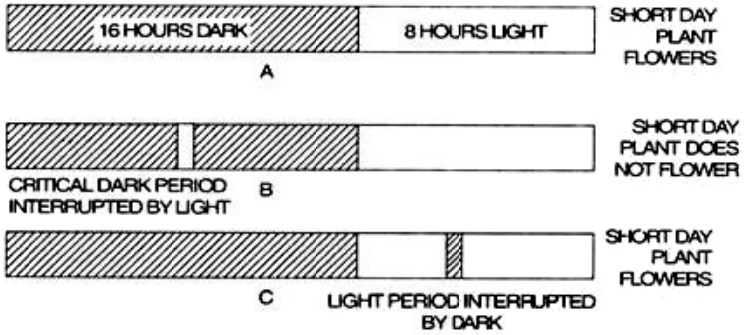


Fig. 18.1. Effect of a brief exposure of red light during dark and interruption of light period by dark on flowering in a short day plant.

ii. Maximum inhibition of flowering with red light occurs at about the middle of critical dark period.

iii. However, the inhibitory effect of red light can be overcome by a subsequent exposure with far-red light (730-735 mu wavelengths).

iv. Interruption of the light period by dark does not have inhibitory effect on flowering in short day plants.

v. Prolongation of the continuous dark period initiates early flowering in short day plants.

(2) Long Day Plants (LDP):

These plants require a longer day light period (usually 14-16 hours) in a 24 hours cycle for subsequent flowering. Some examples of these plants which are also called as short night plants are *Hyoscyamus niger* (Henbane) *Spinacea* (spinach) *Beta vulgaris* (Sugar beet).

i. In long day plants the light period is critical.

ii. A brief exposure in the dark period or the prolongation of the light period stimulates flowering in long day plants.

(3) Day Neutral Plants:

These plants flower in all photoperiods ranging from 5 hours to 24 hours continuous exposure. Some of the examples of these plants are tomato, cotton, sunflower, cucumber and certain varieties of peas and tobacco. During recent years certain intermediate categories of plants have also been recognised. They are,

Long Short Day Plants:

These are short day plants but must be exposed to long days during early periods of growth for subsequent flowering. Some of the examples of these plants are certain species of Bryophyllum.

Short-Long Day Plants:

These are long day plants but must be exposed to short days during early periods of growth for subsequent flowering. Some of the examples of these plants are certain varieties of wheat (*Triticum*) and rye (*Secale*).

Photoperiodic Induction:

Plants may require one or more inductive cycles for flowering. An appropriate photoperiod in 24 hours cycle constitutes one inductive cycle. If a plant which has received sufficient inductive cycles is subsequently placed under un-favourable photoperiods, it will still flower. Flowering will also occur if

a plant receives inductive cycles after intervals of un-favourable photoperiods (i.e., discontinuous inductive cycles). This persistence of photoperiodic after effect is called as photoperiodic induction.

i. An increase in the number of inductive cycles results in early flowering of the plant. For instance Xanthium (a short day plant) requires only one inductive cycle and normally flowers after about 64 days. It can be made to flower even after 13 days if it has received 4-8 inductive cycles. In such cases the number of flowers is also increased.

ii. Continuous inductive cycles promote early flowering than discontinuous inductive cycles.

Some of the example of plants which require more than one inductive cycles for subsequent flowering are Biloxi soybean (SDP) —2 inductive cycles; *Salvia occidentalis* (SDP) — 17 inductive cycles; *Plantago lanceolata* (LDP)—25 inductive cycles.

Perception of the Photoperiodic Stimulus and Presence of a Floral Hormone:

It is now well established that the photoperiodic stimulus is perceived by the leaves. As a result, a floral hormone is produced in the leaves which is then translocated to the apical tip, subsequently causing the initiation of floral primordia.

That the photoperiodic stimulus is perceived by the leaves can be shown by simple experiments on cocklebur (*Xanthium pennsylvanicum*), a short day plant. Cocklebur plant will flower if it has previously been kept under short-day conditions (Fig. 18.2 A). If the plant is defoliated and then kept under short day condition, it will not flower (Fig. 18.2 B). Flowering will also occur even if all the leaves of the plant except one leaf have been removed (Fig. 18.2 C).

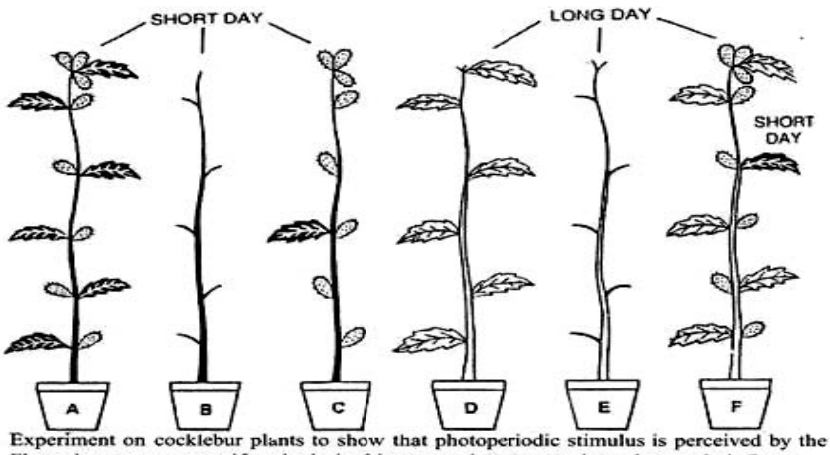


Fig. 18.2. Experiment on cocklebur plants to show that photoperiodic stimulus is perceived by the leaves. Flowering occurs even if a single leaf is exposed to appropriate photoperiod. See text

If a cocklebur plant whether intact of defoliated, is kept under long day conditions it will not flower (Fig. 18.2 D, E). But, if even one of its leaves is exposed to short day condition and the rest are under long day photoperiods, flowering will occur (Fig. 18.2 F).

The photoperiodic stimulus can be transmitted from one branch of the plant to another branch. For example, if in a two branched cocklebur plant one branch is exposed to short day and other to long day photo period, flowering occurs on both the branches (Fig. 18.3 A).

Flowering also occurs if one branch is kept under long day conditions and other branch from which all the leaves except one have been removed is exposed to short day condition (Fig. 18.3 B). However, if one branch is exposed to long photoperiod and the other has been defoliated under short day condition, flowering will not occur in any of the branches (Fig. 18.3 C).

Both the species will flower under a photoperiod of 13 hours. Subjecting LD and SD plants to light and darkness, other than 24 hours, shows that flowering in plants is more of a response to the dark period than to the light.

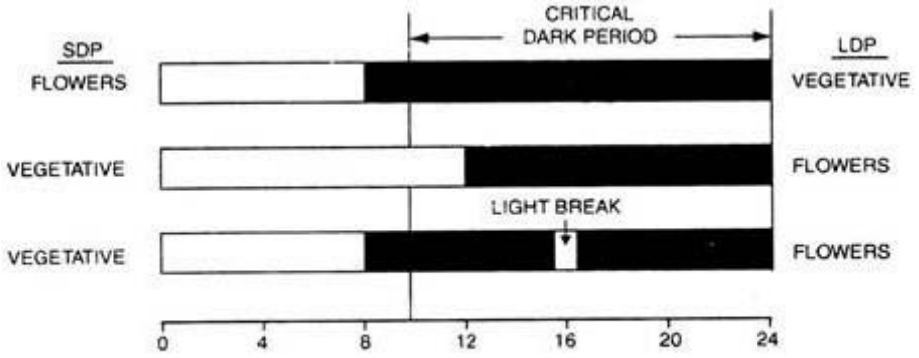


Fig. 22-2. Effect of breaks of light during the dark period on flowering in LDP (long day plant) and SDP (short day plant). Critical dark period is also marked out.

The critical night is that period of darkness, which must be exceeded before SD plants would flower. In LD plants, the dark period must be shorter than the critical night if flowering is to occur. Thus Xanthium, a SD plant, will not flower if the long dark period is interrupted by a single brief flash of light (Fig. 22-3).

On the other hand, interrupting the light period for a short period of darkness had no effect. It may be interpreted to mean that the long dark period which is necessary for flowering in Xanthium, consists of two short dark periods. Most LD plants will flower when so treated.

Alternatively, the SD plants may be called long night plants and LD plants as short night plants. While the length of dark period determines initiation of floral primordia, the length of photoperiod determines their number. In Glycine, the optimum Response is obtained with a photo-cycle comprising 16 hours of darkness and 11 hours of light, respectively.

Table 22-1 gives selective list of some important short day (SD) and long day plants (LD):

Table 22-1. A list of some important SD and LD plants.

Short day plants (SD)	Day Neutral plants	Long day plants (LD)
Cannabis sativus	*Cucumis sativus*	*Lactuca sativa*
Nicotiana tabacum		*Allium cepa*
Xanthium strumarium		*Hyoscyamus niger*
Helianthus tuberosus		*Daucus caryota*
Gossypium hirsutum		*Beta vugaris*
Cosmos bipinnatus		*Spinacea oleracea*
Coffea arabica		*Hordeum vulgare*
Glycine max		*Triticum aestivum*
Oryza sativa		*Raphanus sativus*
Zea mays		*Trifolium species*
Saccharum officinarum		*Pisum sativum*
		Petunia hybrida

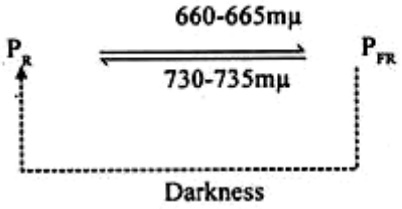
Perception of Photoperiodic Stimulus:

In spinach, a LD plant, it was shown that the leaves are the receptors of the photoperiodic stimulus. It was assumed that something was produced in the leaves in response to photo-inductive cycle and then it was translocated to the apical bud where it initiated flower primordia. The experiments with flowering clearly illustrate this point. If a single leaf of Xanthium is exposed to short photoperiod (16 HD + 8 HL) while the rest of the plant is subjected to a long photoperiod there is formation of flowers. Grafting of photo-induced leaves from one plant to the one with non-inductive cycle also promotes flowering on the receptor plant. The receptor plant is defoliated before the initiation of the experiment to eliminate the antagonistic effect of the natural leaves.

Phytochrome:

It has already been seen that a brief exposure with red light during critical dark period inhibits flowering in short-day plants and this inhibitory effect can be reversed by a subsequent exposure with far-red light. Similarly, the prolongation of the critical light period or the interruption of the dark period stimulates flowering in long-day plants. This inhibition of flowering in short-day plants and the stimulation of flowering in long-day plants involves the operation of a proteinaceous pigment called as phytochrome.

i. The pigment phytochrome exists in two different forms:
(i) Red light absorbing from which is designated as P_R and
(ii) Far-red light absorbing form which is designated as P_{FR}.
ii. These two forms of the pigment are photo chemically inter convertible.
iii. When P_R form of the pigment absorbs red light (660-665mp), it is converted into P_{FR} form.
iv. When P_{FR} form of the pigment absorbs far-red light (730-735mp), it is converted into P_R form.
v. The P_{FR} form of the pigment gradually changes into P_R form in dark.



It is considered that during the day the P_{FR} form of the pigments is accumulated in the plant which is inhibitory to flowering in short-day plants but is stimulatory in long–day plants. During critical dark period in short-day plants, this form gradually changes into P_R form resulting in flowering.

Light Quality and Phytochrome:

If a long light of a photo-inductive cycle for Xanthium is interrupted by a brief flash of light the plant does not flower. The most effective radiation in light break reactions is the red light. Its effect is reversed by far-red light suggesting participation of the phytochrome pigment. The phytochrome exists in two forms, one red absorbing form (Pr) and the other far-red absorbing (Pfr).

According to Borthwick, the far-red absorbing form of phytochrome (Pfr) accumulates in the plant during the day (white light). It is stimulatory to flowering in LD plants and inhibitory to flowering in SD plants.

At the onset of a dark period the Pfr is converted to Pr form which is stimulatory to flowering in SD plants and inhibitory to flowering in LD plants. The interruption of the dark period with red light will return the accumulated Pr form to Pfr form, thus inhibiting flowering in SD plants. If the red light break is followed by a far-red break the red light influence is reversed, since Pfr is converted to pr form, and flowering occurs (Fig 22-5).

The role of red and far-red light on the initiation of flowering in the LD and SD plants is shown in Fig. 22-6. It may be said that the precise role of phytochrome in flower initiation is not clear.

However, it may be added that this pigment is not the flowering stimulus but possibly aids the stimulation of flowering stimulus.

The effect of phytochrome on the flowering of SD plants may be explained as follows. At the end of light period Pfr is in high concentration and Pfr and Pr ratio is such that it prevents formation of flowering stimulus. If the dark period is prolonged, then either Pfr is destroyed or it is reverted to Pr state. At this stage the ratio of Pfr to Pr is such which triggers processes leading to formation of flowering stimulus. Under the situation where dark period is briefly interrupted by red light, there is formation of Pfr from Pr and the ratio of two is altered and therefore formation of flowering stimulus is prevented.

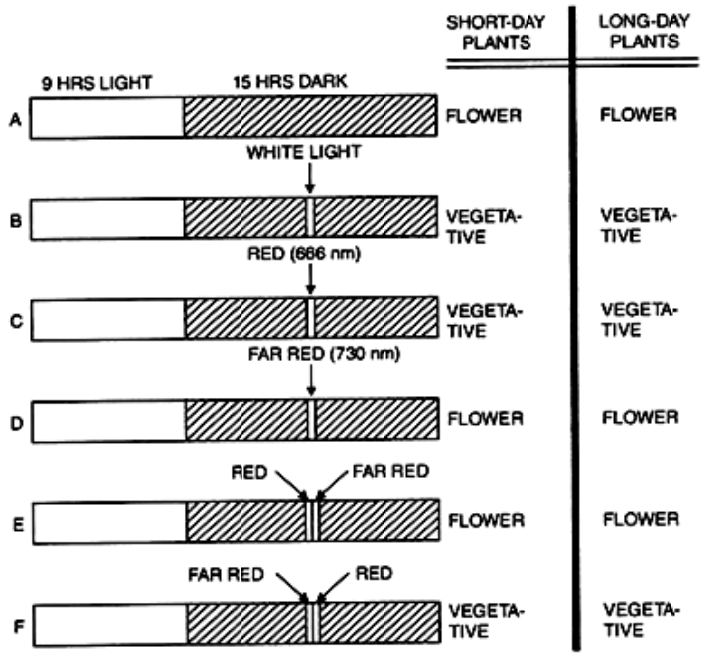


Fig. 22-6. The effect of red and far-red light during the night break on the flowering of LD and SD plants. The light is obtained either from sunlight or high-intensity radiation. Observe contrasting effects of two spectral qualities of light on SD and LD plants.

In the LD plant the role of Phytochrome can be explained as follows long day plants need high ratio of Pfr to Pr to trigger flowering stimulus. This ratio is attained at the end of a long day. Once the night is long then Pfr is reversed back to Pr and the formation of flowering stimulus is prevented.

Under the conditions where night is interrupted by a red light, there Pr is changed to Pfr. Here the ratio of the two pigments is high and flowering stimulus is produced. These explanations, however, fail to provide explanation for the initiation of flowering stimulus as mediated by phytochrome.

Figure 22-7 shows a possible hypothesis regarding photoinduction of a short day plant. According to Hess (1975), day light has more red than far-red light and therefore, concentration of P_{730} in the leaf is high and causes 'chemical inhibition' of flowering hormone. In the absence of light, P_{730} is converted into P_{660}.

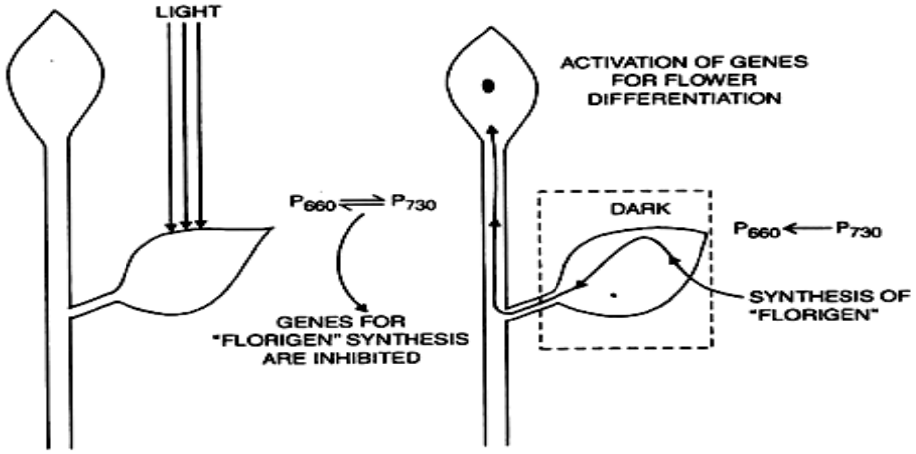


Fig 22.7. Diagrammatic representation of hypothesis regarding photoperiodic induction of a short day plant. Notice synthesis of florigen in the dak and its translocation to the apex for the activation of genes for flower differentiation.

The continued absence of light leaves very little of P_{730} and consequently synthesis of flowering hormone begins. Since antimetabolites inhibit flowering and GA promotes it, a working explanation has been proposed. It is believed that in plants exposed to inductive dark period more of "florigen" is synthesized in the leaf and this is translocate through phloem to the shoot apical flower.

GA and the flowering response. The application of GA to most LD plants will cause them to flower when on a non-inductive cycle but it has no effect on SD plants on a non-insuctive cycle.

Brian has suggested the role of GA in a scheme explaining photoperiodic reactions. According to him, a "GA-like" hormone is produced during the photoperiod as follows:

Red light promotes conversion of the precursor to the GA-like-hormone. During the dark the hormone is reconverted to the precursor. This back reaction is accelerated by far-red light. The GA-like hormone is then converted to florigen.

The steps of conversion are different in LD and SD plants. In LD plant a high level of GA-like hormone is required for the production of florigen. In SD plants, a low level of the GA- like hormone is optimum for a flowering response. However, if enough florigen is produced flowering will occur in both the LD and SD plants.

Genetic Approach to Photoperiod:

The basic approach is to identify the mutant that influences timing at any level, then the wild type gene is isolated and its gene product is analysed for clues to its role in the timing mechanism. In fact in several plant breeding programmes, flowering genes were also sought after. In crop species it is highly desirable to select early flowering photoperiod insensitive genes.

In pea and wheat, genetics of photoperiodic processes have received much attention. Incidentally, both the species are qualitative long-day plants. In pea several genes which affect photoperiodic timing and the onset of flowering have been identified, fsd (flowering short day) is a recessive mutant and makes the plant behave as a qualitative SD plant. When mutant is grafted to a wild-type stock under LD, the mutant will flower.

Recently approach in flowering genes has been made in Arabidopsis where several genes have been identified. This plant is a quantitative LD plant with a critical photoperiod of 8 to 10 hours. Under LD, it flowers with 4 to 7 leaves in the rosette. Under SD, flowering is delayed until 20 leaves are formed.

Flowering is also enhanced when the plant is exposed to blue or FR light suggesting the role of phytochrome in photoperiod phenomenon. Search is made to locate and isolate early and late flowering mutants. Here the flowering time refers to the number of rosette leaves produced before the flowering stem appears.

Incidentally, the mutants that affect phytochrome also affect flowering, hyl mutant is defective in the synthesis of the phytochromechromophore. Hence such mutants show an elongated hypocotyl but they flower earlier than the wild type under both LD and SD. Since the mutants flower under both conditions, they still show a response to photoperiod.

Several mutants which are insensitive to photoperiod and flower either earlier or later than the wild type plants have been identified, elf-3(early flower (-ing 3)—This is an early flowing type. On the contrary co-constants and gi-gigantea are later flowering, day-length insensitive mutants. They flower under LD conditions but SD has no effect.

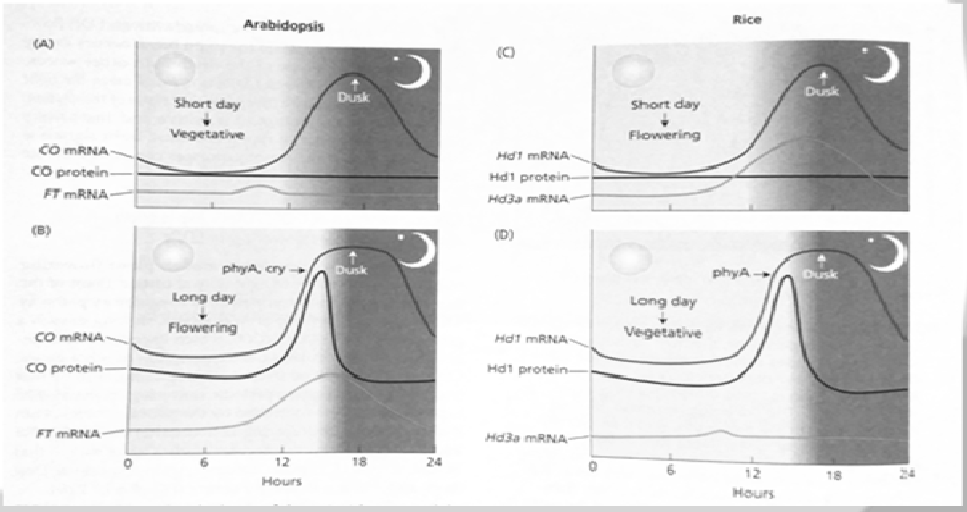
Grafting experiments have shown that wild type genes, GI, operates earlier than CO gene in the same pathway and the floral promotion under LD depends on the amount of COmRNA transcribed. This

mutant is also of special attention since it appears to interact with endogenous clock. elf3 gene affects leaf movement, CAB gene expression besides advancing flowering.

Through traditional plant physiology techniques photoperiodic signal transduction chain and endogenous clock proved difficult to investigate, the molecular genetic approach has provided insight into mechanisms that have eluded researchers for more than seven decades. There are many other instances where modern approaches have solved several of the plant physiology enigmas.

COINCIDENCE MODEL

- **In the coincidence model, flowering is induced in both SDPs and LDPs when light exposure is coincident with the appropriate phase of the oscillator.**
- **CO (in Arabidopsis) and Hd1 (in Rice) regulate flowering by controlling the transcription of floral stimulus genes.**

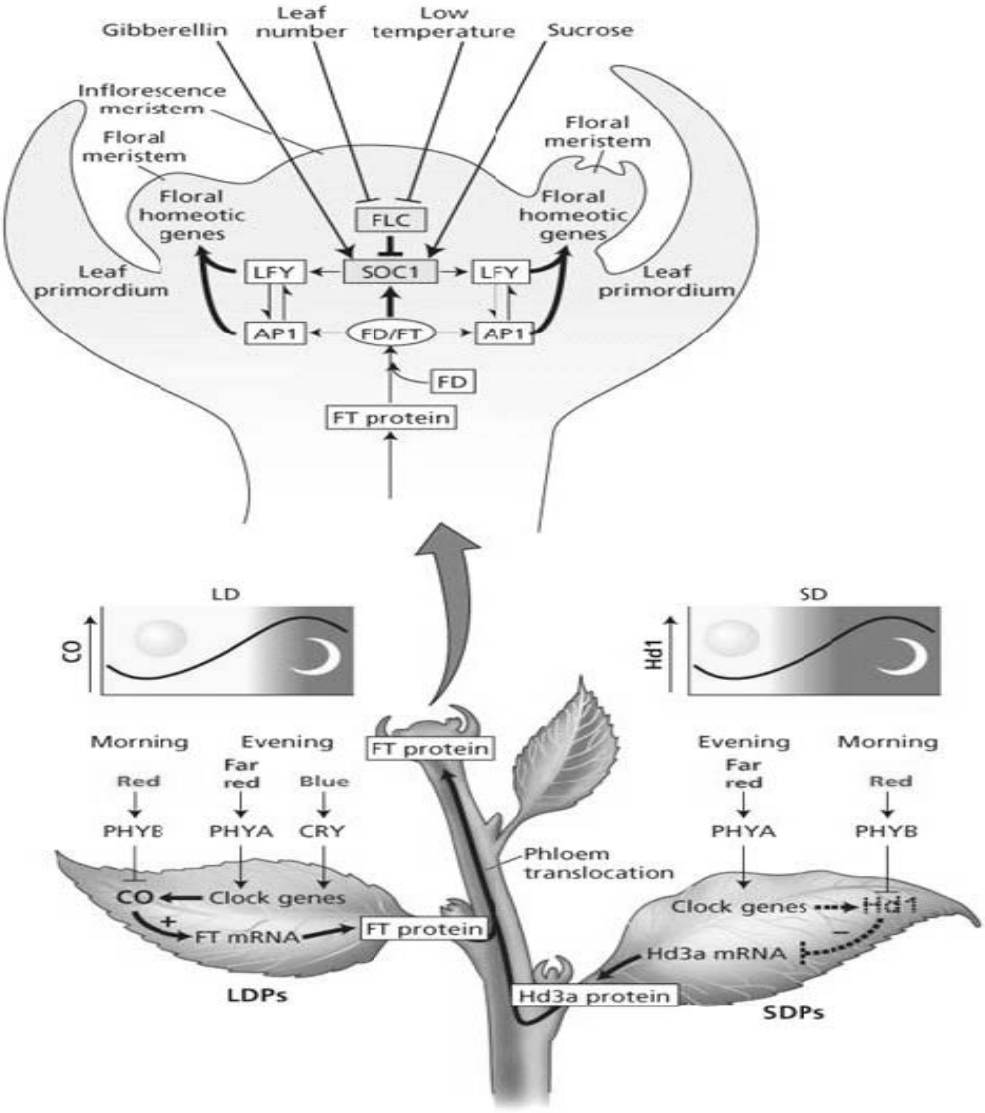


Long-distance signaling involved in flowering

In photoperiodic plants, **a long-range signal** is **transmitted in the phloem** from **leaves** to the **apex** , permitting **floral evocation**.

The discovery of florigen

- **FT** is a small, globular protein that exhibits the **properties** that would be **expected of florigen.**
- **Ft protein moves via the phloem** from the leaves to the shoot apical meristem under inductive photoperiods. In the meristem, **FT forms a complex with the transcription factor FD** forms a complex with the transcription factor **FD** to **activate floral identity genes**.
- **The four distinct pathways** that control flowering coverge to increase the expression of key regulators: **FT** in the vasculature and **SOC1,LFY, and AP1** in the meristem.



Gibberellins and the Flowering Response:

It is now well known that the gibberellins can induce flowering in long-day plants even under non-inductive short days. It is also definite that the gibberellins alone do not constitute the 'florigen', but it is usually held that the gibberellins are in some way connected with the overall process of flowering.

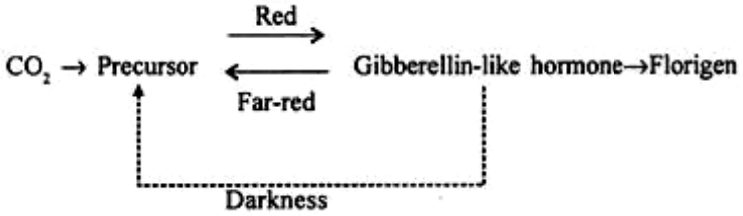
According to a scheme proposed by Brian (1958), a gibberellin like hormone is produced in the leaves during the photoperiod somewhat as follows:

CO_2 → Precursor (P) → Gibberellin-like hormone

The precursor may be slightly stimulatory or inactive or antagonistic to the gibberellin-like hormone. Red irradiations promote the conversion of the precursor to the gibberellin-like hormone. In the dark there is a slow reconversion of the gibberellin-like hormone to the precursor.

This reconversion is accelerated by far-red irradiations. It is further presumed that high concentration of the gibberellin-like hormone leads to the synthesis of florigen in long-day plants. In short-day plants the synthesis of florigen takes place when the level of gibberellin-like hormone is low. But, flowering eventually follows once the florigen synthesis has taken place in both the cases.

The whole scheme is diagrammatically shown below:



Importance of Photoperiodism:

(i) The knowledge of the phenomenon of photoperiodism has been of great practical importance in hybridisation experiments.

(ii) Although the floral hormone 'florigen' has not yet been isolated, the isolation and characterization of this hormone will be of utmost economic importance.

(iii) The phenomenon of photoperiodism is an excellent example of physiological preconditioning (or after-effect) where an external factor (i.e., the photoperiodic stimulus) induces some physiological changes in the plant the effect of which is not immediately visible. It lingers on in the plant and prepares the latter for a certain process (i.e., flowering) which takes place at a considerably later stage during the life history of the plant.

Vernalization:

Many plants do not come to flower before they experience a low temperature. These plants remain vegetative during the warm season, receive low temperature during winter, grow further and then bear flowers and fruits. Requirement of low temperature prevents precocious reproductive development in autumn.

It allows the plant to reach vegetative maturity before reproduction can occur. The condition occurs in winter varieties of some annual food plants (e.g., Wheat, Barley, and Rye), some biennial (e.g., Cabbage, Sugar beet, Carrot) and perennial plants (e.g., Chrysanthemum). The annual winter plants also possess spring varieties. The spring varieties are planted in spring. They come to flower and bear fruits prior to end of growing season. If the winter varieties are sown similarly, they fail to flower and produce fruits before the end of growing season. They are planted in autumn, form seedlings in which form they cover winter. The seedlings resume growth in spring. They bear flowers and fruits in summer.

11. Stress physiology: Response and adaptation to abiotic stress: water stress, temperature stress (heat and cold stress), stress induced gene expression.

Stress

The concept of plant stress is often used imprecisely, and stress terminology can be confusing, so it is useful to start our discussion with some definitions. **Stress** is usually defined as an external factor that exerts a disadvantageous influence on the plant. This chapter will concern itself with environmental or abiotic factors that produce stress in plants, although biotic factors such as weeds, pathogens, and insect predation can also produce stress. In most cases, stress is measured in relation to plant survival, crop yield, growth (biomass accumulation), or the primary assimilation processes (CO_2 and mineral uptake), which are related to overall growth. The concept of stress is intimately associated with that of **stress tolerance**, which is the plant's fitness to cope with an unfavorable environment. In the literature the term *stress resistance* is often used interchangeably with *stress tolerance*, although the latter term is preferred. Note that an environment that is stressful for one plant may not be stressful for another. For example, pea (*Pisum sativum*) and soybean (*Glycine max*) grow best at about 20°C and

30°C, respectively. As temperature increases, the pea shows signs of heat stress much sooner than the soybean. Thus the soybean has greater heat stress tolerance.

Water stress

The water-limited productivity of plants depends on the total amount of water available and on the water-use efficiency of the plant. A plant that is capable of acquiring more water or that has higher water-use efficiency will resist drought better. Some plants possess adaptations, such as the C_4 and CAM modes of photosynthesis that allow them to exploit more arid environments. In addition, plants possess acclimation mechanisms that are activated in response to water stress. **Water deficit** can be defined as any water content of a tissue or cell that is below the highest water content exhibited at the most hydrated state. When water deficit develops slowly enough to allow changes in developmental processes, water stress has several effects on growth, one of which is a limitation in leaf expansion. Leaf area is important because photosynthesis is usually proportional to it. However, rapid leaf expansion can adversely affect water availability.

Response and adaptation to water stress

1. Decreased Leaf Area Is an Early Adaptive Response to Water Deficit

Typically, as the water content of the plant decreases, its cells shrink and the cell walls relax. This decrease in cell volume results in lower turgor pressure and the subsequent concentration of solutes in the cells. The plasma membrane becomes thicker and more compressed because it covers a smaller area than before. Because turgor reduction is the earliest significant biophysical effect of water stress, turgor-dependent activities such as leaf expansion and root elongation are the most sensitive to water deficits.

2. Water Deficit Stimulates Leaf Abscission

The total leaf area of a plant (number of leaves × surface area of each leaf) does not remain constant after all the leaves have matured. If plants become water stressed after a substantial leaf area has developed, leaves will senesce and eventually fall off. Such a leaf area adjustment is an important long-term change that improves the plant's fitness in a water-limited environment. Indeed, many drought-deciduous, desert plants drop all their leaves during a drought and sprout new ones after a rain. This cycle can occur two or more times in a single season. Abscission during water stress results largely from enhanced synthesis of and responsiveness to the endogenous plant hormone ethylene.

3. Water Deficit Enhances Root Extension into Deeper, Moist Soil

Mild water deficits also affect the development of the root system. Root-to-shoot biomass ratio appears to be governed by a functional balance between water uptake by the root and photosynthesis by the shoot. Simply stated, *a shoot will grow until it is so large that water uptake by the roots becomes limiting to further growth*; conversely, *roots will grow until their demand for photosynthate from the shoot equals the supply.* This functional balance is shifted if the water supply decreases. All these factors lead to a preferential root growth into the soil zones that remain moist. As water deficits progress, the upper layers of the soil usually dry first. Thus, plants commonly show a mainly shallow root system when all soil layers are wetted, and a loss of shallow roots and proliferation of deep roots as water in top layers of the soil is depleted. Deeper root growth into wet soil can be considered a second line of defense against drought. Enhanced root growth into moist soil zones during stress requires allocation of assimilates to the growing root tips. During water deficit, assimilates are directed to the fruits and away from the roots. For this reason the enhanced water uptake resulting from root growth is less pronounced in reproductive plants than in vegetative plants. Competition for assimilates between roots and fruits is one explanation for the fact that plants are generally more sensitive to water stress during reproduction.

4. Stomata Close during Water Deficit in Response to Abscisic Acid

The preceding sections focused on changes in plant development during slow, long-term dehydration. When the onset of stress is more rapid or the plant has reached its full leaf area before initiation of stress, other responses protect the plant against immediate desiccation. Under these conditions, stomata closure reduces evaporation from the existing leaf area. Thus, stomatal closure can be considered a third line of defense against drought. Uptake and loss of water in guard cells changes

their turgor and modulates stomatal opening and closing. Because guard cells are located in the leaf epidermis, they can lose turgor as a result of a direct loss of water by evaporation to the atmosphere. The decrease in turgor causes stomatal closure by **hydropassive closure**. This closing mechanism is likely to operate in air of low humidity, when direct water loss from the guard cells is too rapid to be balanced by water movement into the guard cells from adjacent epidermal cells. A second mechanism, called **hydroactive closure**, closes the stomata when the whole leaf or the roots are dehydrated and depends on metabolic processes in the guard cells. A reduction in the solute content of the guard cells results in water loss and decreased turgor, causing the stomata to close; thus the hydraulic mechanism of hydroactive closure is a reversal of the mechanism of stomatal opening. However, the control of hydroactive closure differs in subtle but important ways from stomatal opening. Abscisic acid is synthesized continuously at a low rate in mesophyll cells and tends to accumulate in the chloroplasts. Besides ABA (Sauter et al. 2001), other si gnals, such as pH and inorganic ion redistribution, appear to play a role in long-distance signaling between the roots and the shoots.

5. Water Deficit Limits Photosynthesis within the Chloroplast

The photosynthetic rate of the leaf (expressed per unit leaf area) is seldom as responsive to mild water stress as leaf expansion is because photosynthesis is much less sensitive to turgor than is leaf expansion. However, mild water stress does usually affect both leaf photosynthesis and stomatal conductance. As stomata close during early stages of water stress, water-use efficiency may increase (i.e., more CO_2 may be taken up per unit of water transpired) because stomatal closure inhibits transpiration more than it decreases intercellular CO_2 concentrations. As stress becomes severe, however, the dehydration of mesophyll cells inhibits photosynthesis, mesophyll metabolism is impaired, and water-use efficiency usually decreases. Results from many studies have shown that the relative effect of water stress on stomatal conductance is significantly larger than that on photosynthesis. The response of photosynthesis and stomatal conductance to water stress can be partitioned by exposure of stressed leaves to air containing high concentrations of CO_2. Any effect of the stress on stomatal conductance is eliminated by the high CO_2 supply, and differences between photosynthetic rates of stressed and unstressed plants can be directly attributed to damage from the water stress to photosynthesis.

6. Water Deficit Increases Resistance to Liquid-Phase Water Flow

When a soil dries, its resistance to the flow of water increases very sharply, particularly near the *permanent wilting point*. At the permanent wilting point (usually about –1.5 MPa), plants cannot regain turgor pressure even if all transpiration stops. Because of the very large soil resistance to water flow, water delivery to the roots at the permanent wilting point is too slow to allow the overnight rehydration of plants that have wilted during the day. Rehydration is further hindered by the resistance within the plant, which has been found to be larger than the resistance within the soil over a wide range of water deficits (Blizzard and Boyer 1980). Several factors may contribute to the increased plant resistance to water flow during drying.

As plant cells lose water, they shrink. When roots shrink, the root surface can move away from the soil particles that hold the water, and the delicate root hairs may be damaged. In addition, as root extension slows during soil drying, the outer layer of the root cortex (the hypodermis) often becomes more extensively covered with suberin, a water-impermeable lipid, increasing the resistance to water flow.

Heat stress

Most tissues of higher plants are unable to survive extended exposure to temperatures above 45°C. Non-growing cells or dehydrated tissues (e.g., seeds and pollen) can survive much higher temperatures than hydrated, vegetative, growing cells. Actively growing tissues rarely survive temperatures above 45°C, but dry seeds can endure 120°C, and pollen grains of some species can endure 70°C. In general, only single-celled eukaryotes can complete their life cycle at temperatures

above 50°C, and only prokaryotes can divide and grow above 60°C. Periodic brief exposure to sublethal heat stresses often induces tolerance to otherwise lethal temperatures, a phenomenon referred to as **induced thermo tolerance**. The mechanisms mediating induced thermo tolerance will be discussed later in the chapter. As mentioned earlier, water and temperature stress are interrelated; shoots of most C_3 and C_4 plants with access to abundant water supply are maintained below 45°C by evaporative cooling; if water becomes limiting, evaporative cooling decreases and tissue temperatures increase. Emerging seedlings in moist soil may constitute an exception to this general rule. These seedlings may be exposed to greater heat stress than those in drier soils because wet, bare soil is typically darker and absorbs more solar radiation than drier soil.

Response and adaptation to heat stress

1. High Temperature Reduces Membrane Stability

The stability of various cellular membranes is important during high-temperature stress, just as it is during chilling and freezing. Excessive fluidity of membrane lipids at high temperatures is correlated with loss of physiological function. In oleander (*Nerium oleander*), acclimation to high temperatures is associated with a greater degree of saturation of fatty acids in membrane lipids, which makes the membranes less fluid (Raison et al. 1982). At high temperatures there is a decrease in the strength of hydrogen bonds and electrostatic interactions between polar groups of proteins within the aqueous phase of the membrane. High temperatures thus modify membrane composition and structure and can cause leakage of ions. Membrane disruption also causes the inhibition of processes such as photosynthesis and respiration that depend on the activity of membrane-associated electron carriers and enzymes. Photosynthesis is especially sensitive to high temperature. In their study of *Atriplex* and *Tidestromia*, O. Björkman and colleagues (1980) found that electron transport in photosystem II was more sensitive to high temperature in the cold-adapted *A. sabulosa* than in the heat-adapted *T. oblongifolia*. In these plants the enzymes ribulose-1,5-bisphosphate carboxylase, NADP:glyceraldehyde-3-phosphate dehydrogenase, and phosphoenolpyruvatecarboxylase were less stable at high temperatures in *A. sabulosa* than in *T. oblongifolia*. However, the temperatures at which these enzymes began to denature and lose activity were distinctly higher than the temperatures at which photosynthesis began to decline. These results suggest that early stages of heat injury to photosynthesis are more directly related to changes in membrane properties and to uncoupling of the energy transfer mechanisms in chloroplasts than to a general denaturation of proteins.

2. Several Adaptations Protect Leaves against Excessive Heating

In environments with intense solar radiation and high temperatures, plants avoid excessive heating of their leaves by decreasing their absorption of solar radiation. This adaptation is important in warm, sunny environments in which a transpiring leaf is near its upper limit of temperature tolerance. In these conditions, any further warming arising from decreased evaporation of water or increased energy absorption can damage the leaf. Both drought resistance and heat resistance depend on the same adaptations: reflective leaf hairs and leaf waxes; leaf rolling and vertical leaf orientation; and growth of small, highly dissected leaves to minimize the boundary layer thickness and thus maximize convective and conductive heat loss. Some desert shrubs—for example, white brittlebush (*Encelia farinosa*, family Compositae)—have dimorphic leaves to avoid excessive heating: Green, nearly hairless leaves found in the winter are replaced by white, pubescent leaves in the summer.

3. At Higher Temperatures, Plants Produce Heat Shock Proteins

In response to sudden, 5 to 10°C rises in temperature, plants produce a unique set of proteins referred to as **heat shock proteins** (**HSPs**). Most HSPs function to help cells withstand heat stress by acting as molecular chaperones. Heat stress causes many cell proteins that function as enzymes or structural components to become unfolded or misfolded, thereby leading to loss of proper enzyme structure and activity. Such misfolded proteins often aggregate and precipitate, creating serious problems within the cell. HSPs act as molecular chaperones and serve to attain a proper folding of misfolded, aggregated proteins and to prevent misfolding of proteins. This facilitates proper cell functioning at elevated, stressful temperatures. Heat shock proteins were discovered in the fruit fly (*Drosophila melanogaster*) and have since been identified in other animals, and in humans, as well as in plants, fungi, and

microorganisms. For example, when soybean seedlings are suddenly shifted from 25 to 40°C (just below the lethal temperature), synthesis of the set of mRNAs and proteins commonly found in the cell is suppressed, while transcription and translation of a set of 30 to 50 other proteins (HSPs) is enhanced. New mRNA transcripts for HSPs can be detected 3 to 5 minutes after heat shock (Sachs and Ho 1986). Although plant HSPs were first identified in response to sudden changes in temperature (25 to 40°C) that rarely occur in nature, HSPs are also induced by more gradual rises in temperature that are representative of the natural environment, and they occur in plants under field conditions. Some HSPs are found in normal, unstressed cells, and some essential cellular proteins are homologous to HSPs but do not increase in response to thermal stress (Vierling 1991). Plants and most other organisms make HSPs of different sizes in response to temperature increases. The molecular masses of the HSPs range from 15 to 104 kDa (kilodaltons), and they can be grouped into five classes based on size. Different HSPs are localized to the nucleus, mitochondria, chloroplasts, endoplasmic reticulum, and cytosol. Members of the HSP60, HSP70, HSP90, and HSP100 groups act as molecular chaperones, involving ATP-dependent stabilization and folding of proteins, and the assembly of oligomeric proteins. Some HSPs assist in polypeptide transport across membranes into cellular compartments. HSP90s are associated with hormone receptors in animal cells and may be required for their activation, but there is no comparable information for plants.

Low-molecular-weight (15–30 kDa) HSPs are more abundant in higher plants than in other organisms. Whereas plants contain five to six classes of low-molecular- weight HSPs, other eukaryotes show only one class (Buchanan et al. 2000). The different classes of 15–30 kDa molecular-weight HSPs (smHSPs) in plants are distributed in the cytosol, chloroplasts, ER and mitochondria. The function of these small HSPs is not understood. Cells that have been induced to synthesize HSPs show improved thermal tolerance and can tolerate exposure to temperatures that are otherwise lethal. Some of the HSPs are not unique to high-temperature stress. They are also induced by widely different environmental stresses or conditions, including water deficit, ABA treatment, wounding, low temperature, and salinity. Thus, cells previously exposed to one stress may gain cross-protection against another stress. Such is the case with tomato fruits, in which heat shock (48 hours at 38°C) has been observed to promote HSP accumulation and to protect cells for 21 days from chilling at 2°C.

4. Adaptation to Heat Stress Is Mediated by Cytosolic Calcium

Heat stress causes a reduction in cytosolic pH from the normal slightly alkaline value, probably by inhibiting proton-pumping ATPases and pyrophosphatases that pump protons across the plasma membrane or into the vacuole. Additionally, heat stress effects a change in calcium homeostasis inside the cell by affecting the influx of calcium into the cytosol through either plasma membrane or vacuolar calcium channels, or by action on efflux ATPases or proton cotransporters. This increase in cytosolic calcium leads to the activation of calmodulin (CaM), which binds to glutamate decarboxylase (GAD) converting it from the inactive to the active form. Glutamate conversion to γ− aminobutyric acid (GABA) is then accomplished consuming protons in the process and mediating an increase in cytosolic pH. CAX1 and CAX2 are transport proteins, ACA: Ca_2^+ ATPase.

Cold stress Chilling temperatures are too low for normal growth but not low enough for ice to form. Typically, tropical and subtropical species are susceptible to chilling injury. Among crops, maize, *Phaseolus* bean, rice, tomato, cucumber, sweet potato, and cotton are chilling sensitive. *Passiflora*, *Coleus*, and *Gloxinia* are examples of susceptible ornamentals. When plants growing at relatively warm temperatures (25 to 35°C) are cooled to 10 to 15°C, **chilling injury** occurs: Growth is slowed, discoloration or lesions appear on leaves, and the foliage looks soggy, as if soaked in water for a long time. If roots are chilled, the plants may wilt. Species that are generally sensitive to chilling can show appreciable variation in their response to chilling temperatures. Genetic adaptation to the colder temperatures associated with high altitude improves chilling resistance. In addition, resistance often increases if plants are first hardened (acclimated) by exposure to cool, but noninjurious, temperatures. Chilling damage thus can be minimized if exposure is slow and gradual. Sudden exposure to temperatures near 0°C, called *cold shock*, greatly increases the chances of injury.

Response and adaptation to cold stress

1. Membrane Properties Change in Response to Chilling Injury

Leaves from plants injured by chilling show inhibition of photosynthesis, slower carbohydrate translocation, lower respiration rates, inhibition of protein synthesis, and increased degradation of existing proteins. All of these responses appear to depend on a common primary mechanism involving loss of membrane function during chilling. For instance, solutes leak from the leaves of chillig sensitive *Passiflora maliformis* (conch apple) floated on water at 0°C, but not from those of chilling-resistant *Passiflora caerulea* (passionflower). Loss of solutes to the water reflects damage to the plasma membrane and possibly also to the tonoplast. In turn, inhibition of photosynthesis and of respiration reflects injury to chloroplast and mitochondrial membranes. Why are membranes affected by chilling? Plant membranes consist of a lipid bilayer interspersed with proteins and sterols. The physical properties of the lipids greatly influence the activities of the integral membrane proteins, including H+-ATPases, carriers, and channel-forming proteins that regulate the transport of ions and other solutes, as well as the transport of enzymes on which metabolism depends. As the membranes become less fluid, their protein components can no longer function normally. The result is inhibition of H+-ATPase activity, of solute transport into and out of cells, of energy transduction, and of enzyme-dependent metabolism. In addition, chilling- sensitive leaves exposed to high photon fluxes and chilling temperatures are photo-inhibited, causing acute damage to the photosynthetic machinery.

2. Ice Crystal Formation and Protoplast Dehydration Kill Cells

The ability to tolerate freezing temperatures under natural conditions varies greatly among tissues. Seeds, other partly dehydrated tissues, and fungal spores can be kept indefinitely at temperatures near absolute zero (0 K, or –273°C), indicating that these very low temperatures are not intrinsically harmful. Fully hydrated, vegetative cells can also retain viability if they are cooled very quickly to avoid the formation of large, slow-growing ice crystals that would puncture and destroy subcellular structures. Ice crystals that form during very rapid freezing are too small to cause mechanical damage. Conversely, rapid warming of frozen tissue is required to prevent the growth of small ice crystals into crystals of a damaging size, or to prevent loss of water vapor by sublimation, both of which take place at intermediate temperatures (–100 to –10°C).

3. ABA and Protein Synthesis Are Involved in Acclimation to Freezing

In seedlings of alfalfa (*Medicago sativa* L.), tolerance to freezing at –10°C is greatly improved by previous exposure toncold (4°C) or by treatment with exogenous ABA without exposure to cold. These treatments cause changes in them pattern of newly synthesized proteins that can be resolved on two-dimensional gels. Some of the changes are unique to the particular treatment (cold or ABA), but some of the newly synthesized proteins induced by cold appear to be the same as those induced by ABA or by mild water deficit. Protein synthesis is necessary for the development of freezing tolerance, and several distinct proteins accumulateduring acclimation to cold, as a result of changes in gene expression (Guy 1999). Isolation of the genes for these proteins reveals that several of the proteins that are induced by low temperature share homology with the RAB/LEA/DHN (responsive to ABA, late embryo abundant, and dehydrin, respectively) protein family. As described earlier in the section on gene regulation by osmotic stress, these proteins accumulate in tissues exposed to different stresses, such as osmotic stress. Their functions are under investigation.

4. Numerous Genes Are Induced during Cold Acclimation

Expression of certain genes and synthesis of specific proteins are common to both heat and cold stress, but some aspects of cold-inducible gene expression differ from that produced by heat stress (Thomashow 2001). Whereas during cold episodes the synthesis of “housekeeping” proteins (proteins made in the absence of stress) is not substantially down-regulated, during heat stress housekeeping-protein synthesis is essentially shut down. On the other hand, the synthesis of several heat shock proteins that can act as molecular chaperones is up-regulated under cold stress in the same way that it is during heat stress. This suggests that protein destabilization accompanies both heat and cold stress and that mechanism for stabilizing protein structure during both heat and cold episodes are important for survival.

Stress induced gene expression

Heat stress related gene expression

The five classes of heat shock proteins found in plants

HSP class	Size (kDa)	Examples (Arabidopsis / prokaryotic)	Cellular location
HSP100	100–114	AtHSP101 / ClpB, ClpA/C	Cytosol, mitochondria, chloroplasts
HSP90	80–94	AtHSP90 / HtpG	Cytosol, endoplasmic reticulum
HSP70	69–71	AtHSP70 / DnaK	Cytosol/nucleus, mitochondria, chloroplasts
HSP60	57–60	AtTCP-1 / GroEL, GroES	Mitochondria, chloroplasts
smHSP	15–30	Various AtHSP22, AtHSP20, AtHSP18.2, AtHSP17.6 / IBPA/B	Cytosol, mitochondria, chloroplasts, endoplasmic reticulum

Source: After Boston et al. 1996.

Cold stress related gene expression

More than 100 genes are up-regulated by cold stress. Because cold stress is clearly related to ABA responses and to osmotic stress, not all the genes up-regulated by cold stress necessarily need to be associated with cold tolerance, but most likely many of them are. Many cold stress–induced genes are activated by transcriptional activators called **C-repeat binding factors** (**CBF1**, **CBF2**, **CBF3**; also called DREB1b, DREB1c, and DREB1a, respectively) (Shinozaki and Yamaguchi-Shinozaki 2000). CBF/DREB1-type transcription factors bind to **CRT/DRE elements** (C-repeat/dehydration-responsive, ABA-independent sequence elements) in gene promoter sequences, which were discussed earlier in the chapter. CBF/DREB1 is involved in the coordinate transcriptional response of numerous cold and osmotic stress–regulated genes, all of which contain the CRT/DRE elements in their promoters. CBF1/DREB1b is unique in that it is specifically induced by cold stress and not by osmotic or salinity stress, whereas the DRE-binding elements of the DREB2 type (discussed earlier in the section on osmotic stresses) are induced only by osmotic and salinity stresses and not by cold. The expression of CBF1/DREB1b is controlled by a separate transcription factor, called ICE (*i*nducer of *C*BF *e*xpression). ICE transcription factors do not appear to be induced by cold, and it is presumed that ICE or an associated protein is post transcriptionally activated, permitting activation of CBF1/DRE1b, but the precise signaling pathway(s) of cold perception, calcium signaling, and the activation of ICE are presently under investigation. Transgenic plants constitutively expressing CBF1 have more cold–up-regulated gene transcripts than wild-type plants have, suggesting that numerous cold–up-regulated proteins that may be involved in cold acclimation are being produced in the absence of cold in these CBF1 transgenic plants. In addition, CBF1 tansgenic plants are more coldtolerant than control plants.

Transgenic strategy of stress in plants.

In Arabidopsis, overexpression of cDNA encoding DREB1 and DREB2 triggered expres-sion of many stress tolerance genes under normal growing conditions. Thus, transgenic strat-egy resulted in increased tolerance to drought, salinity and cold stress. Researchers from Japan International Research Center for Agricultural Science (JIRCAS) identified 12 stress-inducible genes which are targeted by DREBIA transcription factor protein.

In the transgene construct DREBIA) was driven under the control of Camv 35S promoter (35S:DREB1A). In the notable observation, all the transgenic plants carrying 35S:DREB1A transgene exhibited severe retar-dation under normal growth condition. This negative effect is probably due to different levels of expression of DREBIA transgenes.

Over production of transcription factor proteins resulted in the expression of all stress inducible target genes, rd29A, rdl7, cor 6.6, cor 15a, erd 10 and kinl. Under normal conditions, plants under this situation suffer heav-ily due to unnecessary drenching of energy for the production of several hundreds of proteins. This problem was effectively tackled by using stress inducible rd 29A promoter.

In an extended transgenic strategy DREBIA gene expressed under the control of stress inducible rd 29A pro-moter provides protection and shows minimal effect on plant growth. In addition, it provides high degree of tolerance to stress condition rather when it is expressed under 35S Camv pro-moter.

Since rd 29A promoter is stress inducible, it did not cause expression of the transcription factor gene (DREB).

Under unstressed conditions, instead, it rapidly enhances the expression of DREBIA transgene only under dehydration, high salt and low temperature conditions (Fig. 15.5). Overall rd 29A promoter may be useful to improve stress tolerance of agriculturally important crops by gene transfer. In the earlier transgenic work, Shinozaki and coworkers have amplified signalling pathway by overexpressing gene containing fusion of a DRE-containing promoter with a DREB gene.

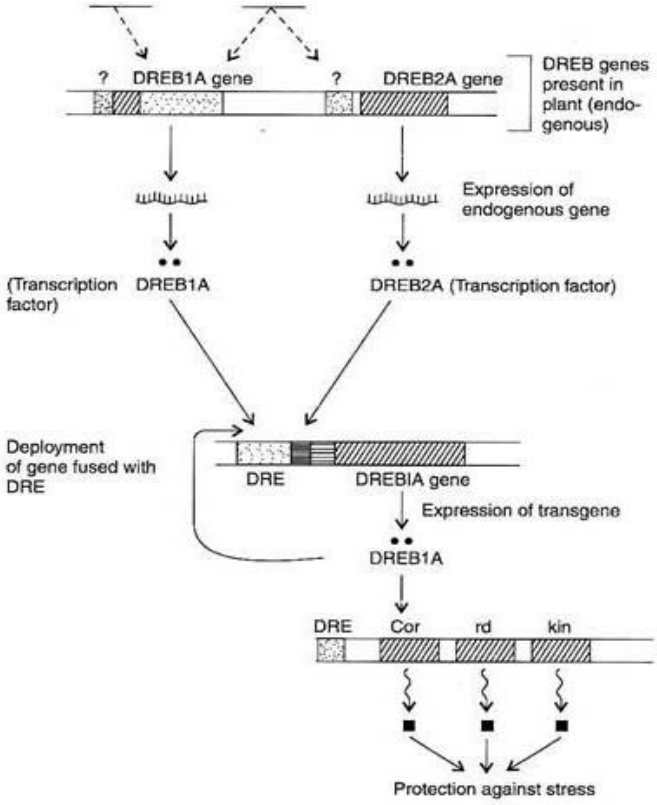


Fig. 15.5 Overexpression of stress inducible transcription factor protein and amplification of signalling pathway by overexpressing the introduced gene (DREB1) fused with DRE-*cis*-acting sequence (after Smirnoff and Bryant, 1999.)

This enhances further DREBIA expression in response to stress. This amplified strategy led to strong induction of DRE-containing target genes. Some contrasting experiments show that DREBIA cannot induce the expression of other drought-responsive genes (such as PSCS, erdl, rd22 and rd 29B) which contain no DRE element in their promoter region.

13. Principle of biophysical chemistry: pH, buffer, reaction kinetics, thermodynamics, law of mass action, acid base reactions, bond energy, energy rich compounds, redox potential, free energy.

A **buffer** solution (more precisely, pH buffer or hydrogen ion buffer) is an aqueous solutionconsisting of a mixture of a weak acid and its conjugate base, or vice versa. Its pH changes very little when a small amount of strong acid or base is added to it. Buffer solutions are used as a means of keeping pH at a nearly constant value in a wide variety of chemical applications. In nature, there are many systems that use buffering for pH regulation. For example, the bicarbonate buffering system is used to regulate the pH of blood.

Principles of buffering

Buffer solutions achieve their resistance to pH change because of the presence of an equilibrium between the acid HA and its conjugate base A−.

$HA \rightleftharpoons H+ + A-$

When some strong acid is added to an equilibrium mixture of the weak acid and its conjugate base, the equilibrium is shifted to the left, in accordance with Le Châtelier's principle. Because of this, the hydrogen ion concentration increases by less than the amount expected for the quantity of strong acid added. Similarly, if strong alkali is added to the mixture the hydrogen ion concentration decreases by less than the amount expected for the quantity of alkali added. The effect is illustrated by the simulated titration of a weak acid with pKa = 4.7. The relative concentration of undissociated acid is shown in blue and of its conjugate base in red. The pH changes relatively slowly in the buffer region, pH = pKa ± 1, centered at pH = 4.7 where [HA] = [A−]. The hydrogen ion concentration decreases by less than the amount expected because most of the added hydroxide ion is consumed in the reaction

$OH- + HA \rightarrow H2O + A-$ and only a little is consumed in the neutralization reaction which results in an increase in pH. $OH- + H+ \rightarrow H2O$

Once the acid is more than 95% deprotonated the pH rises rapidly because most of the added alkali is consumed in the neutralization reaction.

Applications

Buffer solutions are necessary to keep the correct pH for enzymes in many organisms to work. Many enzymes work only under very precise conditions; if the pH moves outside of a narrow range, the enzymes slow or stop working and can denature. In many cases denaturation can permanently disable their catalytic activity.[Industrially, buffer solutions are used in fermentation processes and in setting the correct conditions for dyes used in colouring fabrics. They are also used in chemical analysis and calibration of pH meters.

The majority of biological samples that are used in research are made in buffers, especially phosphate buffered saline (PBS) at pH 7.4. For buffers in acid regions, the pH may be adjusted to a desired value by adding a strong acid such as hydrochloric acid to the particular buffering agent. For alkaline buffers, a strong base such as sodium hydroxide may be added. Alternatively, a buffer mixture can be made from a mixture of an acid and its conjugate base. For example, an acetate buffer can be made from a mixture of acetic acid and sodium acetate. Similarly an alkaline buffer can be made from a mixture of the base and its conjugate acid.

Calculating buffer pH

First write down the equilibrium expression.

$HA \rightleftharpoons A^- + H^+$

This shows that when the acid dissociates equal amounts of hydrogen ion and anion are produced.

To find x, use the formula for the equilibrium constant in terms of concentrations:

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

Substitute the concentrations with the values found in the last row of the ICE table:

$$K_a = \frac{x(x+y)}{C_0 - x}$$

Simplify to:

$$x^2 + (K_a + y)x - K_a C_0 = 0$$

With specific values for C_0, K_a and y this equation can be solved for x. Assuming that pH = $-\log_{10}[H^+]$ the pH can be calculated as pH = $-\log_{10}(x + y)$.

To find x, use the formula for the equilibrium constant in terms of concentrations: To find x, use the formula for the equilibrium constant in terms of concentrations:

Polyprotic acids

Polyprotic acids are acids that can lose more than one proton. The constant for dissociation of the first proton may be denoted as K_{a1} and the constants for dissociation of successive protons as K_{a2}, etc. Citric acid, H_3A, is an example of a polyprotic acid as it can lose three protons.

In chemistry, **pH** is a scale used to specify how acidic or basic a water-based solutionis. Acidic

Calculation of the pH with a polyprotic acid requires a speciation calculation to be performed. In the case of citric acid, this entails the solution of the two equations of mass balance

$$C_A = [A^{3-}] + \beta_1[A^{3-}][H^+] + \beta_2[A^{3-}][H^+]^2 + \beta_3[A^{3-}][H^+]^3$$
$$C_H = [H^+] + \beta_1[A^{3-}][H^+] + 2\beta_2[A^{3-}][H^+]^2 + 3\beta_3[A^{3-}][H^+]^3 - K_w[H^+]^{-1}$$

C_A is the analytical concentration of the acid, C_H is the analytical concentration of added hydrogen ions, β_q are the cumulative association constants

$$\log\beta_1 = pK_{a_3}, \quad \log\beta_2 = pK_{a_2} + pK_{a_3}, \quad \log\beta_3 = pK_{a_1} + pK_{a_2} + pK_{a_3}$$

K_w is the constant for self-ionization of water. There are two non-linear simultaneous equations in two unknown quantities $[A^{3-}]$ and $[H^+]$. Many computer programs are available to do this calculation. The speciation diagram for citric acid was produced with the program HySS.[8]

solutions have a lower pH, while basic solutions have a higher pH. At room temperature (25 °C), pure water is neither acidic nor basic and has a pH of 7.

The pH scale is logarithmic and approximates the negative of the base 10 logarithm of the molar concentration (measured in units of moles per liter) of hydrogen ions in a solution. More precisely it is the negative of the base 10 logarithm of the activity of the hydrogen ion. At 25 °C, solutions with a pH less than 7 are acidic and solutions with a pH greater than 7 are basic. The neutral value of the pH depends on the temperature, being lower than 7 if the temperature increases. Contrary to popular belief, the pH value can be less than 0 or greater than 14 for very strong acids and bases respectively.

pH

pH is defined as the decimal logarithm of the reciprocal of the hydrogen ion activity, a_{H^+}, in a solution.

$$\mathrm{pH} = -\log_{10}(a_{H^+}) = \log_{10}\left(\frac{1}{a_{H^+}}\right)$$

For example, for a solution with a hydrogen ion activity of 5×10^{-6} (at that level, this is essentially the number of moles of hydrogen ions per liter of solution) we get $1/(5\times10^{-6}) = 2\times10^{5}$, thus such a solution has a pH of $\log_{10}(2\times10^{5}) = 5.3$. For a commonplace example based on the facts that the masses of a mole of water, a mole of hydrogen ions, and a mole of hydroxide ions are respectively 18 g, 1 g, and 17 g, a quantity of 10^{7} moles of pure (pH 7) water, or 180 tonnes (18×10^{7} g), contains close to 1 g of dissociated hydrogen ions (or rather 19 g of H_3O^+ hydronium ions) and 17 g of hydroxide ions.

Note that pH depends on temperature. For instance at 0 °C the pH of pure water is 7.47. At 25 °C it's 7.00, and at 100 °C it's 6.14.

This definition was adopted because ion-selective electrodes, which are used to measure pH, respond to activity. Ideally, electrode potential, E, follows the Nernst equation, which, for the hydrogen ion can be written as

$$E = E^0 + \frac{RT}{F}\ln(a_{H^+}) = E^0 - \frac{2.303RT}{F}\mathrm{pH}$$

Reaction kinetics, thermodynamics, law of mass action:

Bioenergetics is the application of thermodynamic laws to the study of energy transformations in biological systems. The energetics of cellular processes can be related to chemical equilibrium and oxidation–reduction potentials of chemical reactions. Whether at the level of molecules, cells, or ecosystems, the flow of energy is central to the maintenance of life. A basic understanding of energy flow is therefore essential to grasping the true beauty, significance, and complexity of biology.

The field of study concerned with the flow of energy through living organisms is called bioenergetics. It is important to note that although the laws of bioenergetics provide critical insight into the driving forces that govern cellular processes, bioenergetics does not provide any insights into the biochemical reaction mechanisms underlying these processes. The purpose of this section is to facilitate an understanding of the laws that govern biological energy transformations by assembling, in the simplest form possible, some basic thermodynamic principles. Thus, a complete understanding of cellular physiology requires an integration of bioenergetics with biochemical reaction mechanisms.

The first law of thermodynamics refers to energy conservation

Biological energy transformations are based on two thermodynamic laws. The first law, commonly known as the **law of conservation of energy**, states that the energy of the universe is constant. This is not a difficult concept to comprehend—it means simply that there is a fixed amount of energy and, while it may be moved about or changed in form, it can all be accounted for somewhere. More to the point, energy is never ‘‘lost’’ in a reaction—an apparent decrease in one form of energy will be balanced by an increase in some other form of energy. In one of the examples mentioned earlier, some of the energy expended in displacing an object appears as work while some appears as heat generated due to friction. In the same way, some of the chemical energy released in the combustion of glucose

will also be found as heat in the environment, while some will be found as bond energy in the product molecules, CO_2 and water.

The second law of thermodynamics refers to entropy and disorder

As biologists we are concerned above all with how much work can be done. But, as suggested earlier, not all energy is available to do work. This brings us to the second law of thermodynamics and the concept of **entropy**. Because it involves the concept of entropy (S), the second law is a bit more difficult to comprehend. What is entropy? Entropy has been variously described as a measure of randomness, disorder, or chaos. However, since entropy is a thermodynamic concept, it is useful to describe it in terms of thermal energy. Temperature is defined as the mean molecular kinetic energy of matter. Thus, any molecular system not at absolute zero (−273◦C, or 0◦K) contains a certain amount of thermal energy—energy in the form of the vibration and rotation of its constituent molecules as well as their translation through space. This quantity of thermal energy and temperature go hand-in-hand: as the quantity of energy increases or decreases, so does temperature. Because temperature cannot be held constant when this energy is given up, it is said to be ‘‘isothermally unavailable’’ (Gr. *isos*, equal). Quantitatively, isothermally unavailable energy is given by the term TS, where T is the absolute temperature and S is entropy.

Since isothermally unavailable energy, and consequently, entropy, are related to the energy of molecular motion, it follows that the more molecules are free to move about, that is, the more random or less ordered or chaotic the system, the greater will be their entropy.

Free energy is related to chemical equilibria

Under appropriate conditions, all chemical reactions will achieve a state of equilibrium, at which there will be no further *net* change in the concentrations of reactants and products. There is a fairly straightforward relationship between free energy and chemical equilibria In a reaction where the reactant A is converted

to product B, K is the equilibrium mass-action ratio—the ratio of concentration of products to the concentration of reactants when the reaction has come to equilibrium. Thus

$$K_{eq} = [B]_{eq}/[A]_{eq} \qquad (5.6)$$

In E= mc^2 the slope of the line represents the change in free energy (_G) when a small amount of the reactant A is converted to the product B. Several useful points can be drawn from this diagram.

1. At equilibrium, the slope of the line is zero. Consequently, *when reactions are at equilibrium*, _G = 0

and no useful work can be accomplished.

2. The further the mass-action ratio is displaced from equilibrium (Keq), the greater the free energy change for conversion of the same small amount of A to B. The free energy change for a reaction is a function of its displacement from equilibrium. Therefore, *the further a reaction is poised away from equilibrium, the more free energy is available as the reaction proceeds toward equilibrium*.

3. As A approaches equilibrium, *Δ*G is negative and free energy is available to do work. However, as the

reaction proceeds past equilibrium toward B, *Δ*G is becomes positive and energy must be supplied. *A system can do work as it moves toward equilibrium*. Note that if the reaction were initiated with pure B, the direction of the arrows would be reversed and work could be done as B approached equilibrium. The relationship between the free energy change (*Δ*G) and the equilibrium constant (Keq) can be expressed

quantitatively as:

$$\Delta G = \Delta G^{\circ\prime} + RT \ln K_{eq} \qquad (5.7)$$

However, at equilibrium, *Δ*G = 0 and Equation 5.7 can be rearranged to give

$$\Delta G^{\circ\prime} = -RT \ln K_{eq} = -2.3RT \log K_{eq} \qquad (5.8)$$

Furthermore, the actual free energy change (*Δ*G) of a reaction *not* at equilibrium is given by:

$$\Delta G = \Delta G^{\circ\prime} + 2.3RT \log \Gamma \qquad (5.9)$$

where R is the universal gas constant, T is the absolute temperature, and *T*equals the observed (i.e., nonequilibrium) mass-action ratio. Equation 5.8 can then be substituted in Equation 5.9 and rearranged to give:

$$\Delta G = -2.3RT \log(K_{eq}/\Gamma) \qquad (5.10)$$

Equation 5.10 reinforces the observation that the value of *Δ*G is a function of the degree to which a reaction is displaced from equilibrium. When _*T*=Keq, the reaction is at equilibrium and *Δ*G = 0 and no useful work can be done. When *T*is less than Keq, *Δ*G < 0 and the reaction will occur spontaneously with the release of energy which can be used to perform useful work. However, when *T*is greater than Keq, *Δ*G > 0 and the reaction will not occur spontaneously, but rather, requires an input of energy to proceed.

Acid–base reaction:

An acid–base reaction is a chemical reaction that occurs between an acid and a base, which can be used to determine pH. Several theoretical frameworks provide alternative conceptions of the reaction mechanisms and their application in solving related problems; these are called the acid–base theories, for example, Brønsted–Lowry acid–base theory.

Their importance becomes apparent in analyzing acid–base reactions for gaseous or liquid species, or when acid or base character may be somewhat less apparent. The first of these concepts was provided by the French chemist Antoine Lavoisier, around 1776.

Lewis definition

The hydrogen requirement of Arrhenius and Brønsted–Lowry was removed by the Lewis definition of acid–base reactions, devised by Gilbert N. Lewis in 1923, in the same year as Brønsted–Lowry, but it was not elaborated by him until 1938. Instead of defining acid–base reactions in terms of protons or other bonded substances, the Lewis definition defines a base (referred to as a Lewis base) to be a compound that can donate an electron pair, and an acid (a Lewis acid) to be a compound that can receive this electron pair.

For example, boron trifluoride, BF3 is a typical Lewis acid. It can accept a pair of electrons as it has a vacancy in its octet. The fluoride ion has a full octet and can donate a pair of electrons.

Acid–base equilibrium:

The reaction of a strong acid with a strong base is essentially a quantitative reaction. For example,

$$HCl_{(aq)} + Na(OH)_{(aq)} \rightarrow H_2O + NaCl_{(aq)}$$

In this reaction both the sodium and chloride ions are spectators as the neutralization reaction,

$$H^+ + OH^- \rightarrow H_2O$$

The equilibrium constant for this reaction can be derived from the acid dissociation constants of adenine and of the dihydrogen phosphate ion.

$$[A^-]\,[H^+] = K_{a1}[AH]$$
$$[HPO_4^{2-}]\,[H^+] = K_{a2}[H_2PO_4^-]$$

The notation [X] signifies "concentration of X". When these two equations are combined by eliminating the hydrogen ion concentration, an expression for the equilibrium constant, *K* is obtained.

$$[A^-]\,[H_2PO_4^-] = K[AH]\,[HPO_4^{2-}]; \qquad K = \frac{K_{a1}}{K_{a2}}$$

Acid–alkali reaction:

An acid–alkali reaction is a special case of an acid–base reaction, where the base used is also an alkali. When an acid reacts with an alkali salt (a metal hydroxide), the product is a metal salt and water. Acid–alkali reactions are also neutralization reactions.

In general, acid–alkali reactions can be simplified to

$$OH^-_{(aq)} + H^+_{(aq)} \rightarrow H_2O$$

by omitting spectator ions.

Acids are in general pure substances that contain hydrogen cations (H^+) or cause them to be produced in solutions. Hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) are common examples. In water, these break apart into ions:

$$HCl \rightarrow H^+_{(aq)} + Cl^-_{(aq)}$$
$$H_2SO_4 \rightarrow H^+_{(aq)} + HSO^-_{4(aq)}$$

The alkali breaks apart in water, yielding dissolved hydroxide ions:

$$NaOH \rightarrow Na^+_{(aq)} + OH^-_{(aq)}$$

Redox potential, free energy:

Photosynthesis and respiration, are electrochemical phenomena. Each operates as a sequence of oxidation–reduction reactions in which electrons are transferred from one component to another. Thus, the oxidation of one component is linked or coupled to the reduction of the next component. Such coupled electron transfer reactions are known as *red*uction–*ox*idation or *redox* reactions. However, it is not clear how to treat the electrons, which have no independent existence. Moreover, our interest in redox couples is more in their tendency to accept electrons from or donate electrons to another couple, a tendency known as **redox potential**.

Redox potentials allow the feasibility and direction of electron transfers between components in a complex system to be predicted. Indeed, in order to understand electron flow in photosynthesis and respiration it is necessary to have a working understanding of redox potential and how it is applied.

The direction of electron transfer between redox couples can be predicted by comparing their midpoint potentials (E*m*). Thermodynamically spontaneous electron transfer will proceed from couples with the more negative (less positive) redox potential to those with the less negative (more positive) redox potential. The energy-transducing membranes of bacteria, mitochondria, and chloroplasts all contain electron-transport

systems involving a number of electron carriers with different midpoint redox potentials.

In addition to allowing us to predict the direction of electron transfer, redox potentials also permit the calculation of Gibbs free energy changes for electron-transfer reactions. This can be done using the following relationship:

$$\Delta G^{\circ\prime} = -n\mathbf{F}\Delta E_m \qquad (5.21)$$

where *n* is the number of electrons transferred and F is the Faraday constant (96 500 coulombs mol−1).

Biological electron transfers may involve either single electrons or pairs, but energy calculations are almost always based on *n*=2._Em is the redox interval through which the electrons are transferred and is determined as Thus, for a coupled transfer of electrons from water (the donor) to NADP+ (the

acceptor) as occurs in photosynthetic electron transport in chloroplasts, ΔEm = (−320) − (+820) = −1140mV = −1.14 V. Substituting this in Equation 5.21, the value of ΔG°_ for a two-electron transfer from H2O to NADP+ is +220 Kj mol−1. Note that the sign of ΔG° is positive, indicating that this electron transfer will not occur spontaneously.
In photosynthesis, light energy is used to drive this coupled endergonic reaction. In contrast, mitochondria transfer electrons from NADH to O_2. For this coupled electron-transfer reaction, the value of ΔEm is +1.14 V and consequentlyΔG°_ is−220 kJ mol−1, indicating that this electron transfer in mitochondria is exergonic and thus will occur spontaneously. It is important to note that the molecular mechanisms by which these electrons are transferred through complex processes such as photosynthesis, respiration, and nitrogen assimilation are not in the purview of bioenergetics.

14. Enzyme: Enzyme kinetics, catalytic reactions and regulatory properties, inhibitions, iso-enzymes, allosterism, ribozyme and abzymes.

Enzymes

Enzymes are proteins that act as catalysts within living cells. **Catalysts** increase the rate at which chemical reactions occur without being consumed or permanently altered themselves. A **chemical reaction** is a process that converts one or more substances (known as reagents, reactants, or substrates) to another type of substance (the product). As a catalyst, an enzyme can facilitate the same chemical reaction over and over again.

Examples

Lipases - a group of enzymes that help digest fats in the gut.

Amylase - helps change starches into sugars. Amylase is found in saliva.

Maltase - also found in saliva; breaks the sugar maltose into glucose. Maltose is found in foods such as potatoes, pasta, and beer.

Trypsin - found in the small intestine, breaks proteins down into amino acids.

Lactase - also found in the small intestine, breaks lactose, the sugar in milk, into glucose and galactose.

Acetylcholinesterase - breaks down the neurotransmitter acetylcholine in nerves and muscles.

Helicase - unravels DNA.

DNA polymerase - synthesize DNA from deoxyribonucleotides.

Properties of enzymes

(1) Enzymes are complex macromolecules with high molecular weight.
(2) They catalyze biochemical reactions in a cell. They help in the breakdown of large molecules into smaller molecules or bring together two smaller molecules to form a larger molecule.
(3) Enzymes do not start a reaction. However, they help in accelerating it.
(4) Enzymes affect the rate of biochemical reaction and not the direction of the reaction.
(5) Most of the enzymes have high turnover number. Turnover number of an enzyme is the number of molecules of a substance that is acted upon by an enzyme per minute under saturated substrate concentration. High turnover number of enzymes increases the efficiency of reaction.
(6) Enzymes are specific in action.

(7) Enzymatic activity decreases with increase in temperature and all enzymes show maximum activity at an optimum kmp of 30-40 0C.
(8) They show maximum activity at an optimum pH of 6 – 8.
(9) The velocity of enzyme increases with increase in substrate concentration and then, ultimately reaches maximum velocity.

Function of enzymes

Enzymes serve a wide variety of functions inside living organisms.

1. They are indispensable for signal transduction and cell regulation, often via kinases and phosphatases.

2. They also generate movement, with myosin hydrolyzing ATP to generate muscle contraction, and also transport cargo around the cell as part of the cytoskeleton. Other ATPases in the cell membrane are ion pumps involved in active transport.

3. Enzymes are also involved in more exotic functions, such as luciferase generating light in fireflies.

4. Viruses can also contain enzymes for infecting cells, such as the HIV integrase and reverse transcriptase, or for viral release from cells, like the influenza virus neuraminidase.

5. An important function of enzymes is in the digestive systems of animals. Enzymes such as amylases and proteases break down large molecules (starch or proteins, respectively) into smaller ones, so they can be absorbed by the intestines. Starch molecules, for example, are too large to be absorbed from the intestine, but enzymes hydrolyze the starch chains into smaller molecules such as maltose and eventually glucose, which can then be absorbed. Different enzymes digest different food substances. In ruminants, which have herbivorous diets, microorganisms in the gut produce another enzyme, cellulase, to break down the cellulose cell walls of plant fiber.

Enzyme kinetics

It is the study of the chemical reactions that are catalysed by enzymes. In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction are investigated. Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme.

Enzymes are usually protein molecules that manipulate other molecules—the enzymes' substrates. These target molecules bind to an enzyme's active site and are transformed into products through a series of steps known as the enzymatic mechanism

$$E + S \rightleftarrows ES \rightleftarrows ES^* \rightleftarrows EP \rightleftarrows E + P$$

Enzyme catalysis

Catalysis is a phenomenon in which the rate of the reaction is altered, and the substance used to accelerate remains unchanged regarding quantity and chemical properties. The substance used to change the speed of the reaction is called a catalyst. Enzymes are a type of catalysts which are

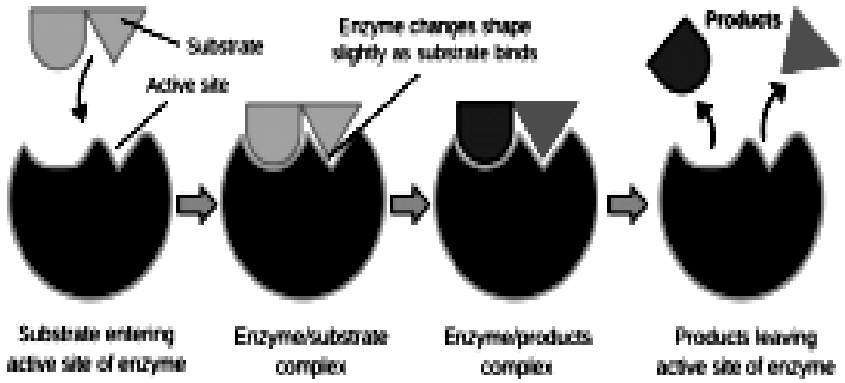
responsible for increasing the rate of reaction in plants and animals. The catalysis in which enzymes act as a catalyst is called enzyme catalysis.

Enzymes are complex compounds which are made up of nitrogen. Animals and plants produce these compounds. Enzymes are proteins which have high molecular mass and form a heterogeneous mixture when dissolved in water. These proteins act very efficiently and are responsible for various reactions which occur in the body of living beings. These proteins can also be called as biochemical catalysts and the catalysis as biochemical catalysis.

Characteristics of enzyme catalysis

- A single molecule of this catalysis can transform a million molecules of the reactant per second. Hence it is highly efficient.
- These biochemical catalysts are unique in nature i.e. the same catalyst cannot be used in more than one reaction.
- The effectiveness of a catalyst is maximum at its optimum temperature. The activity of the biochemical catalysts declines at either side of the optimum temperature.
- Biochemical catalysis is dependent upon the pH of the solution. A catalyst works best at an optimum pH which ranges between 5-7 Ph values.
- The activity of the enzymes usually increases in the presence of a coenzyme or an activator such as Na^+, Ca^{2+} .The rate of the reaction increases due to the presence of a weak bond which exists between the enzyme and a metal ion.

Mechanism of enzyme catalyst:



Catalysis with enzyme as a catalyst

The enzyme consists of a number of cavities which are present on the outer surface. These cavities possess groups such as -COOH, -SH, etc. These centres are called as the active centre of the biochemical particle. The substrate which has the opposite charge of the enzyme fits into the cavities just as a key fits into a lock. Due to the existence of the active groups, the complex formed decomposes to give the products.

Hence this happens in two steps:

Step1: Combining of enzyme and the reactant

E+R→ER

Step 2: Disintegration of the complex molecule to give the product

ER→E+P

Enzyme inhibition

Enzymes play central roles in life processes. It holds for most enzymes that their function is needed only in certain conditions. When those conditions do not apply, the activity of a given enzyme can be futile or even harmful. Accordingly, the activity of most enzymes is under strict control. Enzymes can be regulated at multiple levels, ranging from transcriptional regulation of the expression of the enzyme-encoding gene through the direct regulation of the activity of the enzyme molecule by effector molecules to the controlled proteolytic decomposition of the enzyme. In this chapter, only those inhibitors will be reviewed that reversibly and specifically bind to enzymes through non-covalent interactions and inhibit the substrate-binding and/or catalytic apparatus of the given enzyme. These inhibitors can be classified into three mechanistic groups based on their mechanism of action: competitive, uncompetitive and mixed inhibitors. The type of inhibition can be determined through enzyme kinetic measurements.

Competitive inhibition

Competitive inhibitors compete for the substrate-binding site of the enzyme with the substrate, because the substrate and the inhibitor bind to identical or overlapping sites. Due to the overlapping nature of the binding sites, a ternary complex—in which the substrate and the inhibitor would simultaneously bind to the enzyme—cannot form. Accordingly, in the enzyme-inhibitor complex, the enzyme is completely inactive.

.

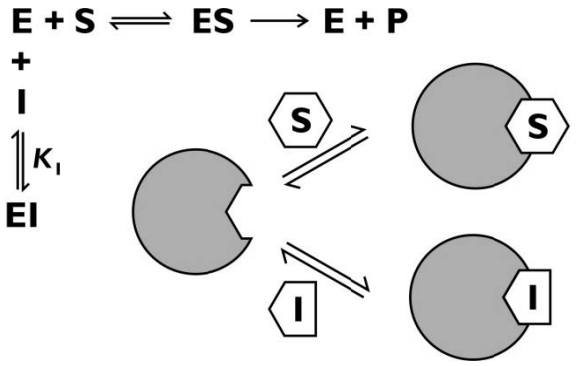


Figure. The scheme of competitive inhibition

Uncompetitive inhibition

Some inhibitors bind only to the ES complex without binding to the free enzyme. This interaction scheme is illustrated in Figure 9.8.

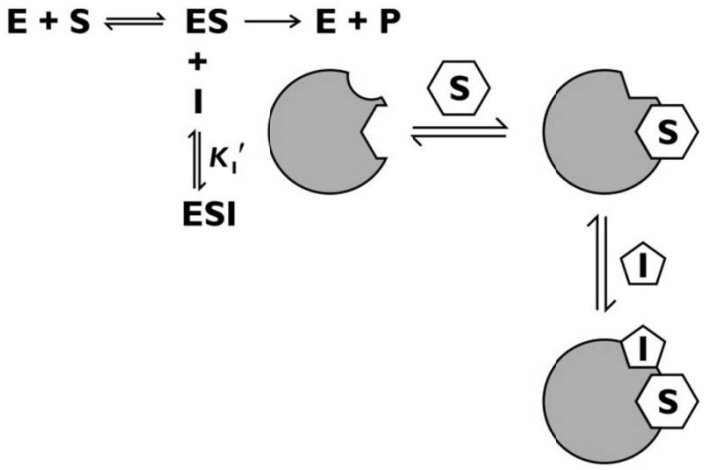


Figure. Uncompetitive inhibition

Mixed or non-competitive inhibition inhibition

There are inhibitors that can bind both to the free enzyme as well as to the ES complex.

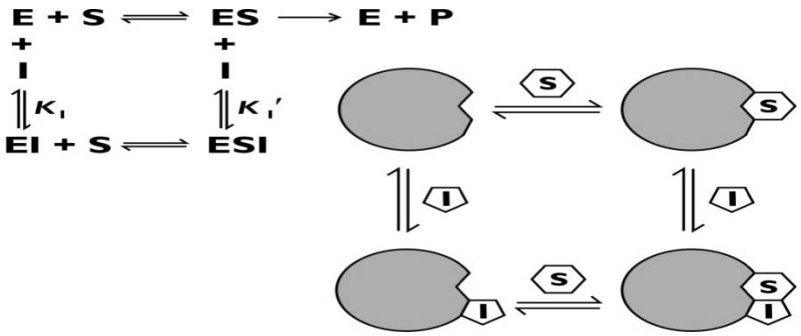


Figure. Mixed inhibition

Isozymes

These were first described by R. L. Hunter and Clement Markert (1957) who defined them as different variants of the same enzyme having identical functions and present in the same individual. This definition encompasses enzyme variants that are the product of different genes and thus represent different loci (described as isozymes) and enzymes that are the product of different alleles of the same gene (described as allozymes).

Isozymes are usually the result of gene duplication, but can also arise from polyploidisation or nucleic acid hybridization. Over evolutionary time, if the function of the new variant remains identical to the original, then it is likely that one or the other will be lost as mutation accumulate, resulting in a pseudogene. However, if the mutations do not immediately prevent the enzyme from functioning, but instead modify either its function, or its pattern of gene expression, then the two variants may both be favoured by natural selection and become specialised to different functions. For example, they may be expressed at different stages of development or in different tissues.

Allosterism

Allosteric regulation (allosterism) is the modulation of an enzyme's activity via the binding of an effector molecule (ligand) to a site *other* than the enzyme's active site (an allosteric site)

Allosteric binding causes a conformational change in the enzyme's structure which affects the enzyme's affinity for substrate

Allosteric regulation can be either positive (activation) or negative (non-competitive inhibition)

Positive Allosterism

An example of allosteric activation is seen in the binding of oxygen molecules to haemoglobin

Haemoglobin is composed of four distinct subunits and can bind up to four oxygen molecules (HbO8)

As each oxygen molecule binds, it changes the conformation of haemoglobin and increases its affinity for oxygen

This ensures that haemoglobin will transport the maximum amount of oxygen from oxygen-rich areas (i.e. the lungs)

Conversely, the release of an O2 molecule decreases haemoglobin's affinity for oxygen – promoting its release in the tissues

Negative Allosterism

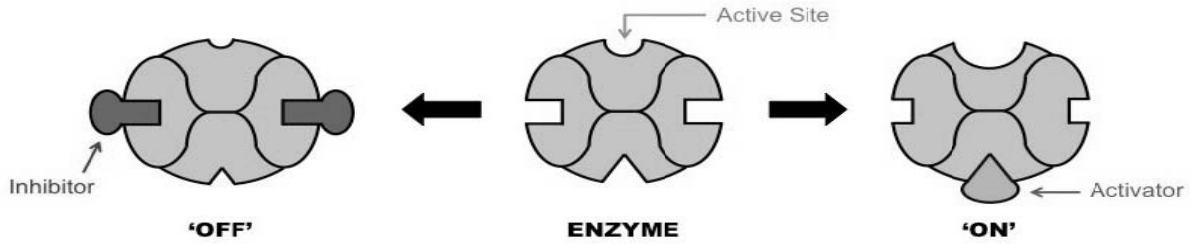
An example of allosteric inhibition can be seen in any example of non-competitive inhibition

Phosphofructokinase (PFK) is an enzyme involved in the breakdown of glucose during glycolysis (to make ATP)

ATP binds to an allosteric site on PFK and inhibits its activity – preventing glycolysis from occurring

Thus ATP prevents the further production of more ATP when energy stocks are high (end-product inhibition)

When energy stocks are low, there is insufficient ATP to inhibit PFK and glycolysis will be able to proceed



Allosteric Regulation

Ribozymes (ribonucleic acid enzymes)

They are RNA molecules that are capable of catalyzing specific biochemical reactions, similar to the action of protein enzymes. The 1982 discovery of ribozymes demonstrated that RNA can be both genetic material (like DNA) and a biological catalyst (like protein enzymes), and contributed to the

RNA world hypothesis, which suggests that RNA may have been important in the evolution of prebiotic self-replicating systems. The most common activities of natural or in vitro-evolved ribozymes are the cleavage or ligation of RNA and DNA and peptide bond formation. Within the ribosome, ribozymes function as part of the large subunit ribosomal RNA to link amino acids during protein synthesis. They also participate in a variety of RNA processingreactions, including RNA splicing, viral replication, and transfer RNA biosynthesis. Examples of ribozymes include the hammerhead ribozyme, the VS ribozyme, Leadzyme and the hairpin ribozyme.

Ribozymes have been proposed and developed for the treatment of disease through gene therapy. One major challenge of using RNA based enzymes as a therapeutic is the short half-life of the catalytic RNA molecules in the body. To combat this, the 2' position on the ribose is modified to improve RNA stability. One area of ribozyme gene therapy has been the inhibition of RNA-based viruses.

A type of synthetic ribozyme directed against HIV RNA called gene shears has been developed and has entered clinical testing for HIV infection. Similarly, a ribozyme has been designed to target the hepatitis C virus RNA. The ribozyme is able to cleave the conserved regions of the virus's genome which has been shown to reduce the virus in mammalian cell culture. Despite these efforts by researchers, these projects have remained in the preclinical stage.

Abzyme

Abzyme is a monoclonal antibody with catalytic activity. Abzymes are usually raised in lab animals immunized against synthetic haptens, but some natural abzymes can be found in normal humans (anti-vasoactive intestinal peptide autoantibodies) and in patients with autoimmune diseases such as systemic lupus erythematosus, where they can bind to and hydrolyze DNA. They are, however, subjects of considerable academic interest. Studying them has yielded important insights into reaction mechanisms, enzyme structure and function, catalysis, and the immune system itself.

Enzymes function by lowering the activation energy of the transition state of a chemical reaction, thereby enabling the formation of an otherwise less-favourable molecular intermediate between the reactant(s) and the product(s). If an antibody is developed to bind to a molecule that's structurally and electronically similar to the transition state of a given chemical reaction, the developed antibody will bind to, and stabilize, the transition state, just like a natural enzyme, lowering the activation energy of the reaction, and thus catalyzing the reaction. By raising an antibody to bind to a stable transition-state analog, a new and unique type of enzyme is produced.

So far, all catalytic antibodies produced have displayed only modest, weak catalytic activity. The reasons for low catalytic activity for these molecules have been widely discussed. Possibilities indicate that factors beyond the binding site may play an important, in particular through protein dynamics. Some abzymes have been engineered to use metal ions and other cofactors to improve their catalytic activity.

Vitamins co factors and coenzymes

Vitamins are organic compounds that are required in small amount (µg or mg/day) for normal growth metabolism and proper functioning of the body.

Vitamins are also known as accessory food factors or food hormones. Animal can't synthesize vitamins, and therefore depends upon plants and microbes for their supply.

Any deficiency of vitamins for a longer period or presence of anti-vitamins in the diet causes deficiency disease or avitaminosis.

However, treatment with antibiotics often results in deficiency of B-Complex vitamins because the intestinal bacteria that synthesize them get killed by such drugs.

For this reason, doctors recommend vitamin B-complex during antibiotic therapy. Excess intake of vitamins may cause some pathological conditions called hyper-vitaminosis. However, it is rare and occurs in case of fat soluble vitamins which can be stored in considerably high amounts.

Types of Vitamins:

On the basis of solubility, vitamins are of two types:

(a) Fat soluble vitamins: Vitamins A, D, E & K.

(b) Water soluble vitamins: Vitamins C and B-complex (B_1, B_2, B_6 etc.)

Vitaminoids:

These are the organic compounds having similar biological properties like vitamins but required in larger amounts.

They are of following types:

(a) Fat soluble vitaminoids:

Essential Fatty acids and ubiquinone's.

(b) Water soluble vitaminoids:

Biotin (Vit-B), Choline (Vit-B_4), Inositol (Vit-B_8), bioflavonoids, N- Lipoic acid etc.

History:

Lunin (1881) first discovered them and Funk (1912) coined the term 'vitamins'. Later, Drummond (1920) proposed the term vitamin by dropping the final 'e'

Pseudovitamin, Antivitamin and Provitamin:

Pseudovitamins (false vitamins) are certain organic compounds that are structurally similar to some vitamins but don't show the physiological actions of the vitamin. For example, methylcobalmine is a pseudovitamin of cyanocobalamine (Vit – B_{12}).

Antivitamin is a compound which resembles structurally to a vitamin but inhibits or antagonize the function of the vitamin. For example, pyrithiamine, galactoflavin and avidin are antivitamins of thiamine, riboflavin and biotin respectively. Provitamin is the precursor of a vitamin which when taken through diet is converted into the vitamin in the body, e.g. (3-carotene (provitamin (A), ergosterol (Provitamin D) etc.

Fat Soluble Vitamins:

Vitamin A:

Clinical name:

Axerophthol, antixeropthalmic or antinyctalopic vitamin.

Chemical name:

Chemically, vitamin A ($C_{20}H_{29}OH$) is an unsaturated alcohol called retinol. Vitamin A exists in two isomeric forms: vitamin A (retinol) is a trans-isomer and occurs naturally, whereas vitamin A_2(dehydroretinal or retional2) is a cisisomer and has 30 – 40% of vitamin A actively.

Sources:

Plant sources like green vegetables, fruits, and cereals supply pro vitamin A (β-carotene) in diet. Animal sources of vitamin A are liver, milk, butter, egg yolk etc. Liver of fresh water fish contain A_2.

Functions:

(i) Retinol (vitamin A aldehyde) combines with lysine residues of opsin protein to form rhodopsin pigments of rod cells of retina, so it is essential for night vision,

(ii) Retinoic acid (vitamin A acid) has some anticancer effects,

(iii) Vitamin A maintains the integrity of epithelial cells; permeability of cell membranes as well as the membranes of organelles,

(iv) In young animals vitamin A causes growth, formation of bones and teeth.

Deficiency Symptoms:

(i) Vitamin A deficiency causes the defective night vision called night blindness (nyctalopia or Henerolopia).

(ii) In children deficiency of vitamin A_2 causes xerophthalmia (drying of conjunctiva) and keratomalacia ulceration and softening of cornea) that may lead to complete blindness,

(iii) Toad's skin is another detectable early symptom of vitamin A where the skin becomes dry and rough particularly in the lateral part of forearms and sides of thigh.

Hypervitaminosis A:

In acute cases of hypervitaminosis A headache, nausea, vomiting and drowsiness noticed. But in chronic cases (prolonged intake) the symptoms develop are anorexia (loss of appetite), alopecia (loss of hair), cracking of lips, dry itchy skin, pain in bones and joints.

Vitamin D:

Clinical name:

Clinically, Vitamin D is called antirachitic vitamin because it possesses the property of curing or preventing ticket.

Chemical name:

Chemically Vitamin D is a steroid which is related to calcium metabolism. Hence it is called calciferol. Vitamin D exists in 2 forms i.e. Vitamin D_2 (ergocalciferol) and Vitamin D_3 (cholecalciferol). They are synthesized from the provitamins by the action of UV-rays of sunlight. Because of it, vitamin D is called sunshine vitamin. The provitamin D_2 (ergosterol) occurs in plants and the provitamin D_3 (7-dehydrocholesterol) occurs in animals. The term vitamin D_1 is no longer used.

Sources:

Vit. D_3 obtained from fish liver oil, egg, milk, butter, ghee etc.

Functions:

(i) Vitamin D_3 is also considered as a prohormone which gives rise to a hormone colcitriol (1, 2, 5 Dihydroxy cholecalciferol) by various metabolic changes. Calcitriol has a role in calcium and phosphate metabolism,

(ii) Vitamin D activates the transcription of mRNA for calcium binding protein,

(iii) It helps in the growth and development of bone and teeth,
(iv) Increase the excretion of phosphate.

Deficiency symptoms:

Vitamin D deficiency leads to rickets in children and osteomalacia in adult. Rickets is characterized by bowlegs, knock knees, bending of ribs leading to pigeon's breast, enlargement of ankles, knees, wrists, elbow etc. In osteomalacia the bones become weak and fragile instead of being soft due to decalcification of bones.

Hypervitaminosis D:

Excess intake of vitamin D cause increase level of Ca and P. As a result, kidney, arteries, muscles, etc. become calcified. The early symptoms cause anorexia (loss of appetite), thrist, constipation and polyuria followed by nausea, vomiting and diarrhea.

Vitamin E:

Clinical name:

It is called antisterility vitamin or fertility vitamin because of its requirement in proper functioning of reproductive system.

Chemical name:

Tocopherol (tokes = child birth; phero = to bear, ol = alcohol)

Source:

Vegetable oil, leafy vegetables, milk, cheese, egg, meat etc.

Function:

(i) It acts as an antioxidant and prevents oxidation of vitamin A, K, essential fatty acids.
(ii) It keeps the skin glowing by reducing keratinization. Hence, it is also called as beauty vitamin,
(iii) It helps in the normal functioning of skeletal muscles, gonads and renal tubules.

Deficiency symptoms:

(i) Causes sterility and miscarriage,
(ii) Causes muscular weakness and dystrophy (degeneration.)

Vitamin K:

Clinical name:

It is called antihemorrhagic vitamin or vitamin for blood clotting or coagulation vitamin.

Chemical name:

Chemically vitamin K is a naphthoquinone derivative. Naturally it occurs in two forms, i.e. vitamin K_1 (phylloquinone) and vitamin K_2(menaquinone or farnoquinone). The vitamin K_3 (mendione) is a synthetic product.

Source:

Green leafy vegetables, soybean, carrots, potatoes, milk, fish and meats etc.

Dietary requirement:

140 – 200 mg.

Function:

(i) Act as co-enzyme Q and participate in oxidative phosphorylation in ETC.,
(ii) Acts as a co-factor of carboxylase,
(iii) Required for fat absorption.

Deficiency symptoms:

(i) Delay in blood clotting,

(ii) Cause hemorrhagic disease of newborn,

(iii) Defective functioning of liver.

Hypervitaminosis K:

Develop hyperbilirubinemia.

Water Soluble Vitamin

Vitamin C:

Clinical name:

It is called antiscorbutic acid because it prevents scurvy. It is also known as anti- rabies or and cancer vitamin.

Chemical name:

Chemically it exists in L-ascorbic acid and L-dehydroascorbic acid (ascorbone). The L-ascorbic acid is ay-lactone (with internal esterification) that is synthesized in plants and most animals except primates and guinea pigs.

Source:

Citrus fruits (lemons, oranges), grapes, apple, papaya, guava, vegetables etc.

Daily requirement:

40 – 50 mg.

Functions:

(i) It is required for absorption of iron,

(ii) It keeps gums and capillary walls healthy.

(iii) It gives resistance against cold and viruses. Hence, it is often called as anti-viral vitamin,

(iv)It is necessary for wound healing,

(v) It acts as co-enzyme for hydroxylation and oxidation -reduction reactions. Thus, it helps in metabolism of amino acids, collagen synthesis etc.

Deficiency symptoms: It causes scurvy (Sailor's disease) characterized by bleeding gums, loosening of teeth, fragile capillaries, failure in wound healing, anemia, general weakness etc.

Vitamin B-Complex:

It includes at least 10-11 different water soluble vitamins. They are grouped together as B-complex because all the members' acts as coenzymes and their actions are closely related.

Thousands of chemical reactions proceed very rapidly at any given instant within all living cells of an organism. Virtually all of these reactions are mediated by remarkable molecular devices called enzymes. That is, the enzymes are central to every biochemical reaction and are called the catalysts of biological systems (biocatalysts).

They in organized sequences and catalyse the hundreds of stepwise reactions by which nutrient molecules are degraded, chemical energy is conserved and transformed, and biological macromolecules are made from simple precursors. Through the action of regulatory enzymes, metabolic pathways are highly coordinated to yield a harmonious interplay among the many different activities necessary to sustain life.

Enzymes catalyse an enormous diversity of biochemical reactions due to their capacity to specifically bind a very wide range of molecules. By utilizing the full repertoire of intermolecular forces, enzymes

bring substrates together in an optimal orientation, the prelude to making and breaking chemical bonds.

They catalyse reactions by stabilizing transition states, the highest energy-species in reaction pathways. By selectively stabilizing a transition state, an enzyme determines which one of several potential biochemical reactions actually takes place.

Until 1980s, all enzymes were believed to be proteins. Then, Tom Cech and Sidney Altman independently discovered that certain RNA molecules may function as enzymes may be effective biocatalysts. These RNA biocatalysts have come to be known as ribozymes.

An enzyme is a protein that is synthesised in a living cell and catalyses or speeds up a thermodynamically possible reaction so that the rate of the reaction is compatible with the biochemical process essential for the maintenance of the cell. It is sometimes called as organic catalyst or biocatalyst. Over 90% of enzymes are simple globular proteins (Fig. 8.14). The remainder is conjugated proteins, which have a non-protein fraction called the prosthetic group. Many enzymes have relative molecular mass of between 10,000 and 50,000da.

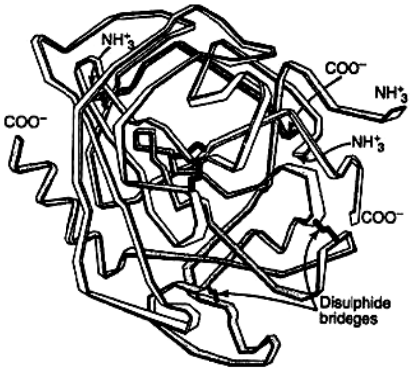


Fig. 8.14 : Three-dimensional globular structure of chymotrypsinogen. It is an inactive precursor of pancreatic chymotrypisn

The first enzyme discovered was amylase, which catalyses the conversion of starch to maltose, in 1833 by two French chemists Payen and Persoz. However, it was not well-known until 1876 when Wilhelm Kuhne, the distinguished German biochemist, proposed the term enzyme.

Unit of Enzyme:

The actual molar amount of the enzyme in an enzyme-catalyzed reaction is not known in many situations. In such cases the amount of enzyme can be expressed in terms of the enzyme activity observed.

The International Commission on Enzymes established by International Union of Biochemistry defines One International Unit of enzyme as the amount of enzyme that catalyzes the formation of one micromole of product in one minute.

In determining the One International Unit the conditions of assay must be specified because enzymes are very sensitive to factors such as pH, temperature, and ionic strength. Another definition for units of enzyme is the ‘katal’. One katal is defined as the amount of enzyme that catalyses the conversion of one mole of substrate to product in one second. Thus, one katal equals 6 x 107 international units.

Chemical Nature of Enzymes:

All enzymes are globular proteins with the exception of recently discovered RNA enzymes. Some enzymes may additionally contain a non-protein group. Accordingly there are two types of enzymes, simple and conjugate.

Simple Enzyme:
It is an enzyme which is wholly made up of protein. Active site is formed by specific grouping of its own amino acids. Additional substance or group is absent, e.g., pepsin, trypsin, urease.

Conjugate Enzyme:
It is an enzyme which is formed of two parts— a protein part called apoenzyme (e.g., flavoprotein) and a nonprotein part named cofactor. The complete conjugate enzyme, consisting of an apoenzyme and a cofactor, is called holoenzyme. Active site is formed jointly by apoenzyme and cofactor.
Cofactor is small, heat stable and dialysable part of conjugate enzyme. It may be inorganic or organic in nature. Organic cofactors are of two types, coenzymes and prosthetic groups.
Coenzymes are easily separable non-protein organic cofactors. Prosthetic groups are non-protein organic cofactors firmly attached to apoenzymes, e.g., heme (=haem), biotin, pyridoxal phosphate. Heme (= haem) is iron containing prosthetic group in cytochromes, haemoglobin, myoglobin, catalase and peroxidase.
The last two cause breakdown of hydrogen peroxide to water and oxygen. FMN and FAD are considered prosthetic groups by some workers while others consider them to be coenzymes.
Both coenzyme and prosthetic group take part in group transfer reactions. Prosthetic group requires a single apoenzyme for picking up the group and transferring the same. Coenzyme requires two Apo enzymes, one for picking up the group and the second for transferring the group, e.g., NAD^+, $NADP^+$, CoA.

Active Site or Active Spot:
The whole of enzyme molecule is not active in catalysing a chemical reaction. Only a small portion of it is active. It is called active site or active spot. An enzyme may have one to several active sites. An active site or spot is an area of the enzyme which is capable of attracting and holding particular substrate molecules by its specific charge, size and shape so as to allow the chemical change.
It fails to recognise other molecules. Active site consists of a few amino acids and their side groups which are brought together in a particular fashion due to secondary and tertiary folding of a protein molecule (Fig. 9.27) and its association with the cofactor, if any.
For example, the active site for aldolase is glycine-histidine-alanine while that of pyruvic oxidase is aspartic acid-cysteine-alanine. The remaining amino acids help maintain the shape of the enzyme molecule.

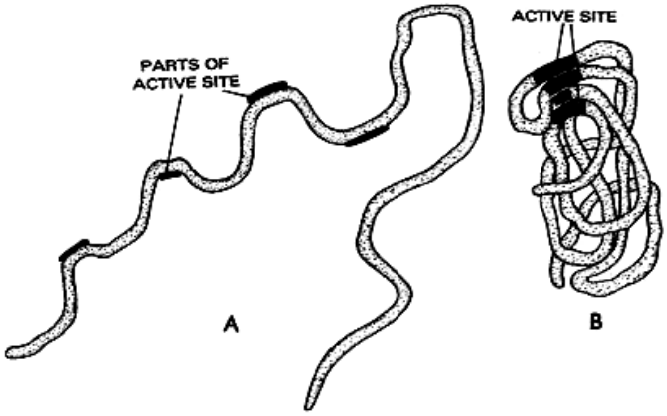


Fig. 9.27. Grouping of amino acids of a polypetide during the formation of tertiary structure to produce an active site.

Characteristics of Enzymes:

All enzymes are proteins, but a functional enzyme has different components and these components are named differently, viz.,

Holoenzyme:
A conjugated protein and functional enzyme.

Apoenzyme:
Polypeptide segment of the enzyme, which is catalytically inactive.
Coenzyme:
The non-protein organic moiety, which can frequently be separated from the apoenzyme.
Prosthetic group:
If a substance is firmly (covalently) attached to the protein part of the enzyme, it is referred to as a prosthetic group. It is the non-protein portion of any conjugated protein. So coenzyme is a specific example of prosthetic group.
Activator:
There are many metalloprotein enzymes in which the metal ion (e.g. Mg^{++}, Mn^{++}, and Zn^{++}) is bonded either to the apoenzyme or to the coenzyme. The metal is usually designated as activator. They form a co-ordination complex between the enzyme and the substrate, and activate the substrate by prompting electronic shifts.
Pro-enzyme or Zymogens:
They are simple protein enzymes, which are secreted, in an inactive form.

Modes of Enzyme Action:

There are two view points by which enzymes are supposed to bring about chemical reaction.
i. Lock and Key Hypothesis:
It was put forward by Emil Fischer in 1894. According to this hypothesis, both enzyme and substrate molecules have specific geometrical shapes. 'In the region of active sites the surface configuration of the enzyme is such as to allow the particular substrate molecules to be held over it. The active sites also contain special groups having —NH2, —COOH, —SH for establishing contact with the substrate molecules.
The contact is such that the substrate molecules or reactants come together causing the chemical change. It is similar to the system or lock and key. Just as a lock can be opened by its specific key, a substrate molecule can be acted upon by a particular enzyme. This also explains the specificity of enzyme action.
After coming in contact with the active site of the enzyme, the substrate molecules or reactants form a complex called enzyme-substrate complex. In the complexed state the molecules of the substrate undergo chemical change.
The products remain attached to the enzyme for some time so that an enzyme-product complex is also formed. However, the products are soon released (Fig. 9.34) and the freed enzyme is able to bind more substrate molecules.

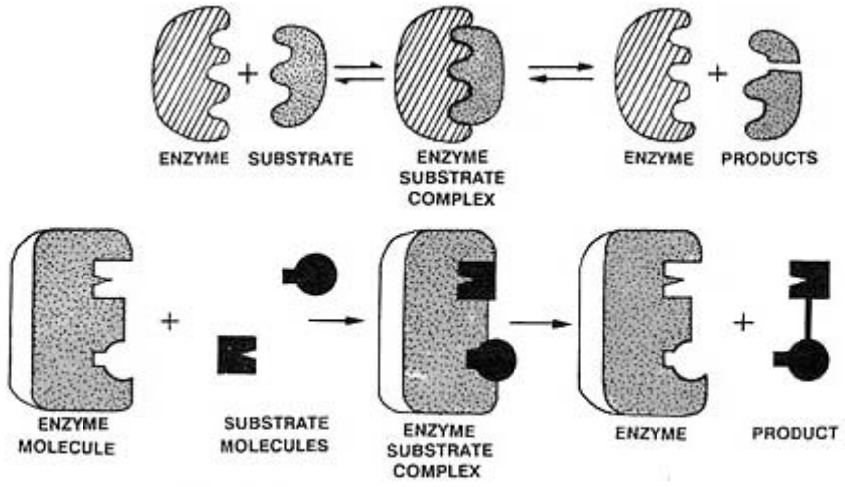


Fig. 9.33. Lock and key theory of enzyme action.
Upper Series – Breakdown Reaction Lower Series – Biosynthetic Reaction

Enzyme + Substrate ⇋ Enzyme – Substrate Complex
Enzyme – Substrate Complex ⇋ Enzyme – Products Complex
Enzyme – Products Complex ⇋ Enzyme + Products
Thus we see that the chemical reactants do not cause any alteration in the composition or physiology of the enzyme.

The same enzyme molecule can be used again and again (Fig. 9.35). Hence, enzymes are required in very small concentrations.

Evidences:

1. Blow and Steitz (1970) have found the formation of complex between the enzyme chymotrypsin and its substrate.
2. Keilen and Maun have observed that the absorption spectra of the same enzyme are different in the free state and in the presence of the substrate.
3. The theory explains how a small concentration of enzyme can act upon a large amount of the substrate.
4. Lock and key theory explains how the enzyme remains unaffected at the end of chemical reaction.
5. It is able to predict the increase in the rate of chemical reaction on the addition of more enzyme or substrate.
6. The theory explains how a substance having a structure similar to the substrate can work as competitive inhibitor.

ii. Induced-Fit Theory (Fig. 9.35):

It is modification of lock and key hypothesis which was proposed by Koshland in 1959. According to this theory the active site of the enzyme contains two groups, buttressing and catalytic. The buttressing group is meant for supporting the substrate. The catalytic group is able to weaken the bonds of reactants by electrophilic and nucleophilic forces.

The two groups are normally at a distance. As soon as the substrate comes in contact with the buttressing group, the active site of the enzyme undergoes conformational changes so as to bring the catalytic group opposite the substrate bonds to be broken.

Catalytic group helps in bringing about chemical reaction. The substrate is converted into product. The product is unable to hold on the buttressing site due to change in its structure and bonds. Buttressing group reverts to its original position. The product is released.

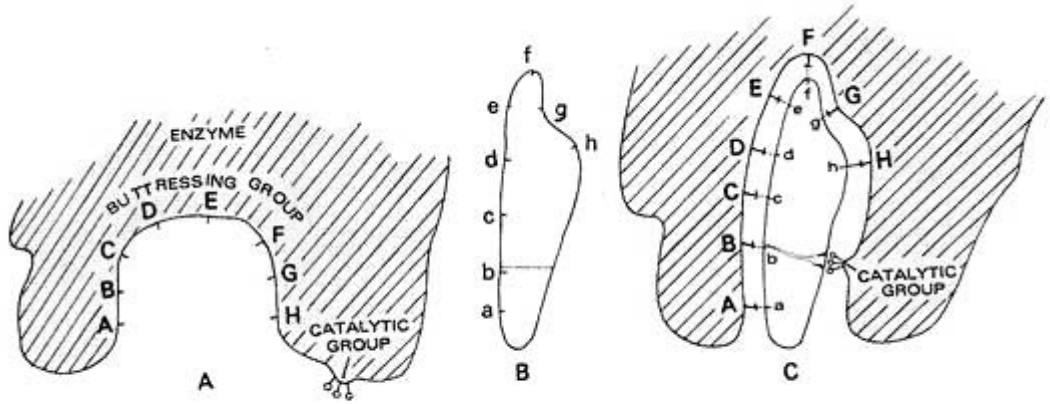


Fig. 9.35. Induced-fit theory of enzyme action. A, active site of enzyme. B, substrate molecule. C, enzyme-substrate complex with conformational changes so as to bring the catalytic group against the substrate bonds to be broken.

Inhibition of Enzyme Action:

Four common types of enzymes inhibition are as follows:

i. Protein Denaturation:

Enzyme activity is dependent upon the maintenance of tertiary structure of the protein moiety. The latter is destroyed by several factors like heat, high energy radiations and salts of heavy metals.

ii. Competitive inhibition:

It is the inhibition of enzyme activity by the presence of a chemical that competes with the substrate for binding to the active site of the enzyme. The inhibitor chemical is also called substrate analogue or competitive inhibitor.

It resembles the substrate in structure and gets bound up to the active site of the enzyme without getting transformed by the latter (Fig. 9.37). As a result, the enzyme cannot participate in catalytic change of the substrate. This is similar to the jamming of a lock by a key similar to original one.

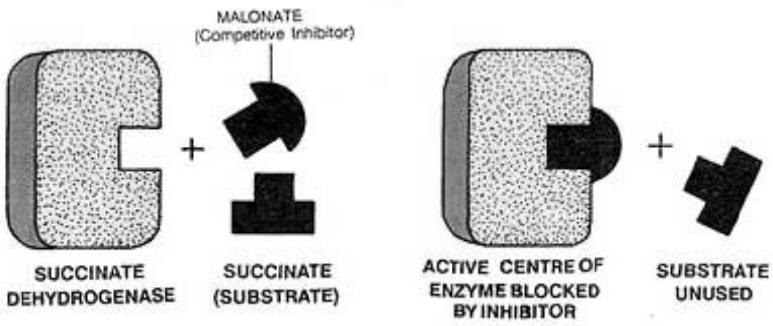


Fig. 9.37. Competitive inhibition of enzyme action.

Equilibrium constant for inhibitor binding is called K_i. A high $K_i$reduces enzyme activity while a low K_i allows enzyme activity to continue though at a reduced rate. Classical example of competitive inhibition is reduction of activity of succinate dehydrogenase by malonate, oxaloacetate and other anions which resemble succinate in their structure.

Competitive inhibition is usually reversible since the addition of more substrate tends to reduce the effect of the inhibitor.

The inhibition is important in that:

(i) It gives evidence for lock and key hypothesis of enzyme action,
(ii) Substrate analogues are not metabolized by enzymes,
(iii) Control of bacterial pathogens has been effected through competitive inhibition.
Sulpha drugs (e.g., sulphanilamide) inhibit the synthesis of folic acid in bacteria by competing with p-amino benzoic acid (PABA) for the active site of enzyme. Preformed folic acid is obtained by animal cells. Therefore, sulpha drugs do not harm them.

iii. Non-competitive Inhibition:

It is an irreversible inhibition of enzyme activity by the presence of a substance that has no structural similarity with the substrate. It is of two types, reversible and irreversible.

The irreversible non-competitive inhibitor destroys or combines irreversibility with a functional group of enzyme that is essential for its catalytic function. Cyanide inhibits the activity of cytochrome oxidase by combining with its metallic ions.

It has no structural similarity with the substrate of the enzyme, namely cytochrome c. Cytochrome oxidase is a respiratory enzyme. In its inhibition, the animal is unable to perform the respiration properly and gets killed. Di-isopropyl fluorophosphates (DFP, a nerve gas) prevents impulse transfer by combining irreversibly with amino acid serine of acetylcholine esterase.

It also poisons a number of other enzymes like trypsin, chymotrypsin, phosphoglucomutase, elastase, etc. lodoacetamide inhibits enzymes having sulphahydryl (—SH) or imidazole group.

iv. Allosteric Modulation or Feed Back Inhibition:

It is a type of reversible inhibition found in allosteric enzymes. The inhibitor is non-competitive and is usually a low molecular intermediate or product of a metabolic pathway having a chain of reactions involving a number of enzymes. It is, therefore, also called end product or feedback inhibition.

The inhibitor is also called modulator. Modulator is a substance that attaches with an allosteric enzyme at a site other than catalytic one but influences the latter, either inhibiting or activating the same. An example of feed back or allosteric inhibition is stoppage of activity of enzyme hexokinase (glucokinase) by glucose-6-phosphate, the product of reaction catalysed by it (Fig. 9.38).

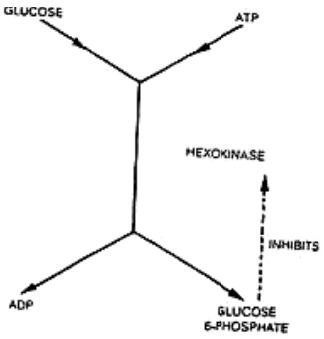


Fig. 9.38. Feedback or allosteric inhibition of hexokinase.

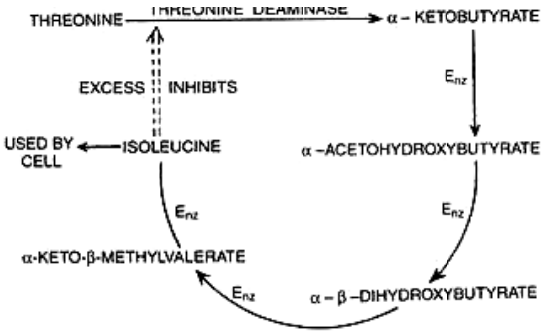


Fig. 9.39. Feedback or end product in case of isoleucine.

Another example is inhibition of threonine deaminase by isoleucine (Fig. 9.3). Amino acid isoleucine is formed in bacterium Escherichia coli in a 5-step reaction from threonine. Each step requires a separate enzyme. When isoleucine accumulates beyond a threshold value, its further production stops. Isoleucine added to the medium of bacterium also stops its internal production showing that its excess prevents some step of the reaction. The latter was found out to be enzyme threonine deaminase which is involved in the first step of the reaction (threonine to a-ketobutyrate).

Importance:

(i) It has a regulatory role on enzyme activity,
(ii) Enzyme inhibitors have been used in the study of metabolic pathways,
(iii) Some inhibitors are used in controlling pathogenic activity, e.g., sulpha drugs,
(iv) Use of inhibitors have shown the mechanism of enzyme action.

Feedback Inhibition of Enzymes:

Feedback inhibition (also called end-product inhibition or allosteric modulation) is one in which the end- product of the reaction acts as inhibitor and inhibits the activity of regulatory enzyme, usually, enzyme of the first step of a biosynthetic pathways. In multi-enzyme system synthesis of a product is completed in a number of steps, each step being catalyzed by a specific enzyme.

In some of such systems, the regulatory enzyme is specifically inhibited by the end-product of the pathway whenever the concentration of the end-product exceeds the cell's requirement. When the reaction catalyzed by the regulatory enzyme is slowed, all subsequent enzymes act at reduced rates due to the depletion of their substrates.

The rate of production of the pathway's end-product is thereby brought into balance as per the requirement of the cell. Feedback inhibition is beautifully illustrated by biosynthesis of L-isoleucine from L-threonine (Fig. 10-12).

In this system, the first enzyme, threonine dehydratase, is inhibited by L-isoleucine. No other intermediate in the sequence inhibits threonine dehydratase, nor is any other enzyme of the system inhibited by L-isoleucine.

L-isoleucine binds not to the active site, but to regulatory site on the enzyme molecule; this is called allosteric modulation. However, when the concentration of the end-product drops sufficiently, the enzyme reactivates and the end-product is resynthesized.

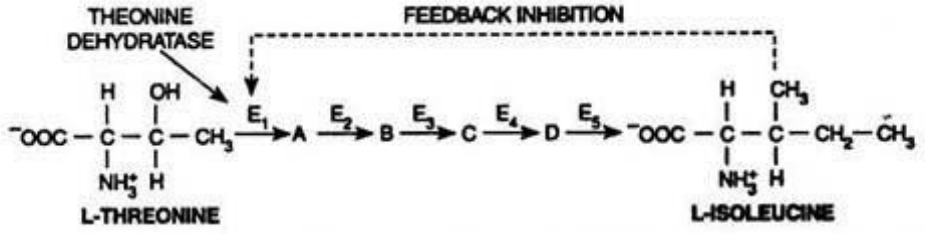


FIG. 27.12. Feedback inhibition. The conversion of L-threonine to L-isoleucine is a multistep system catalyzed by a sequence of five enzymes (E_1 to E_5). Threonine dehydratase (E_1) is specifically inhibited by L-isoleucine, the end-product of the pathway.

Regulation of Enzyme:

Biochemical reaction studies have shown that the pace of a chemical reaction in a biological system is maintained by the activities of the enzymes. Enzymes are rather unstable molecules and are synthesized and degraded simultaneously. Their activities may be regulated either through their synthesis or by modifying the existing enzyme molecules.

The activities of enzyme molecules are regulated by several ways which are the following:

I. Allosteric Regulation:

Allosteric regulation is a fine mechanism of controlling a reaction through the enzyme activity. Some enzymes (called allosteric enzymes), show sigmoidal curve between the substrate concentration and the activity. The activity of these enzymes is modified by several metabolites. The effect of different concentrations of 'activator' and 'inhibitor' on these enzymes is also sigmoid.

These effector molecules have a structure different from the substrate molecules. In most of the cases, allosteric inhibitors are the end products of the reaction; inhibiting the first enzyme in the series.

Thus, this kind of inhibition is called feedback inhibition, end product inhibition or retro-inhibition. The allosteric activators arc normally one of the substrates or cofactors of the enzyme. The effect of the allosteric 'inhibitor' or 'activator' on the enzyme is reversible.

When they are withdrawn, the enzyme resumes the original activity:

i. Allosteric Inhibition:

Inhibition of threonine deaminase by isoleucine is an example of allosteric inhibition. Threonine deaminase deaminates threonine to α-ketrobutyrate. The final product of the reaction is isoleucine.

Whenever the accumulation of isoleucine occurs, conversion of threonine to α-ketobutyrate and consequently formation of other intermediaries in the biosynthesis of isoleucine is stopped. When isolcucine is used up, threonine deaminase is reactivated and reactions for the biosynthesis of isoleucine start again.

ii. Allosteric Activation:

Activation of glycogen synthetase by glucose-6-phosphate is an example of allosteric activation. Another example of allosteric regulation (of both inhibitory and activating type) is observed during Pasteur effect. Pasteur effect is the inhibition of glycolysis and fermentation by oxygen. The molecular basis of this effect is the allosteric inhibition of enzyme phosphofructokinase by ATP and citrate and its activation by AMP.

Like many others, this kind of regulation is also of adaptive significance. As the level of AMP increases due to increased use of ATP in the cell, glycolysis is increased by the activation of phosphofructokinase with the result of more formation of ATP. When ATP level exceeds normal requirement of the cell, inhibition of glycolysis occurs through the same enzyme phosphofructokinase and ATP synthesis is stopped.

iii. Mechanism of Allosteric Regulation:

Regarding the mechanism of allosteric regulation, it is proposed that allosteric enzymes have two active centers; one for the substrate and the other for effector. These two sites lie either on same or on two different subunits. Binding of a effector molecule to one type of subunit changes the structure of the enzyme molecule in such a way that binding of the substrate to the other subunit is affected.

To explain the mechanism, an example of allosteric regulation of aspartate transcarbamylase may be cited. Asparate transcarbamylase contains two types of subunits. These two types of subuntis may be split apart by treatment with mercurials; with one type retaining the ability to bind with the substrate, whereas the other to recognize the inhibitor.

When these two species of subunits are together (active enzyme molecule), binding of the inhibitor to one type of subunit changes the structure of other subunits in such a way that the binding of the substrate is inhibited. When the subunits containing binding sites for the inhibitor are removed enzyme is not affected by the inhibitor.

Further, it gives a typical Michaelis-Menten curve with the substrate concentration. Similarly, the binding of activator may change the molecular structure in such a way that the binding of substrate is facilitated.

15. Chemistry of plant products: structure and properties of carbohydrates, lipids, amino acids, proteins, nucleic acids, secondary metabolites.

Carbohydrates are a group of organic compounds consisting of C, H, O usually in the ratio of 1: 2: 1 and include such well known compounds as sugars, starch, cellulose etc. Previously, the carbohydrates were regarded as hydrates of carbon and corresponded to general formula ($C.H_2O$)

But the group name 'carbohydrates' was sometimes found misleading because:

(i) Some organic compounds e.g., formaldehyde (HCHO), acetic acid (CH_3COOH), lactic acid ($C_3H_6O_1$) inositol ($C_6H_{12}O_6$) etc. correspond to the general formula but are not carbohydrates.

(ii) Some carbohydrates e.g., rhamnose ($C_6H_{12}O_5$), rhamnohexose ($C_7H_{14}O_6$), digitoxose ($C_6H_{12}O_4$) do not correspond to the above general formula.

(iii) Besides containing C, H and O, some carbohydrates also contain nitrogen and sulphur.

Therefore, the carbohydrates are more appropriately referred to as polyhydroxyaldehydes or polyhydroxyketones and their derivatives or the substances which yield these on hydrolysis. But, the group name 'carbohydrates' is still retained traditionally.

The metabolism of carbohydrates is of utmost importance to organisms individually and collectively. Fundamentally, all organic foodstuffs are ultimately derived from the synthesis of carbohydrates through photosynthesis. The catabolism of carbohydrates provides the major share of the energy requirement for maintenance of life and preformation of work. Moreover carbohydrates act as energy reservoirs and serve architectural functions and are important constituents of nucleic acids.

Classification of the Carbohydrates:

Depending upon their complexity and behaviour on hydrolysis, the carbohydrates are classified into following 3 categories (Fig. 13.1).

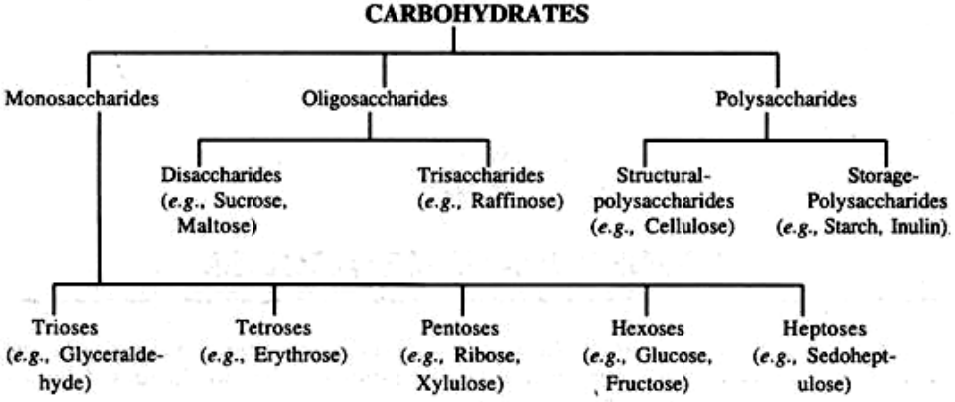


Fig. 13.1. Classification of the Carbohydrates

1. **Monosaccharide's (Simple sugars):**
2. i. These are simplest of carbohydrates and are known as sugars.
3. ii. These are the building units of complex carbohydrates.
4. iii. These cannot be hydrolysed.
5. iv. These are sweet-tasting, crystalline and soluble in water.
6. v. They have a potential aldehyde or keto group and hence, are reducing in nature.
7. vi. Aldehyde group or the reducing centre always lies at C No. 1 of the monosaccharide molecule. Such sugars are known as aldoses or aldose sugars.
8. vii. Monosaccharide's having keto group are known as ketoses or ketose sugars. In such sugars the keto group or the reducing centre always lies at C No. 2.

   Monosaccharides are polyhydroxyaldehydes or polyhydroxyketones containing 3-7 carbons. Monosaccharide's are the basic units, of carbohydrate which cannot be further hydrolyzed into simpler saccharides.

   On the basis of carbonyl group, monosaccharides are of two types i.e. aldoses (contain terminal aldehyde, —CHO) and ketoses (contain subterminal ketone, > C= O). Sugars with 3,4,5,6 and 7 carbon atoms are called trioses, tetroses, pentoses, hexoses and heptoses respectively.

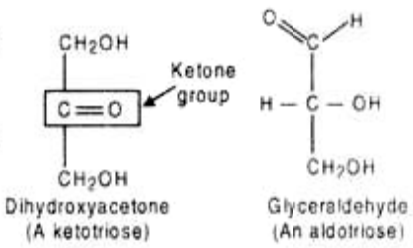
   Depending upon the number of the C atoms, the monosaccharide's are further classified as follows:

   (i) Triose Sugars, $C_3H_6O_3$ (e.g., glyceraldehyde, dihydroxyaeetone)

   (ii) Tetrose Sugars, $C_4H_8O_4$ (e.g., erythrose)

   (iii) Pentose Sugars, $C_5H_{10}O_5$ (e.g., ribose, ribulose, xylose, xylulose, arabinose).

Carbon atoms	Aldoses	Ketoses
Trioses, $(CH_2O)_3$ –	Glyceraldehyde	Dyhydroxyacetone
Tetroses, $(CH_2O)_4$ –	Erythrose, Threose	Erythrulose
Pentoses, $(CH_2O)_5$ –	Ribose, Arabinose, Xylose, Lyxose	Ribulose, Xylulose
Hexoses, $(CH_2O)_6$ –	Glucose, Mannose, Galactose	Talose, Fructose
Heptoses, $(CH_2O)_7$ –	Glucoheptose, Galactoheptose	Sedoheptulose



   (iv) Hexose Sugars, $C_6H_{12}O_6$ (e.g., glucose, fructose, galactose mannose).

   (v) Heptose Sugars, $C_7H_{14}O_7$ (e.g., sedoheptulose).

Properties of monosaccharides:

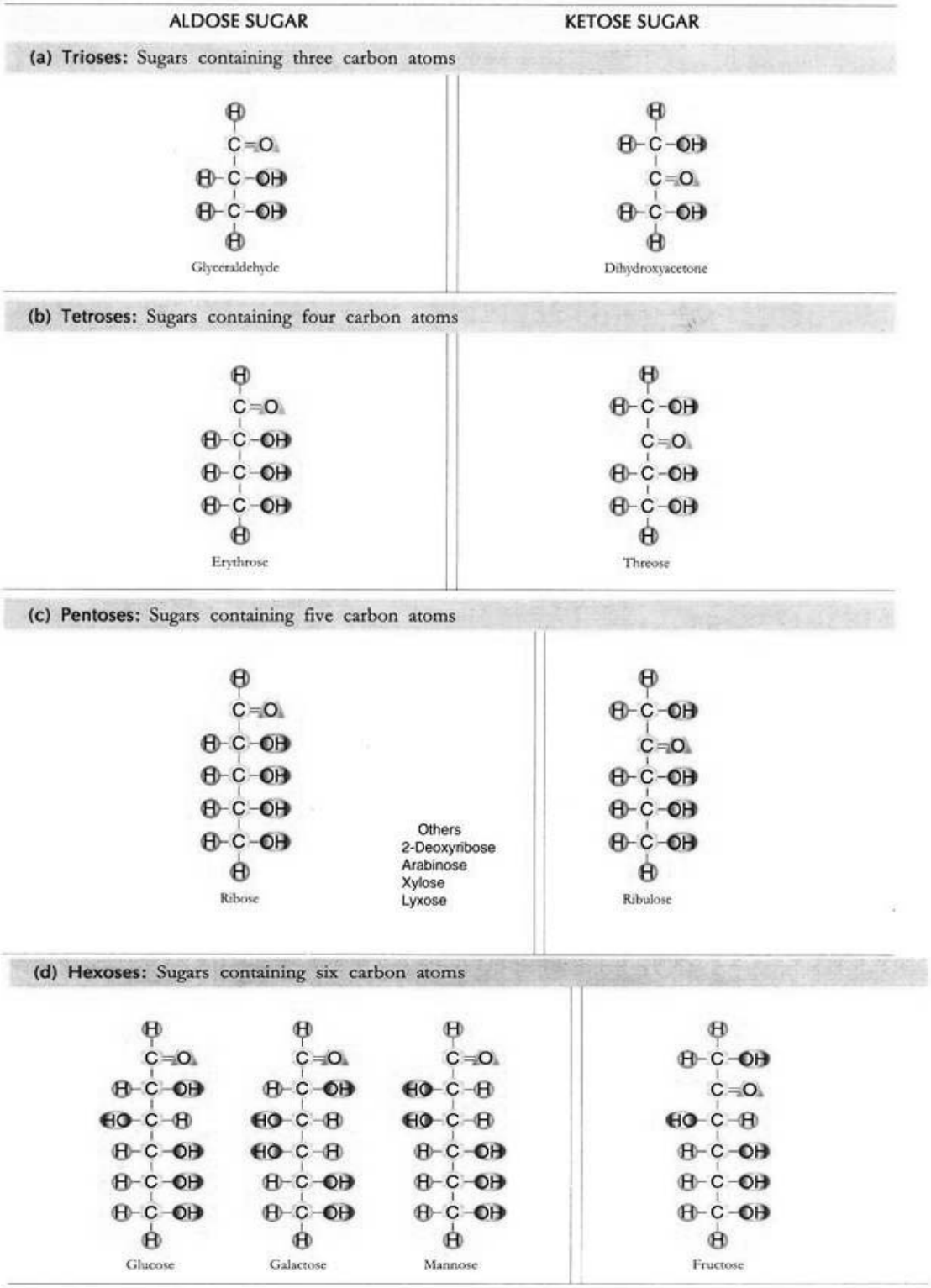
Physical properties:

All monosaccharides are colourless, crystalline, water soluble, sweet in taste and low MW organic compounds.

1. Isomerism:

Except dihydroxyacetone, all monosaccharides have asymmetric or chiral carbon atoms (carbon atoms attached to four different atoms). This allows the formation of stereoisomers or in short, isomers. Stereoisomers have the same structural formula but differ in spatial configuration. The number of possible isomers depends on the number of asymmetric carbon atoms (n) and is equal to 2^n. For glucose n = 4, so there are 16 isomers. Ketoses have one less asymmetric carbon atom than do aldoses with same number of carbon atoms.

Depending upon the number of carbon atoms, aldoses and ketoses are further classified as:

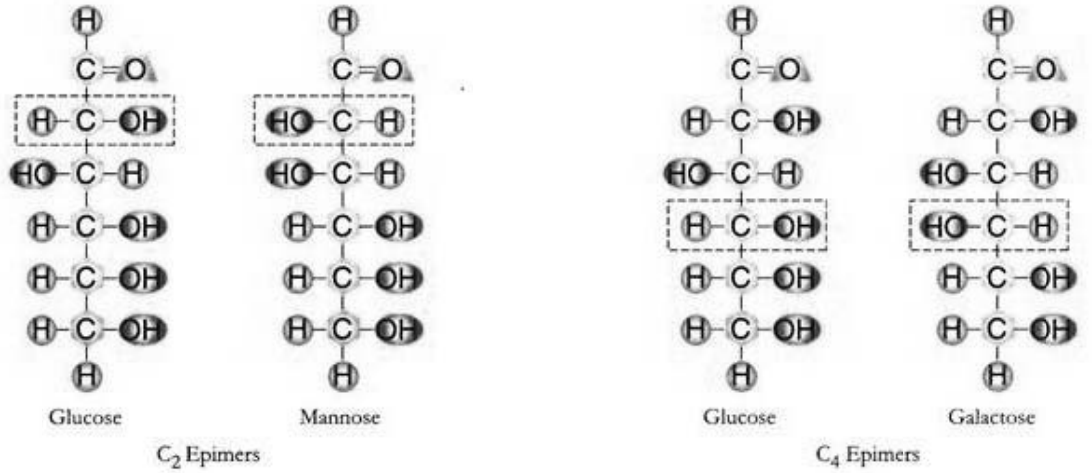


(e) **Heptoses:** Sugars containing seven carbon atoms: An aldo-heptose is sedoheptose and a keto-heptose is sedoheptulose.

The important isomers of monosaccharide's are as follows:

(i) Epimers:

When sugars differ only in the configuration around one specific carbon atom they are called epimers. e.g., Glucose and mannose are epimers at C_2 whereas glucose and galactose are epimers at C_4.



(ii) D- and L-isomers:

D-sugars and L-sugars are optical isomers. In biological systems all sugars are found in D-forms. The optical activity is due to the asymmetric carbon atoms which can be able to rotate the plan of polarized light to either left (levorotatory) or right (dextrorotatory). Hence the mono saccharides are two types i.e. L-sugars and D-sugars. The D-sugars can be represented by the orientation of-OH group of asymmetric carbon farthest from aldehyde or ketone group is on right. When it is on left, it is a member of L-sugars. As the D-sugars are mirror images of L-sugars, they are called enantiomers. For example, D- and L-glycerol, D- and L- glucose, etc.

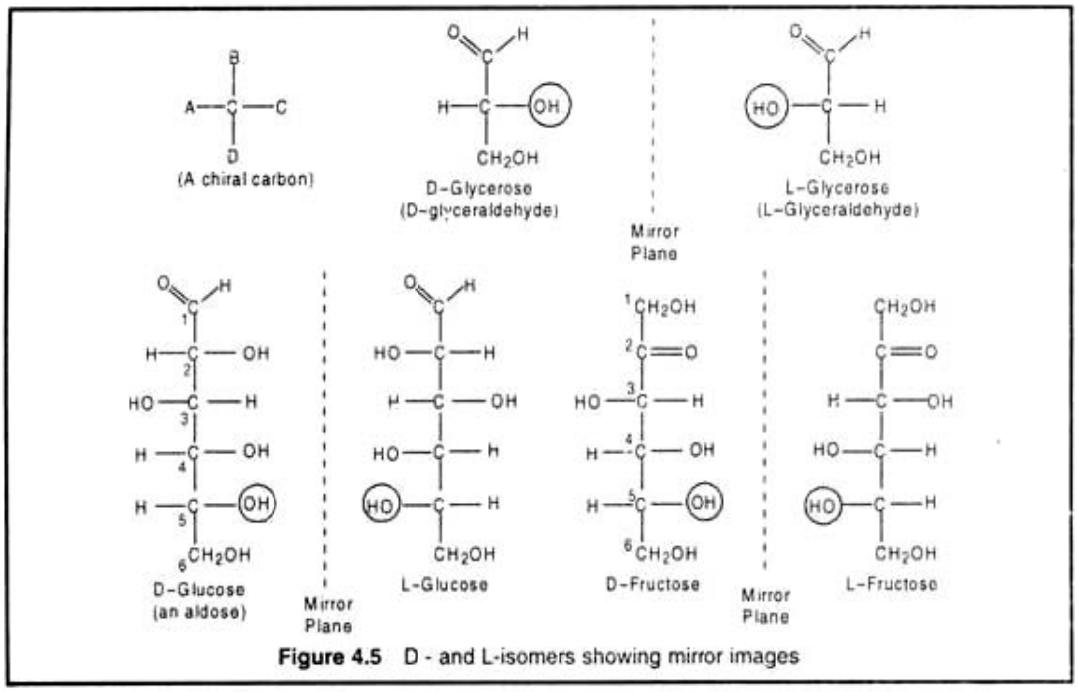


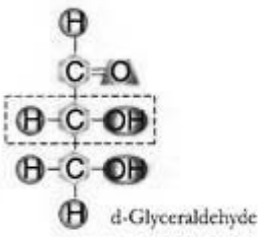
Figure 4.5 D - and L-isomers showing mirror images

(iii) Enantiomers:

Non super-imposable mirror images are known as enantiomers. e.g., D and L sugars.

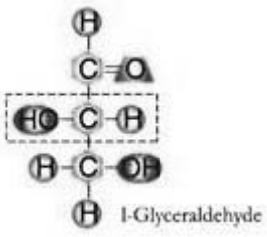
Explanation:

When white light (which is a mixture of different wavelengths) is passed through a Nichol prism, then the emerging light will be of a single wavelength and this is known as plane polarized light. When this plane polarized light is passed through a solution containing carbohydrate, the light is deflected either towards the right or left, which depends upon the configuration of atoms around the chiral centre.



When the solution containing glyceraldehyde with the configuration in the figure given below around the chiral centre is taken, wherein the —OH group on the asymmetric carbon atom is towards right, when written on paper in the straight line projection form, then the light is deflected towards right. Hence this glyceraldehyde is known as dextrorotatory sugar or compound and is designated as d-sugar (+ sugar).

When the solution containing glyceraldehyde with the following configuration around the chiral centre is taken, wherein the —OH group on the asymmetric carbon atom is towards left, when written on paper in the straight line projection form, then the plane polarized light is rotated towards left. Hence this glyceraldehyde is known as levorotatory sugar or compound and is designated as 1-sugar (— sugar).

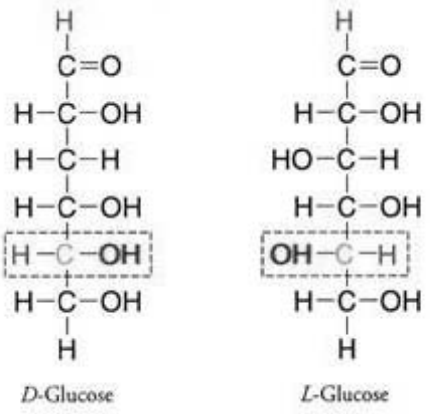


Glyceraldehyde has only one chiral centre or asymmetric carbon atom, whereas tetroses, pentose's and hexoses have more than one chiral centre. In such cases the rotation of the plane polarized light is dependent upon many factors, viz., the configuration around each of the chiral centre, the solvent in which it is present, temperature, etc.

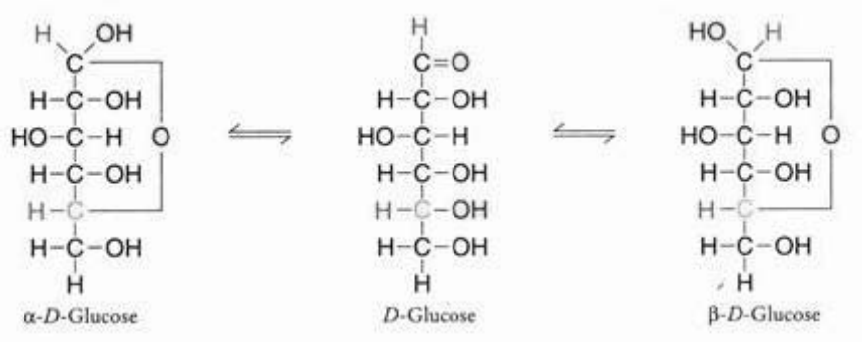
Under such circumstances there is no relation between the configurations of the sugar to the rotation of the plane polarized light. Hence other compounds (sugars, amino acids, etc.) are grouped into two categories namely D series compounds and L series compounds.

D series compounds are those compounds that contain the reference group on the right side of the last chiral centre from the functional group. If glucose is taken, the functional group is the aldehydic group (—CHO) and the chiral centre furthermost from it is the 5th carbon atom and the reference group -OH is present on the right side of the straight chain.

Hence it is known as D-glucose. This glucose may or may not be dextrorotatory. It may also be levorotatory. If this glucose is dextrorotatory then it is designated as D-(+)-glucose and if this glucose is levorotatory then it is designated as D-(-)-glucose.



L series compounds are those compounds that contain the reference group on the left side of the last chiral centre from the functional group. If the -OH group is present at the left side on 5th carbon of the straight chain form of glucose then it is known as L-glucose. This glucose may or may not be levorotatory. It may also be dextrorotatory. If this glucose is dextrorotatory, then it is designated as L-(+)-glucose and if this glucose is levorotatory then it is designated as L-(-)-glucose.



Racemic mixture:

A solution containing equal number of d (+) & l (-) forms of a sugar is known as a racemic mixture.

(iv) Anomers:

Sugars differing at the anomeric carbon atom are known as anomers.

Explanation:

When an aldehydic group (or carbonyl carbon) reacts with an alcoholic group, then it results in the formation of a hemiacetal. Carbohydrates contain both aldehydic (carbonyl) and alcoholic groups within the molecule. Hence it is possible that the aldehydic group present at the 1st carbon atom of the sugars can react with any of the alcoholic groups present on the other carbon atoms, thus resulting in the creation of an additional chiral centre at the 1st carbon atom and this chiral centre is now known as the anomeric carbon atom. Sugars differing at this anomeric carbon atom are known as anomers.

Two anomers for each of the sugars are possible. If the -OH group on the anomeric carbon atom is towards the right then it is known as alpha (α) anomer. If the —OH group on the anomeric carbon atom is towards the left, then it is known as beta (β) anomer or β-sugar.

(v) Pyranose and Furanose rings:

All sugars exist in linear o open-chain form. But in solution pentoses and nexoses cyclize into ring-forms. Ring-forms are two types, pyranose and furanose. Pyranose is 6-membered sugar ring similar to pyran with five carbon atom and one oxygen atom. While furanose is 5-membered sugar ring similar to furan with four carbon atom and one oxygen atom. The sugar rings are represented by Haworth projections. In Haworth projection (Haworth, 1928) the plane of sugar ring can be imagined as perpendicular to the plane of the paper, with the heavy line on the ring closest to the reader.

(iii) α-and β-anomers:

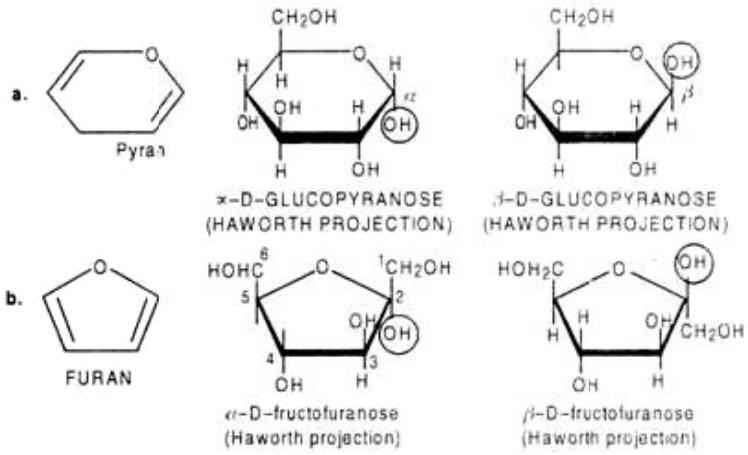
In a sugar ring an additional asymmetric carbon atom is created called anomeric carbon. For example, in glucose (aldose) C-l becomes anomeric and in fructose (ketose) C-2 becomes anomeric. The sugar isomers on anomeric carbon are called anomers. In α-anomer, the -OH group of anomeric carbon is written below and in β -anomer it is written above. The interconvertion of α- and β-anomers in solution is called mutarotation. In solution, glucose exist in 3 inter convertible forms- open-chain form (0.02%), α-D-fructofuranose (36%) and β- D-glucopyranose (67%).

Mutarotation:

Change in the specific rotation of an optically active compound without any change in its other properties is knows as mutarotation.

Explanation:

Glucose crystallized from cold water is α-D-glucose and it shows a specific rotation of $\{\alpha\}^{20}_{D}$ =



+112.2°. If it is dissolved in water, the specific rotation gradually changes with time and reaches a stable value of 52.7°.

This change in specific rotation is because α-D-glucose isomerizes to β-D-glucose via a straight chain intermediate and finally an equilibrium mixture of about 1/3rd of α-D-glucose, 2/3rds of β-D- glucose and a little of straight chain form is formed. This change in specific rotation is known as mutarotation.

Similarly, β-D-glucose, which can be obtained on crystallization from pyridine shows a specific rotation of $\{\alpha\}^{20}_{D}$ = +19°. When this is dissolved in water its rotation gradually changes and finals to 52.1°. This is again due to mutarotation and formation of α, β and straight chain forms of glucose in an equilibrium of 1/3: 2/3: 0/1 (n).

Chemical properties:

1.Reducing nature:

All monosaccharides are reducing. Disaccharides like lactose and galactose are also reducing, except sucrose. The reducing nature is due to presence of free aldehyde or ketone group which can reduce cupric ion (Cu^{2+}) to cuprous form (Cu^{+}).This is the basis of Benedict's test & Fehling's test for sugar detection in urine.

$C_6H_{12}O_6 + 2Cu(OH)_2$—» $C_6H_{12}O_7 + Cu_2O + H_2O$

Glucose Blue Gluconic acid red

In Toliens reagent (an ammonia solution), a reducing sugar reduce Ag^{+} of silver oxide (Ag_2OT txr a metallic silver (Ag) lining in the reaction vessel.

$C_6H_{12}O_6 + Ag_2O$—> $C_6H_{12}O_7 + 2Ag$

Silver oxide Silver

In alkaline medium, the aldehydic or ketonic group of sugars can reduce a number of substances (metals) like copper, silver, mercury and bismuth. Copper salts are reduced to cuprous hydroxide or oxide in solution. The sugars are identified in the urine and blood based upon this principle. Benedict's reagent is commonly used for the detection of sugars in urine.

(a) Reducing sugars:

Sugars having a free aldehydic or ketonic group are known as reducing sugars, e.g., glucose, fructose, galactose and all other monosaccharide's. Among disaccharides maltose and lactose are reducing sugars.

Benedict's test:

This is a semi quantitative test most commonly used for the detection of the percentage of sugar in urine. Benedict's test is carried out in a mild alkaline media. Hence weak reducing agents like uric acid and creatinine in urine cannot reduce Benedict's reagent. Therefore, this test is very specific for glucose or other reducing sugars in urine.

(b) Non-reducing sugars:

Sugars that do not have a free aldehydic or ketonic group are called as non-reducing sugars, e.g., sucrose and trehalose. Note: Though polysaccharides have at least one free aldehydic or ketonic group, but still they are non- reducing sugars owing to their larger molecular size and complexity of the structure. Hence the aldehydic or ketonic group is not available for the reducing action.

2.Glycosidic bond:

Glycosidic bond is a covalent bond formed by the condensation reaction between a sugar and the -OH group of a second compound which may or may not be the sugar. Glycosidic bonds are of two types i.e. O- glycosidic bond (-C-0- C) and N-Glycosidic bond (-C-N-C). Sugar can be joined to each other by O-glycosidic bonds to form disaccharides, oligosaccharides and polysaccharides. N-glycosidic bond formed when the anomeric carbon of a sugar liked to the nitrogen atom of an amine, as in nucleosides.

3. Formation of osazones:

Phenyl hydrazine reacts with reducing sugars to form osazones. It involves carbonyl carbon and the adjacent carbon. Osazone is a crystalline compound and is used as an identification test for sugars.

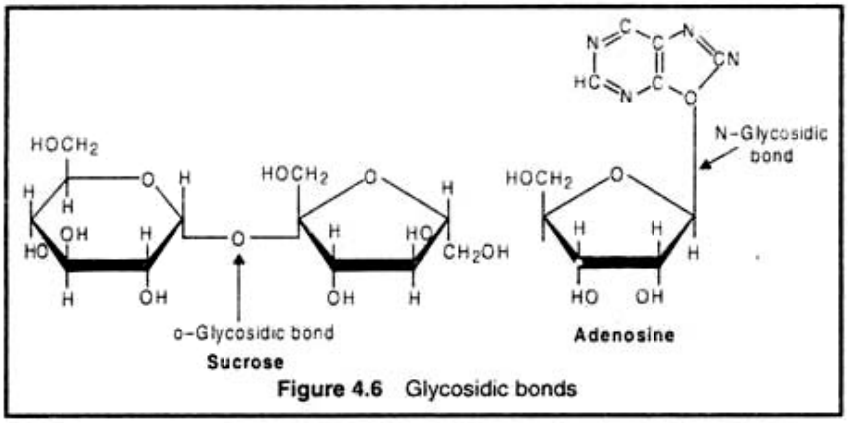
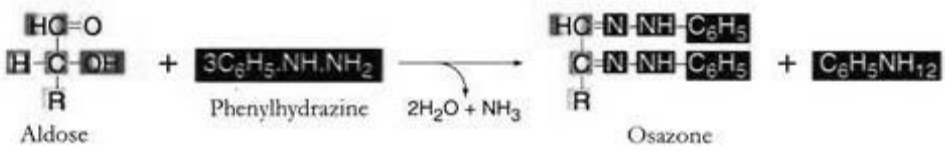


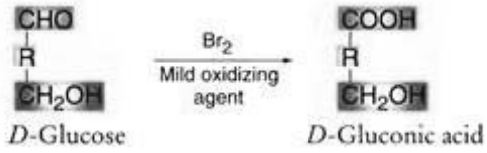
Figure 4.6 Glycosidic bonds



Fructose and glucose forms a broom stick shaped crystal in 3 and 5 minutes respectively. Maltose forms star shaped crystals in 20 minutes whereas lactose forms puff shaped crystals in 30 minutes time.

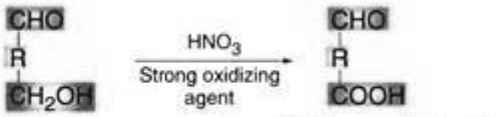
4. Oxidation of sugars:

(a) Mild oxidizing agent like bromine oxidizes the aldehydic group of carbohydrates converting it to an acid group.



D-Glucose D-Gluconic acid

(b) Strong oxidizing agent like nitric acid oxidizes the primary alcohol of the carbohydrates forming saccharic acids.

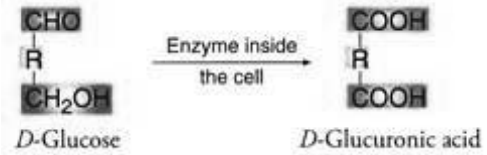


D-Glucose D-Glucosaccharic acid

Galactose forms mucic acid, which in insoluble in water. This forms an identification test for galactose known as mucic acid test.

(c) Enzymes:

Inside the cell, the enzymes oxidize both the aldehydic and primary alcoholic groups of the carbohydrates forming uronic acids.



D-Glucose D-Glucuronic acid

D-glucuronic acid is a component of structural materials like chondroitin sulphate, mucoitin sulphate and glycoproteins (proteoglycons). It plays an important role in detoxification of bile pigments. L-glucose forms ioduronic acid.

5. Dehydration with strong acids:

Concentrated H_2SO_4 removes the adjacent —OH groups as water (H_2O) forming furfural form pentose's and hydroxymethyl furfural from hexoses.



Furfural Hydroxymethyl Furfural

Furfural condenses with α-naphthol in presence of alcohol forming a purple violet coloured complex. This is the principle of Molisch's test which is a common identification test for all carbohydrates.

6. Derived sugars:

Substances formed from sugars on oxidation, reduction or addition/replacement of any group are called derived sugars.

(a) Amino sugars:

The hydroxyl group at the second carbon of a sugar is replaced by an amino group to form an amino sugar, e.g., glucosamine, galactosamine.

(b) Deoxy sugars:

These sugars are formed due to removal of one of the oxygen from the alcoholic group, e.g., 2-Deoxy-ribose, here the 'O' of the 2nd alcoholic group is removed. It is present in DNA. L-fucose is 6-Deoxy L-galactose, L-rhamnose is 6-Deoxy L-mannose.

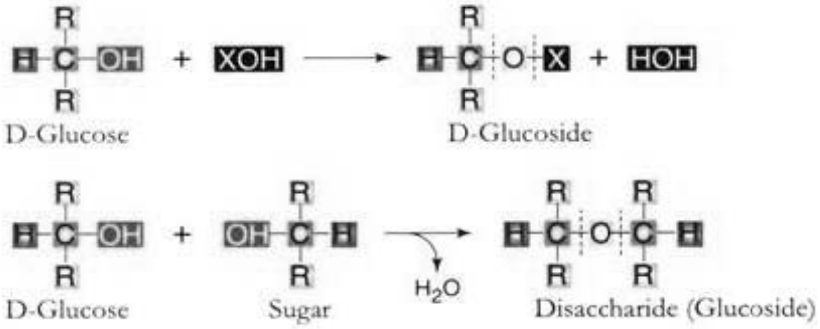
(c) Oxidation products of carbohydrates:

Uronic acids and saccharic acids are also derived sugars.

6. Formation of glycosides with alcohol:

When two alcoholic groups react with each other, a glycoside is formed. Carbohydrates contain many alcoholic groups. Hence two carbohydrates can react with alcoholic groups of one another sugars,

forming glycosides. Union of two carbohydrates is known as a disaccharide, three is trisaccharide and many is a polysaccharide.



Monosaccharide derivatives:

Some other important modified monosaccharide's are as follows-

(a) Deoxyribose:

The deoxygenation of ribose at C-2 position produces deoxyribose. It is important component of deoxyribonucleotides of DNA.

(b) Phosphorylated sugars:

The transfer of phosphorylated group from ATP to -OH group of a sugar produces phosphorylated sugar, e.g. glucose-6-phosphate. The phosphorylated sugars are anionic which prevent their escape from cell and also acts as reactive intermediates.

(c) Sugar alcohol:

It is formed by reduction of aldehyde and ketone group of sugar, e.g., mannitol, glycerol, sorbitol etc. Mannitol is a sugar alcohol in brown algae, some fruits and honey. It is used as a medicine for patients with brain edema. Glycerol required in fat synthesis. Sorbitol, a sugar alcohol commercially obtained from reduction of glucose and fructose, is used in diets of diabetics because it is absorbed very slowly and metabolized after conversion to glucose, but it does not raise blood sugar.

(d) Sugar acids:

Ascorbic acid or vitamin-C is a sugar acid required for collagen synthesis. Glucouronic acid and iduronic acid are components of mucopolysaccharides.

(e) Amino sugars (hexosamines):

Glucosamine forms chitin and hyaluronic acid.

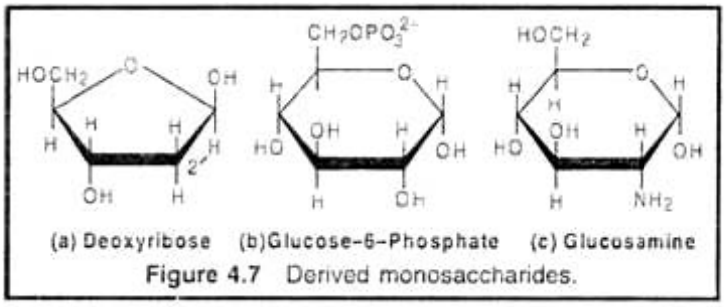


Figure 4.7 Derived monosaccharides.

2. Oligosaccharides:

i. These consist of more than one but fewer number of monosaccharide molecules joined together by glycosidic bonds.

ii. On hydrolysis, they yield the monosaccharide units which may be similar or dissimilar.

iii. These are also sweet tasting, crystalline, soluble sugars.

iv. These may or may not have a free -OH group at the reducing centre and accordingly may or may not be reducing.

Depending upon the number of the monosaccharide molecules which constitute them, the oligosaccharides are grouped in following categories:

(i) Disaccharides. $C_{12}H_{22}O_{11}$ (e.g., sucrose, maltose, lactose etc.)

(ii) Trisaccharides, $C_{18}H_{32}O_{16}$ (e.g., raffinose, gentianose etc.)

- **Disaccharides (Double sugars):**

Disaccharides are composed of 2 monosaccharide's linked by O-glycosidic bond.

The physiologically important disaccharides are:

Maltose (malt sugar) = Glucose + Glucose

Lactose (milk sugar) = Galactose + Glucose

Sucrose (cane sugar) = Glucose + Fructose

Maltose is a reducing disaccharide composed of two glucose units linked by α1,4-glycosidic bond. The reducing property of maltose is due to free aldehyde group of C-l of second glucose. Maltose is an enzymatic hydrolysis product of starch. It is formed in the germinating cereals by enzyme diastase (an amylase) and also formed during starch digestion by α-amylases of salivary and pancreatic juices.

Lactose is also a reducing disaccharide consists of galactose joined to glucose by a β 1,4 glycosidic bond. Its reducing nature is due to free aldehyde of C-l of glucose residue. Lactose present naturally in milk of mammals where its concentrations vary from 0-7%, depending on species.

Souring of milk occurs when bacteria convert lactose into lactic acid. Lactose is nutritionally important because it provides galactose which is essential for development of nervous tissue in the young ones.

Sucrose is a non-reducing disaccharide as it lacks any free reducing group in contrast to other sugars. It is commercially extracted from sugarcane and beet roots. It is the principal form of carbohydrate transported from source (leaves) to sink (site of utilization) in higher plants. Sucrose is dextrorotatory but on hydrolysis by sucrase produces a mixture of equal amount of glucose and fructose which is laevorotatory.

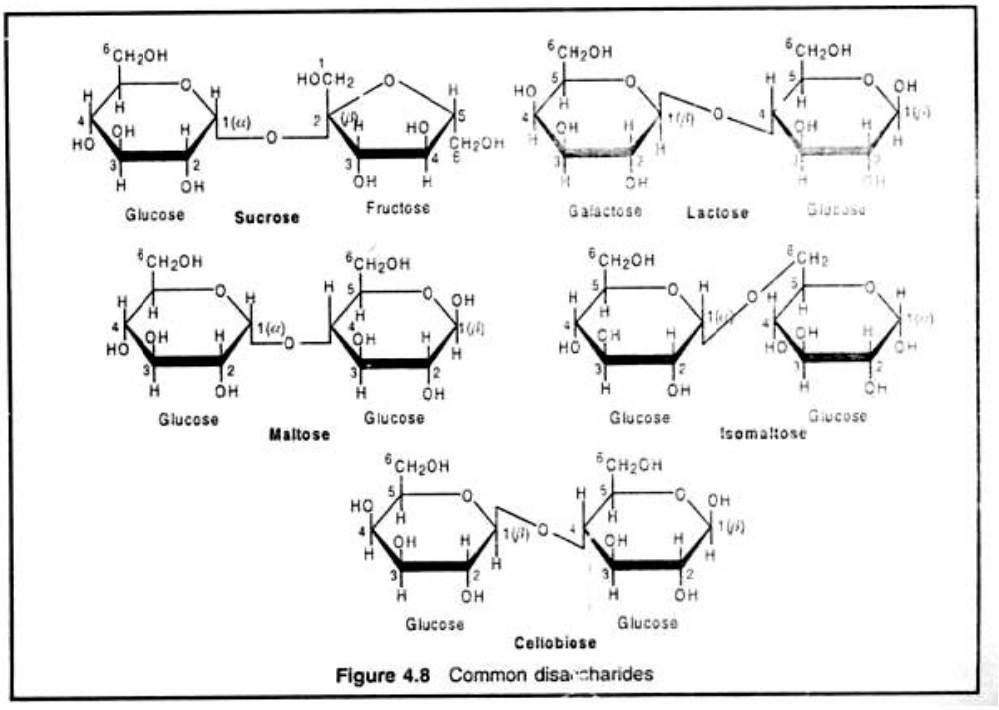


Figure 4.8 Common disaccharides

Cellobiose is the repeating disaccharide of cellulose in which β-glucosidic bond present.

Oligosaccharides may be composed of 3-9 monosaccharide units linked by O-glycosidic bonds. They may be referred as trisaccharides, tetrasaccharides etc. When linked with proteins and lipids they form glycoproteins and glycolipids respectively.

Gentianose (glucose-glucose-glucose), raffinose (galactose-glucose-fructose) are found in some plants. Webb and Burley (1964) found that stachyose (galactose-galactose-glucose) is a transport carbohydrate in plants like Frcixinusaыепсапа, Cucurbitci pepo and Verbascum thapus.

Functions of oligosaccharides:

i. Transport form of Sugar:

Although glucose is the common transport sugar for many animals, sugars are transported in plants and other organisms as disaccharides.

ii. Storage Food:

Sucrose is the reserve storage product in sugarcane and sugar beet.

iii. Recognition Point and other Functions in Cells:

Oligosaccharides attached to cell membranes form cell coat of glycocalyx.

They are important for:

(a) Cell recognition,
(b) Cell attachment,
(c) Receptor molecules (for receiving and responding to external stimuli),
(d) Antigen specificity for human blood groups (A, B, Rh),
(e) Components of antibodies which are large molecules with attached carbohydrates,
(f) Glycoproteins of some viral coats for attaching to and invading host cell.
(g) Lactose (a disaccharide) is the natural sugar of milk.

3. Polysaccharides (Glycans):

Polysaccharides are macromolecules polysaccharides consist of at least more than ten monosaccarbohydrates. They may be linear or branched chains. Chemically, polysaccharides are of 2 types, homopolysaccharides and heteropolysaccharides. Homopolysacccharides are polymers of similar type of monosaccharides residue.

They may be glucosan or glucan (polymer of glucose) fructosan or fructan (polymer of fructose), galactosan or galactan (polymer of galactose), pentosan (polymer of pentose), hexosan (Polymer of hexoses), araban (polymer of arabinose, a pentose), xylan (polymer of xylose, a pentose) etc. Heteropolysaccharides are polymers of 2 or more type of monosaccharides residues, e.g., glycosamionoglycans (also called mucopolysaccharides), pectin, hemicelluloses etc. Heteropolysaccharide and structural polysaccharides secreted outside the cell for functioning.

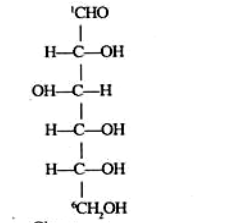
Structural Features:

Open Chain and Ring Forms of Monosaccharides:

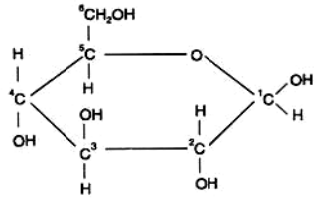
Many monosaccharide's e.g ribose, glucose, fructose etc. exist both in open straight chain and ring form. If the ring is 5-membered it is called as furanose sugar. Sugar with a 6-membered ring is called as pyranose sugar e.g.,

(The names pyranose and furanose are derived from six and five membered cyclic ethers called pyran and furan respectively with which these sugars bear a formal resemblance).

If the highest numbered asymmetric carbon atom of the sugar molecules contains—OH group on right-hand side in open chain structure, the sugar is known as D-Sugar and if on left side it is known as L-Sugar. For example, in open chain structure of glucose carbon number 2, 3, 4 and 5 are asymmetric. The highest

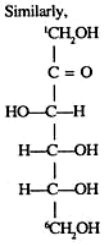


Glucose
(Open Chain Structure)
or Fischer's projection formula)*

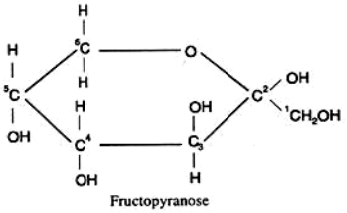


Glucopyranose
(Six membered ring structure of Glucose)

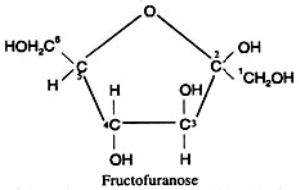
Similarly,



Fructose
(Open Chain Structure)



Fructopyranose
(6-membered ring structure of Fructose)



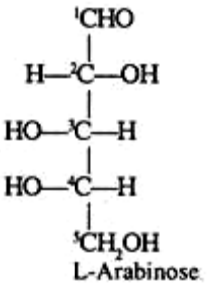
Fructofuranose
(5-membered ring-structure of Fructose)

numbered asymmetric C-atom is therefore, 5. As shown below, it bears -OH group on right-hand side and hence, glucose is called a D-sugar and written as D-Glucose or D-Glucopyranose (in case of 6-membered ring structure).

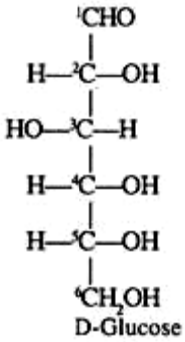
In arabinose (a pentose sugar), the highest numbered asymmetric carbon atom is 4 which bears—OH group on left-hand side, therefore, this is a L-Sugar and called as L-Arabinose.

(The carbon atom whose 4 valancies are satisfied by four different groups or atoms is called as asymmetric carbon atom).

i. Almost all the sugars in plants are D-Sugars.



L-Arabinose



D-Glucose

ii. Symbols D and L should not be confused with the optical activity. If the compound is optically active and rotates the plane polarised light to right, it is called as dextrorotary and is denoted by small italic letter d or + sign. But, if it rotates the plane to left side it is called as laevorotatory and is denoted by I or – sign. For example, D-Glucose is dextrorotatory and D- Fructose leavorotatory and are written as:-

D (+) Glucose or D (d) Glucose

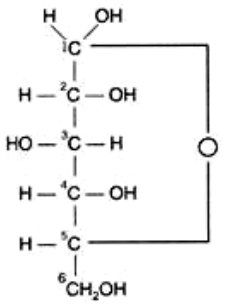
D (-) Fructose or D (l) Fructose

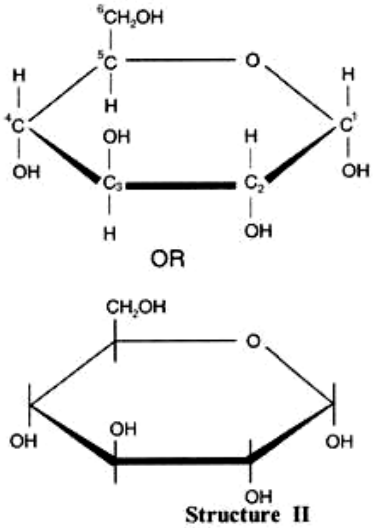
iii. The ring form of sugars is in fact a cyclic hemi-acetal or hemiketal structure formed by combination of the carbonyl group (i.e., aldehyde group at C_1 in aldose sugars and keto group at C_2 in ketose sugars) and one of the hydroxyl groups (usually at the highest numbered asymmetric C atom) of the sugar molecule in open chain structure.

The hemi acetal or hemiketal bond formation creates a new asymmetric centre at C_1 in aldose sugars and C_2 in ketose sugars which is now called as anomeric carbon atom. Taking for example a-D-Glucopyranose, the ring form of the sugars is usually depicted by following two structural formulas:

Although structure I indicates the basic features of a-D-Glucopyranose, it provides little insight as to the actual shape of the molecule and the spatial relationship of the various functional groups to one another. The latter features are clearly represented by structure II which is also called as Haworth's formula.

iv. In Haworth's perspective formula, the plane of the pyranose ring is considered to be perpendicular to that of the page on which it is written with the substituents (-H, -OH and -CH_2OH groups) either above or below the plane of the ring. The thin bonds of the ring are behind the plane of the paper while the thick bonds in front of it.


Structure I



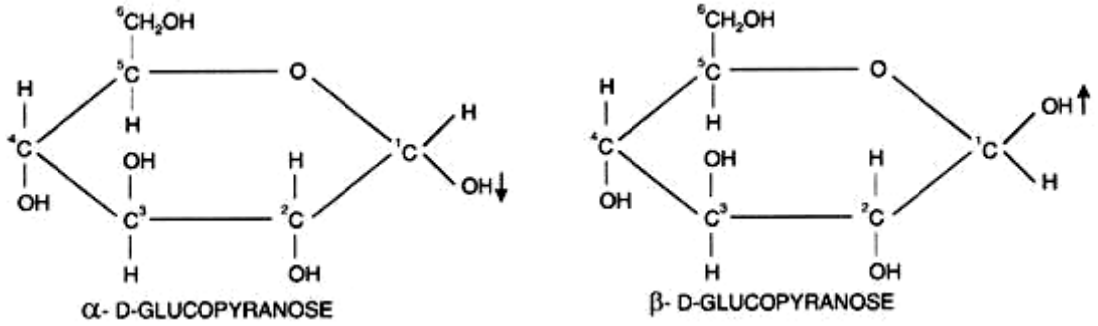
Structure II

α-D-Glucopyranose

(As a rule, the substituents written to the right side of the C chain in structure I are shown below the plane of the ring in Haworth's representation while those on left side above the plane of ring. However, opposite rule applies to that C atom whose—OH group is involved in the formation of cyclic hemiacetal. Thus, the CH_2OH group is written in an above position and the H atom on the same carbon written below despite the fact that it is on the left side in structure I.

In practice, however, the carbon atoms of the ring and the H atoms are omitted and often the thick bonds of the ring are not shown. The formula may also be inverted or twisted in oligo and polysaccharides to show the linkages between various monosaccharide molecules more clearly.)

If in the Haworth representation of the sugars the—OH group at the reducing centre (i.e., C No. 1 in aldose sugars and C No. 2 in ketose sugars) is present below the plane of the ring, the sugar is said to be in a-form. And if it is present above the plane of the ring, the sugar is said to be β-form. For example,



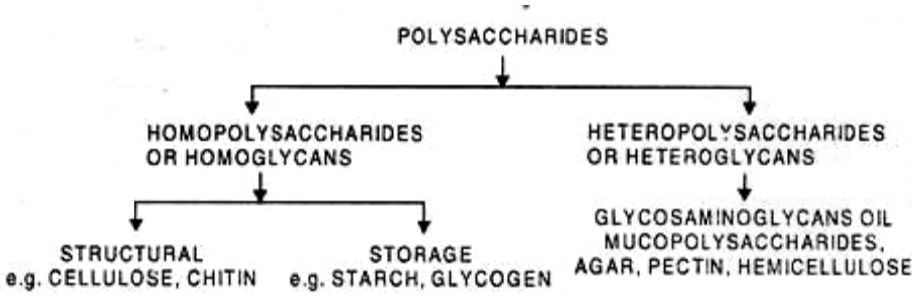
When α and β-forms change into one another the phenomenon is called as mutarotation.

There is an equilibrium between these two forms of ring compound. For example, the equilibrium in the mutarotation of a synthetic sample of D-Glucopyranose corresponds to a mixture of 36% of the a-form and 64% of the β-form.

α and β-forms of a given sugar are anomers of each other.

(The proportion of the open chain and ring forms of sugars in solution differs with different sugars. For most of the aldohexoses and aldopentoses the ring form is predominant one. Fructose (a ketose sugar) and most of the aldopentoses exist predominantly in the furnose form while glucose and other aldohexoses exist mainly in pyranose form. Ketopentoses such as ribulose usually prefer open chain form).

Functionally, polysaccharides are of 3 types – storage, structural and mucosubstances.



(A) Structural Polysaccharides:

They are the macromolecules that perform architectural functions in plant cell wall and skeleton of animals.

Cellulose:

Cellulose is the most abundant organic compound in the biosphere. It is estimated that about 10^{15} kg of cellulose are synthesized and degraded annually. Cellulose is the structural polysaccharide of plant cell walls, some protists and tunic of urochordates.

Each cellulose chain is a linear glucan of about 2000-25,000 D-glucose residues joined by β1→4 glycosidic linkages. The adjacent monomers lie at an angle of 180° and the straight chain is stabilized by hydrogen bonds. The individual chain is about 1-5 mm (micrometer) long.

Nearly 100 individual cellulose chains lie side by side and bonded to each other to make a crystalline domain or micelle. About 2.0-40 micelles embedded in an amorphous matrix to form a cellulose micro fibril. Micro-fibrils are synthesized on the plasma membrane by protein complexes called particle rosettes. About 250 micro-fibrils arranged in a bigger bundle called macro-fibrils.

Vertebrates don't have celluloses (P-amylase /β1 → 4) and therefore, can't digest cellulose. However, the rumen (1st stomach chamber) of herbivores, gut of termites contains symbiotic microorganisms that secretes cellulase and thus can digest cellulose.

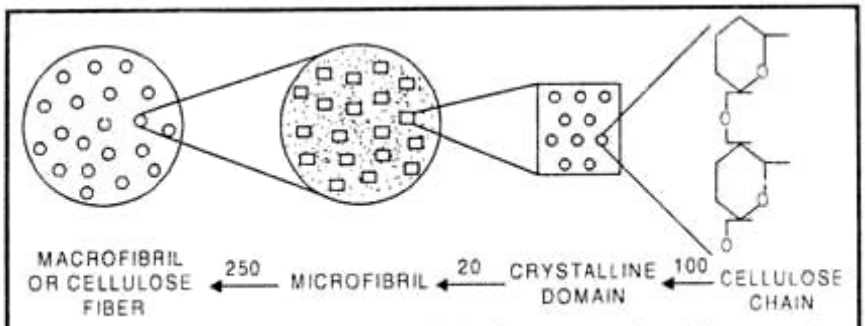


Figure 4.9 Association of cellulose chains in cross section, diagrammatic.

Chitin (fungal cellulose):

Chitin is the main structural polysaccharides of the exoskeletons of arthropods and also in the cell wall of most fungi and algae. Chitin is a linear polymer of N-acetyl-glucosamine (NAM) residues in/β→4 linkage. Structurally chitin is similar to cellulose. It becomes hard when impregnated with $CaCO_3$ and proteins. Chitin is soft leathery and provides strength and elasticity.

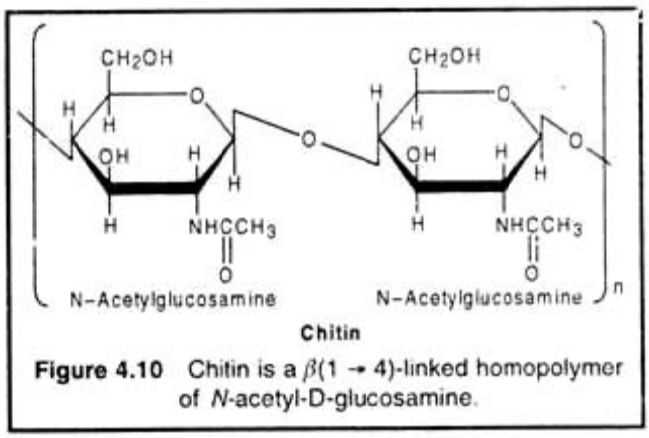


Figure 4.10 Chitin is a β(1 → 4)-linked homopolymer of *N*-acetyl-D-glucosamine.

(B) Storage Polysaccharides:

These polysaccharides serve as reserve food. At the time of need, they are hydrolyzed to release sugars available to the living cells for respiration and biosynthesis.

Followings are some important food storage

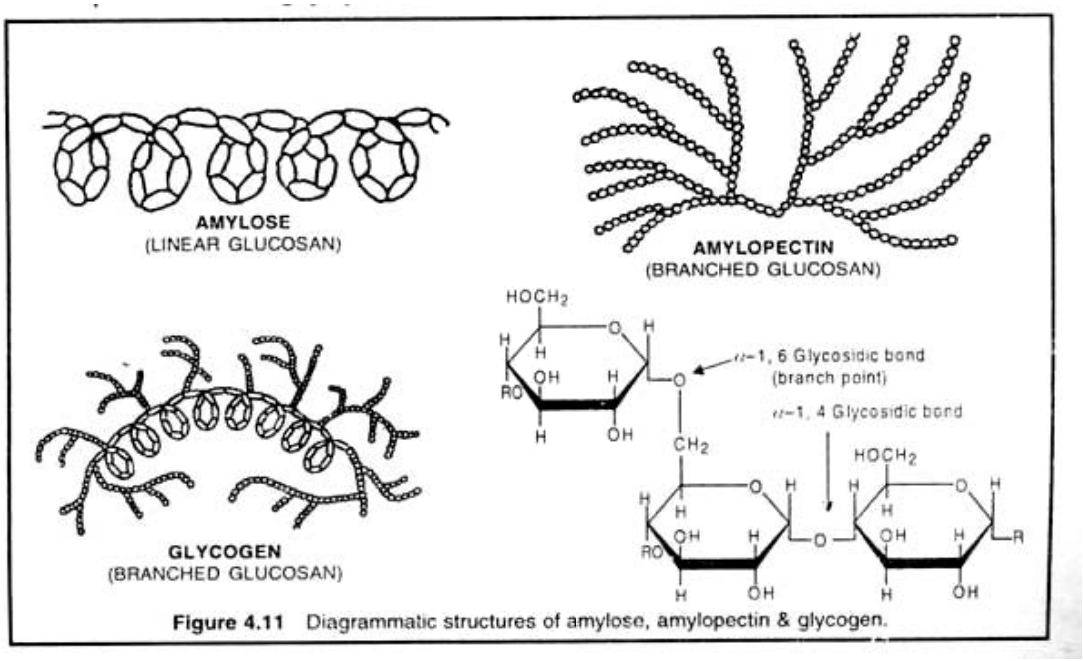


Figure 4.11 Diagrammatic structures of amylose, amylopectin & glycogen.

polysaccharides:

(i) Dextran:

It is the storage polymer in bacteria and yeasts. Dextran is a glucosan where the glucose resides are linked by αl-6 linkage with occasional branches occur by αl-4 linkages. Dextrans are non-toxic hence used chemically as substitute of plasma proteins.

(ii) Levan:

It is the storage polysaccharides in bacteria. Levan is a fructosan composed of fructose residue joined by 2-6 linkages.

(iii) Starch (=amylum):

Starch is a mixture of glucans that synthesized as end product of photosynthesis. It is stored in form of insoluble granules (starch grains) in chloroplasts, amyloplasts and cytoplasm of plant cells. Starch grain occur singly (simple) or in groups (compound). Starch grains have characteristic shape which differs in plant species. Each starch grain has a common point called hilum surrounded layer of starch ring.

Each starch grain consists of a mixture of two type of glucans i.e. amylose (20-30%) and amylopectin (70-80%). Amylose generally found in core while amylopectin in outer part of starch grain. Amylase is a linear but coiled polymer of about 200-1000 D-glucose residues linked by α1 →4 linkages.

Amylopectin is a branched structure of at least 80 chains each with 24-30 glucose residues, inter linked byal →6 linkages. Each branch found at an interval of 24-30 glucose residues. Thus amylopectin is like a glycogen except for its lower degree of branching. Amylose gives blue-black colour with iodine solution while amylopectin gives red-violet colour.

Cooking or boiling causes swelling of amylopectin component of starch grain which ruptures the cell wall and facilitates digestion. But amylase dissolves in water and separates. Partial hydrolysis of starch produce oligosaccharides called dextrins which are water soluble. Initially larger dextrins formed called erythrodextrin which give red colour to iodine and later smaller dextrins produce called achrodextrins which give no colour with iodine.



Dextrins also produce when flour in browned or bread is toasted. However, dextrins may be found naturally in honey and plant leaves.

(iv) Inulin (Dahlia starch):

Inulin is a water soluble fructosan consists of 25-35 /β-D fructose residues joined by/β1-2 linkages. It is a storage homoglycan deposited in the form of fan-shaped crystals in root tubers of Dahlia, Dandelion, stem tubers of Chicory, bulbs of onion and garlic etc. It gives no colour in iodine. Insulin is not metabolized in human body and therefore, used for the determination of rate of glomerular filtration (GFR) in kidney. Insulin is smallest polysaccharide.

(v) Glycogen (animal starch):

Glycogen in the storage polysaccharide of animals that present in all cells but most prevalent in liver and muscles where it occurs as cytoplasmic granules usually associated with SER. It is also found in yeasts and fungi but not in green plants. In liver and muscles, excess glucose convert into glycogen by glycogenesis and during starvation glycogen hydrolyzed to release glucose by glycogenosis.

Glycogen is water soluble and gives red colour to iodine. Structurally, a glycogen molecule resembles amylopectin of starch but glycogen is more branched with branch points occur every 8-12 glucose residues. Each glycogen consists of 5000-30,000 glucose residues linked by αl-4 glycosidic bands with αl, 6-linkage of branch point.

(C) Mucosubstances:

Mucopolysaccharides (glucosaminoglycans), mucoproteins (glycoproteins) are mucosubstances forming slime, mucus or mucilaginous compounds in both plant and animal body. They have structural and protective functions. Mucopolysaccharides are heteropolysaccharides present in the matrix of cell wall and the ground substances of connective tissues in animals. They are characterized by the presence of amino sugars and uronic acids.

Some mucopolysaccharides are as follows:

(i) Hemicelluloses and Pectins:

These are heterogeneous group of polysaccharides that found in the amorphous matrix of plant cell wall. Hemicelluloses are flexible cellulose-linking glycans. They are not the modified cellulose as the name indicates. Some common hemicelluloses are xyloglucan, xylan and glucomannan.

Pectins are linear or highly branched polysaccharides that form a hydrophilic gel in which hemicellulose-cellulose network is embedded. The most common pectins are homoglactouronan (pectic acid), arabinans, arabinogalactan, rhamoglactouronan etc. Pectins of cell wall determine the hydration and orientation of cellulose microfibrils. In wood, the matrix contains lignin, a phenolic polymer.

(ii) Agar (Agar -agar):

It is a cell wall mucopolysaccharide of some red algae (Gelidium, Gracilaria). It is used in culture medium, Cosmetics, leather, laxative, baked goods, meat industry and an emulsifier in dairy products.

(iii) Carrageenin:

It is related to agar and found in red algae Chordrus crispus. It is used as a component of tooth pastes, deodorants, cosmetics etc.

(iv) Hyaluronic acid:

It is a linear polymer of 250 to 25,000 disaccharide units that composed of D- glucuronic acid and N-acetylglucosamine joined by β l → 4 linkages. It occurs in the ground substance of connective tissue, synovial fluid and vitreous humor of eye.

The other glycosaminoglycans of ground substance of connective tissue are chondroitin sulphate, dermatan sulphate and keratin sulphate. But heparin is a sulfated glycosaminoglycan present in most cells of connective tissue and acts as anti-coagulant.

(v) Peptidoglycan (Murein, Mucopeptide):

It is made of heteropolysaccharide chains cross- linked by short peptides (generally tetrapeptide). Heteropolysaccharide chains are formed of two alternate amino-sugar molecules, N-acetylglucosamine (NAG) and N-acetylmurainic acid (NAM).

(vi) Lipopolysaccharides:

It is a complex of lipid and polysaccharides which forms the outer membrane of Gram negative bacteria. Lipopolysaccharide induces fever, shock and other toxic effects.

Functions of Polysaccharides:

Some of the important functions of Polysaccharides are listed below:

1. Starch and glycogen are the major storage foods of organic world.
2. On hydrolysis storage carbohydrates provide both energy and carbon chains.
3. Chitin is the structural carbohydrate of fungal walls and exoskeleton of arthropods.
4. Cellulose is the structural substance of cell walls in most of the plants.
5. Cellulose is economically important in the production of furniture, shelter, fuel, paper, textiles, ropes, rayon, cellophane, plastics, shatter proof glass, propellant explosives, emulsifier and raw material for several fermentation products.
6. For human beings cellulose has a roughage value but it is a food for ruminants, snails and termites.
7. Mucilage present as a protective coating around aquatic plants, bacteria, blue-green algae and some animals is derived from polysaccharides.
8. Mucopolysaccharides in human body have several functions— lubrication of ligaments and tendons, formation of some types of body fluids like cerebrospinal fluid, synovial fluid, vitreous humor, providing strength and flexibility to skin, connective tissue and cartilage, binding of proteins in cell walls and holding water in interstitial spaces.
9. Heparin prevents blood clotting inside blood vessels of animals.
10. Some mucopolysaccharides have medicinal and other commercial importance, e.g., husk of Plantago ovata, mucilage of Aloe, and alginic acid, agar, carragenin of marine algae.
11. Pectin’s form matrix of cell wall. As calcium pectate they produce middle lamella or cementing layer between adjacent plant cells. Pectin’s are commercial jellying agents.

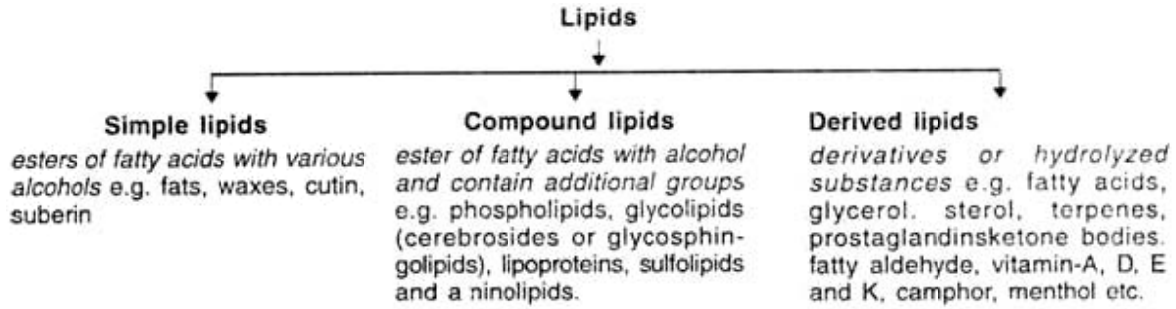
Lipids

Lipids are heterogeneous group of water insoluble compounds which are oily or greasy in consistency but soluble in non-polar solvents like ether, chloroform, benzene etc.

For examples, fatty acids, fats, oils, waxes, certain vitamins and hormones are considered as lipids. Lipids are composed of C, H, O, like carbohydrates but poor in oxygen and therefore require more oxygen for oxidation to release energy.

Classification:

Bloor (1943) coined the term 'lipid' and classified them into three types: Simple, Compound and



Derived.

1. SIMPLE LIPIDS

(A) Fatty acids:

Fatty acids are monocarboxylic acids (R-COOH) with long hydrocarbon chains. Fatty acids don't occur free in nature; rather occur as esters in natural fats and oils. The fatty acid is called an acyl group when it is a part of ester. In biological systems, fatty acids usually contain an even number of carbon atoms, typically between 14 and 24. The most common fatty acids have 16-18 carbon and 0-3 double bonds. For example, plamitic (C16), stearic (C18), oleic (C18), linoleic (C18) etc.

Fatty acids are amphipathic molecules because their carboxylic groups (-COOH) are hydrophilic or polar and the hydrocarbon chains are hydrophobic or non-polar. In physiological conditions, fatty acids found in ionized forms. For example, palmitic acid exist in form of palmitate and so on.

The carbon atoms of fatty acids are numbered from the carboxyl carbon (C-l). The C-2 and C3 are called α-carbon and/β-carbon respectively. The methyl carbon at the end is called ω-carbon. Fatty acids are of 2 types, saturated and unsaturated. Saturated fatty acids contain no double bonds (saturated in hydrocarbon chain, e.g., $CH_3(CH_2)_{14}COOH$ (palmitic acid), $CH_3(CH_2)_{16}COOH$ (stearic acid), $CH_3(CH_2)_{18}$ carboxylic COOH (arachidonic acid) etc. They have higher group melting points and are solid at room temp. Unsaturated fatty acids contain one or more double bonds (unsaturated) in hydrocarbon chain. A double bond in an unsaturated fatty acid has two possible configurations, cis or Trans. The double bonds in most unsaturated fatty acids have the cis orientation that introduces a bend or kink in the hydrocarbon side chain.

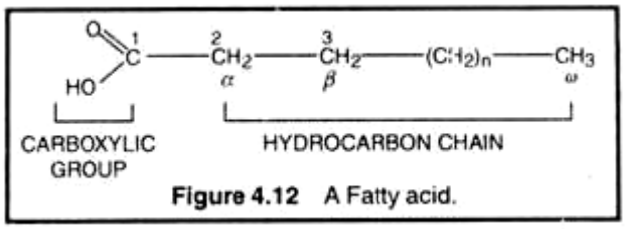
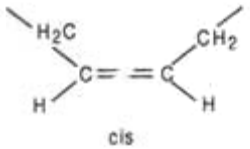
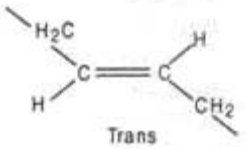


Figure 4.12 A Fatty acid.





Essential Fatty acids (EFA; Durr and Burr, 1930):

Bacterial and plants can synthesize all the required fatty acids. But animals can't synthesize 3 polyunsaturated fatty acids – linoleic, linolenic and arachidonic acids – that are required for growth and synthesis of prostaglandins. If linoleic acids are sufficiently available in the diet then other two EFA can be synthesized from it. EFAs are popularly known as Vitamin F.

Importance of Fatty acids:

1. Fatty acids are fuel molecules, stored as triglycerides in the fat-cells (adipose cells). Under the influence of hormone adrenaline adipose cells hydrolyze the triglycerides into free fatty acids that are

released into the blood. The complete oxidations of 3 fatty acids of a triglyceride release 9 Kcal/g, in contrast to carbohydrate and protein that release 4 Kcal/g.

2. They are the building blocks of phospholipids, glycolipids and lipoproteins that found in the biological membranes.

3. Eicosanoids (prostaglandins, thromboxane's and leukotriene's) are the polyunsaturated fatty acids, which serve as hormones.

4. Fatty acid derivatives act as intracellular messengers, e.g. IP_3(inositol 1, 4, 5-triphosphate) and DAG (diacylglycerol).

5. Deficiency of EFA (essential fatty acids) in human diet causes sterility, kidney failure, skin lesions like phrenoderma (hard skin), eczema etc.

6. Provide the insight to many diseases like obesity atherosclerosis etc.

7. Hydrogenation of unsaturated fatty acids converts oil to solid fat at room temperature. This is the basis of manufacturing edible vanaspati ghee or margarine from inedible and cheap cotton seed oil.

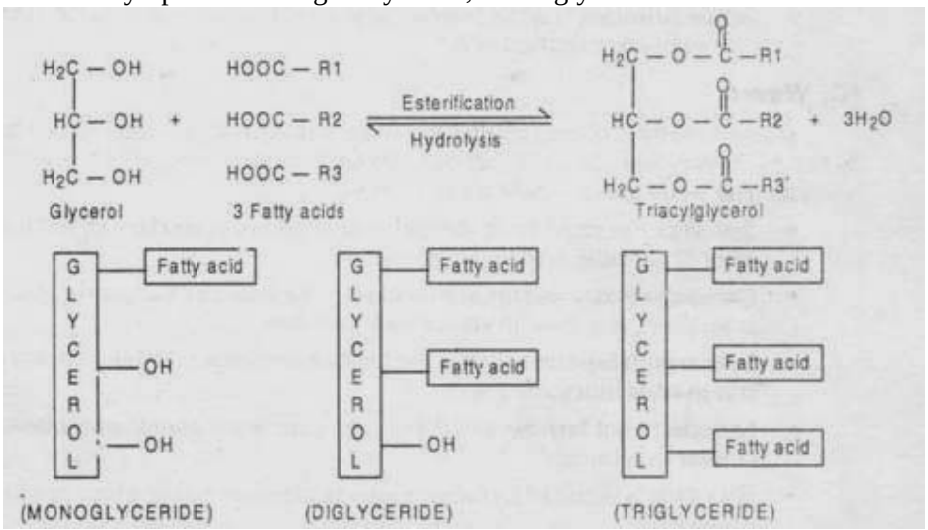
8. Physicians recommend taking PUFA to those having high blood cholesterol or cardiovascular disease.

9. The cleaning action of soap is due to reduction of surface tension of water by fatty acids

(B) Fats (= True fats, neutral fats, triglycerides or triacylglycerol)

They are the unchanged ester of 3 fatty acids and a gtycerol. When 2 or 1 fatty acids esterified with glycerol, then they are called mono-glycerides respects. In pure fats all the 3 fatty acids are similar e.g tripalmitin, tristearin. In mixed fat (triglyceride) all the three or at least one or 2 fatty adds are dissimilar e.g. butter.

Triglycerides are high efficiency storage fuel. In mammals, triglycerides are deposited in the form of large fat globules in the cytoplasm. In migratory birds, the triglycerides are stored under the skin, in



muscle, in abdominal cavity & in liver. About two-thirds of this stored fat is consumed in the long flight over water. Triglycerides store six time as much energy as does glycogen because they are deposited in highly reduced and anhydrous form.

Hence, in evolutionary process triglycerides were selected over glycogen as the major energy reservoir. In a typical 70kg man, triglycerides constitute about 11kg of total body weight with a fuel

reserve of 10^5kcal. If this amount of energy is stored in the form of glycogen, his total body weight would be 55kg greater.

- **Saturated Fatty Acids:**

Their general formula is: $CH_3 — (CH_2)_n — COOH$. The most frequent are palmitic acid (C_{10}) and stearic acid (C_{18}). In lower concentration are found the fatty acids with 14 or 20 carbon atoms. Longer fatty acids (up to 36 carbon atoms) are present in numerous cells (bacteria, unicellular eucaryotes, plants, vertebrates).

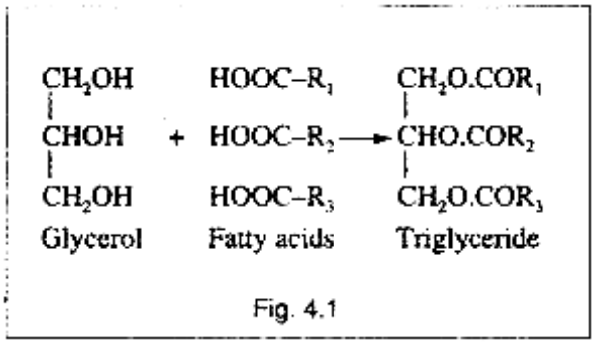


Fig. 4.1

They are generally present in some types of lipids. Milk on the contrary, is rich in short-chain fatty acids. Besides the even-carbon fatty acids, are generally found small quantities of fatty acids having 15, 17 or 19 carbon atoms.

- **Unsaturated Fatty Acids:**

Fatty acids are numbered from the terminal carboxyl (carbon 1) to the CH_3 group (carbon n). The double bond is indicated by the sign Δ, accompanied by the number corresponding to the first carbon atom participating in the double bond. The sign: is being increasingly used; it is followed by the number of double bonds, the position of the latter being indicated within brackets.

There is also a biochemical nomenclature. In this case carbon 1 is the terminal methyl. The place of the last double bond is indicated by ω followed by the number of atoms of the carbon existing up to this double bond. In practically all biological unsaturated fatty acids, the double bond, has a cis isomerism.

The principal unsaturated fatty acids are:

A. Monounsaturated Fatty Acids (1 Double Bond):

Oleic acid (C_{18}), double bond between carbon atoms C_9 and C_{10} , abbreviated as: (C_{18}, Δ^9 or 18 :1(9) or 18 ω 9).

CH_3-$(CH_2)_7$-CH = CH-$(CH_2)_7$-COOH

B. Polyunsaturated Fatty Acids (Several Double Bonds):

In the most common of such acids, the non-conjugated double bonds are separated by a methylene group. Plants can however contain fatty acids with conjugated double bonds, for example, eleostearic acid.

Linoleic acid (C_{18}, $\Delta^{9,12}$ or 18:2 (9,12) or 18 ω 6)

CH_3-$(CH_2)_4$-CH = CH-CH_2-CH = CH-$(CH_2)_7$-COOH

Linolenic acid (C_{18}, $\Delta^{9,12,15}$ or 18 : 3 (9,12,15) or 18 ω 3)

CH_3—CH_2—CH = CH—CH_2—CH = CH—CH_2—CH = CH—$(CH_2)_7$—COOH

Arachidonic acid (C_{20}, $\Delta^{5,8,11,14}$).

Docosahexaenoic acid (C_{22} $\Delta^{4,7,10,13,16,19}$).

Eleostearic acid (C_{18}, $\Delta^{9,11,3}$).

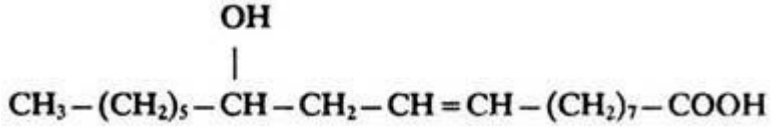
In mammals, polyunsaturated fatty acids can have up to 22 carbon atoms and 6 double bonds, but in plants these acids do not exceed 18 carbon atoms and 4 double bonds.

An important physical property of fatty acids is their melting point; it decreases with increasing number of double bonds. For example, the melting point of stearic acid (18: 0) is 70°C, whereas that of oleic acid (18 :1) is 13°C, that of linoleic acid (18: 2), -5.8°C and that of arachidonic acid (20: 4), –49.5°C.

- **Hydroxylated Fatty Acids:**

Plants can synthesize a series of hydroxylated fatty acids like ricinoleic acid for example:

Some of these hydroxylated fatty acids lead to the formation of cutin.
Other types of hydroxylated fatty acids are found in mammals. Some glycolipids contain large



quantities of α-hydroxylated acids (OH on carbon 2) with 22, 23, 24 and 25 carbon atoms. Moreover, cells of the epiderm have lipids containing very long-chain ω hydroxylated acids which play a role in the structure of this particular tissue.

- **Branched Fatty Acids:**

Example: 15 methylhexadecaenoic acid
$(CH_3)_2 - CH - (CH_2)_{13} - COOH$
The above type of fatty acid is particularly abundant in $Gram^+$bacteria.

Properties of Fats:

1. The fats of animal and plant origin are mixtures of different triglycerides. Plant fats have more unsaturated fatty acids and animal fats have saturated fatty acids. For commercially used fats are different into hard fats (animal fats) and oils (vegetable fats). Fats are solid at room temperature (20°C) but when liquid at 20°C called oils.
2. The property of fat depends upon chain length and their degree of saturation. Short chain length and un-saturation of fatty acids lower the melting point and increase the fluidity.
3. Hydrolysis: Lipases hydrolase the ester bonds so that a triglyceride forms 3 fatty acids and 1 glycerol. It is the basis of fat digestion.
4. Oxidation: On the outer mitochondrial membrane fatty acids first linked to coenzyme A by the enzyme fatty acid thiokinase. This is called fatty acid activation. Subsequently the activated fatty acids enter into the mitochondrial matrix where /β-oxidation (oxidation at /β-carbon) takes place to liberate energy.
5. Saponification: It is the process of formation of soap (metallic salts of fatty acids) by boiling a fat or oil with alkali like KOH, NaOH etc.
6. Saponification number: It is the number of milligrams of KOH required to saponify 1 gm of a given fat or oil. Saponification gives an idea about the molecular weight or chain length of the fatty acids of a fat. A fat having smaller fatty acids have higher saponification.
7. Rancidity: It is the development of unpleasant odour and taste of a fat or oil when kept for a long time, or exposed to air, moisture, heat etc. Rancidity is caused by microbial degradation of fatty acids, hydrolysis of a fat and formation of peroxides at the double bonds. Vegetable fats can be preserved for longer period than animal fat. This is because vegetable fats (oils) contain antioxidants like Vitamin E, Phenols etc. which prevent rancidity.
8. Acid Number: It is the number of milligrams of KOH required to neutralize the free fatty acids present in 1 gm of fat. The acid number indicates the degree of rancidity of a given fat. Higher the acid number, greater is the rancidity or fat.
9. Iodine Number: It is the number of grams of iodine absorbed by 100 gm. of fat. It indicates the degree of un-saturation of fat.

Identification of Fats and Oils:

(a) Hydrolysis:

1. Hydrolysis of triacylglycerol takes place by lipases producing fatty acids and glycerol.
2. Phospholipases attack the ester linkage of phospholipids.

(b) Saponification:

1. Boiling with an alcoholic solution of strong metallic alkali hydrolyses triglycerides into glycerol and fatty acids —this is called saponification.

2. The products are glycerol and the alkali salts of the fatty acids which are called soaps.
3. Fats, phospholipids, glycolipids and waxes are called saponifiable lipids.
4. Steroids, polyisoprenoids and higher alcohols are grouped as un-saponifiable lipids because they cannot give rise to soap.

(c) Saponification number:

1. The number of milligrams of KOH required to saponify 1 gram of fat or oil.
2. The amount of alkali needed to saponify a given quantity of fat will depend upon the number of-COOH group present. It is inversely proportional to the average molecular weight of the fatty acids in the fat i.e. the fats containing short chain fatty acids will have more -COOH groups per gram than long chain fatty acids—this will take up more alkali and, hence, will have higher saponification number.

Example:

Butter—containing a larger proportion of short chain fatty acids such as butyric and caproic acids, has relatively high saponification number 220 to 230.

(d) Acid number:

1. The number of milligrams of KOH required to neutralize the free fatty acids of 1 gram of fat.
2. Significance: The acid number indicates the degree of rancidity of the given fat.

(e) Iodine number:

1. This is the amount (in grams) of iodine absorbed by 100 grams of fat.
2. This is the measure of the degree of unsaturation of a fat.
3. Significance: If the fat contains higher number of unsaturated fatty acids, it becomes essential for the protection of heart disease. These unsaturated fatty acids, combined with the cholesterol, are oxidized in the liver—producing bile acids, bile salts, vit., D, gonadotrophin hormones. They prevent atherosclerosis.

(f) Acetyl number:

1. The number of milligrams of KOH required to neutralize the acetic acid obtained by saponification of 1 gram of fat after it has been acetylated.
2. This is a measure of the number of hydroxy acid groups in the fat.

(g) Polenske number:

1. The number of milliliters of 0.1 (N) KOH required to neutralize the insoluble fatty acids from 5 grams of fat.

(h) Reichert-Miessl number:

1. This is same as the Polenske number except that the soluble fatty acids are measured by titration of the distillate obtained by steam distillation of the saponification mixture.

Significance: It measures the amount of volatile soluble fatty acids.

(i) Halogenation:

1. Chlorine, bromine and iodine atoms may be added to the double bonds of unsaturated fatty acids containing fats.

(j) Rancidity:

1. Nearly all natural fats are oxidized when exposed to air, light, moisture, particularly, if warm, it develops an unpleasant odour and taste. The enzyme lipase—in the presence of moisture and warm temperature— bring about hydrolysis rapidly.
2. This happens due to the formation of peroxides at the double bonds of unsaturated fatty acids.
3. Vitamin E is an important natural antioxidant and prevents development of rancidity.

(B) Soaps:

1. Soaps are metallic salts of fatty acids.
2. Soaps are formed by adding alkalis to fatty acids.
3. Soaps of unsaturated fatty acids are softer and more water soluble than those of saturated fatty acids.
4. Potassium soap of an acid is more water-soluble and softer than the sodium soap; calcium and magnesium soaps are far less soluble.

(B) Waxes:
1. They are esters of fatty acids with higher alcohols other than glycerol.
2. In the human body, the commonest waxes are esters of cholesterol.
3. They are mainly three types:
(a) True waxes are esters of higher fatty acids with acetyl alcohol or other higher straight chain alcohols.
(b) Cholesterol esters are esters of fatty acid with cholesterol.
(c) Vitamin A and vitamin D esters are palmitic or stearic acid esters of vitamin A (Retinol) or vitamin D, respectively.

The well known waxes are as follows:

i. Bee-Wax:
Secreted from the abdominal glands of worker bees to build honeycombs. It is the ester of palmitic acid and hexacosonol.

ii. Carnauba wax:
It occurs as a coating on the leaves of Carnauba palm tree of Brazil (Copernicia prunifera). It is used in automobile polishes.

iii. Spermaceti (Sperm oil):
It is the hardest known wax obtained from the head of sperm whale. It is used as lubricating wax.

iv. Lanolin (wool fat):
Secreted from the cutaneous glands of fur bearing animals. It is closing similar to sebum.

v. Wax-D:
It is secreted by tuberculosis and leprosy causing bacteria. It is a measure cause of the diseases.

vi. Cerumen (ear wax):
Secreted from the ceruminous glands of external auditory canal.

vii. Paraffin wax:
It is obtained from petroleum. Candles are prepared from paraffin wax and stearic acid.

(C) Cutin:
It is formed by cross-esterification and polymerization of hydroxyl fatty acids. Along with wax (cuticular wax), cut in form cuticle on leaf epidermis which check transpiration. The cuticle is frequently covered by a layer of epicuticular wax.

(D) Suberin:
It is composed of glycerol and phellonic acid. It is found in the walls of cork cells and from Casparian strip of root endodermis. It is water impermeable and checks infection in plants.

2. Compound Lipids:

A. Phospholipids (phosphatides):
(i) They are esters of fatty acids with glycerol containing an esterified phosphoric acid and a nitrogen base.
(ii) They are present in large amounts in nerve tissue, brain, liver, kidney, pancreas and heart.

Biological functions of phospholipids:
(i) They increase the rate of fatty acid oxidation.
(ii) They act as carriers of inorganic ions across the membranes.
(iii) They help blood-clotting.
(iv) They act as prosthetic group to certain enzymes.
(v) They form the structures of membranes, matrix of cell wall, myelin sheath, microsomes and mitochondria.

Classification:
It is based on the type of alcohol present in the phospholipid.

There are three types:
1. Glycerophosphatides — In this, glycerol is the alcohol group.

Example:
(i) Phosphatidyl ethanolamine (cephalin).
(ii) Phosphatidyl choline (Lecithin).
(iii) Phosphatidyl serine.
(iv) Plasmalogens.
(v) Phosphatidic acid.
2. Phosphoinositides — In this, inositol is the alcohol.
Example:
Phosphatidyl inositol (Lipositol).
3. Phosphosphingosides — In this, sphingosine is an amino alcohol.
Example:
Sphingomyelin.
The phospholipids include the following groups:
1. Phosphatidic acid and phosphatidyl glycerol's:
Phosphatidic acid is important as an intermediate in the synthesis of triacylglycerol's and phospholipids.
Cardiolipin:
(a) It is formed from phosphatidyl glycerol.
(b) Chemically, it is di-phosphatidyl glycerol.
(c) It is found in inner membrane of mitochondria and bacterial wall.

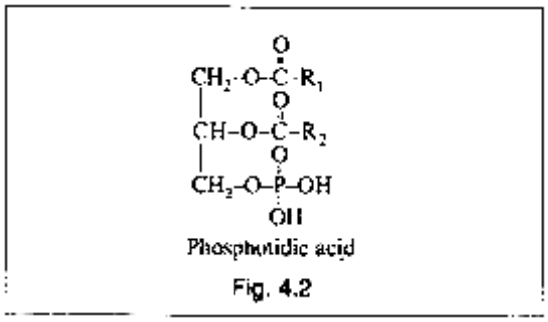


Fig. 4.2

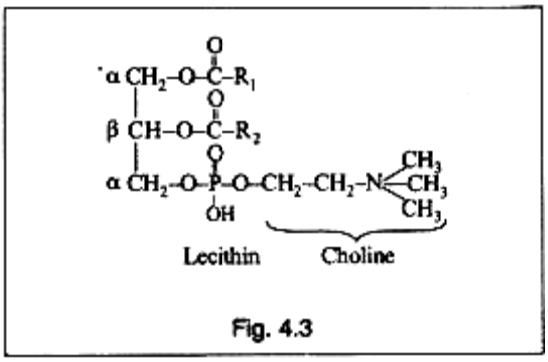


Fig. 4.3

2. Lecithin (Phosphatidylcholine):
The lecithin's contain glycerol and fatty acids, phosphoric acid and choline (nitrogenous base). Lecithin's generally contain a saturated fatty acid at α position and an unsaturated fatty acid at β position. They can exist in α or β forms.
Physical Properties:
(i) Lecithin's are waxy, white substances but become brown soon when exposed to air
(ii) They are soluble in ordinary fat solvents except acetone.
(iii) They decompose when heated.
(iv) They constitute valuable agents for the emulsifications of fats and oils.
Chemical Properties of Lecithin:
(i) When aqueous solution of lecithin's are shaken with H_2SO_4, choline is split off, forming phosphatidic acid.
(ii) When lecithin's are boiled with alkalis or mineral acids, not only choline is split off, but phosphatidic acid is further hydrolyzed to glycerophosphoric acid and 2 molecules of fatty acids:

$$\text{Lecithin} \xrightarrow{H_2SO_4} \text{Phosphatidic acid} +$$

choline.

Lecithin → H_2SO_4 Phosphatidic acid + choline.
Phosphatidic acid → Glycerophosphoric acid + fatty acids (2 mol)

Physiological Functions of Lecithin:

(i) It facilitate the combinations with proteins to from lipoproteins of plasma and cells.

(ii) Acetylcholine formed from choline has an important role in the transmission of nervous impulses across synapses.

(iii) Choline is the most important lipotropic agent as it can prevent formation of fatty liver.

(iv) Lecithin lowers the surface tension of lung alveoli. Dipalmityl lecithin is a major constituent of "lung surfactant" which prevents the adherence of the inner surface of the alveoli of the lungs (preventing the collapse of the alveoli) by its surface tension lowering effect. The absence of this in the alveolar membrane of some premature infants causes the respiratory distress syndrome in them.

(v) It lowers the surface tension of water molecule and helps in the emulsification of fat.

Difference of Lecithin and Cephalin:

Cadmium chloride compound of cephalin is soluble but cadmium chloride compound of lecithin is insoluble.

3. Cephalitis (Phosphatidyl ethanolamine):

They always occur in the tissues in association with lecithin's and are very similar in properties. The only difference is the nitrogenous base.

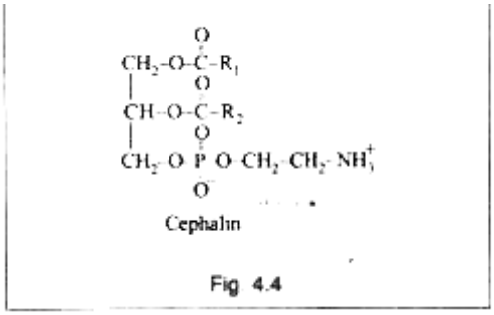


Fig 4.4

4. Phosphatidyl Serine:

A cephaline like phospholipid is found in tissues.

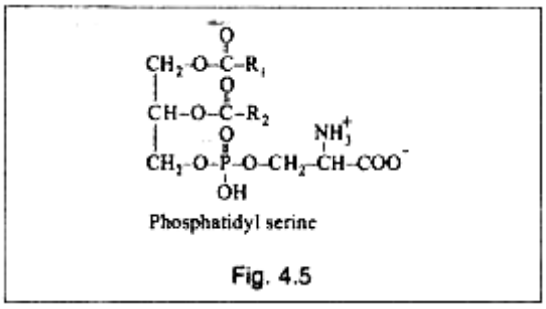


Fig. 4.5

5. Phosphatidyl inositol (Lipositol or Phosphoinositides):

(i) It acts as second messenger in Ca^{++} dependent hormone action.

(ii) Some signals must provide communication between the hormone receptor on the plasma membrane and intracellular Ca^{++} reservoirs.

(iii) They are more acidic than the other phospholipids.

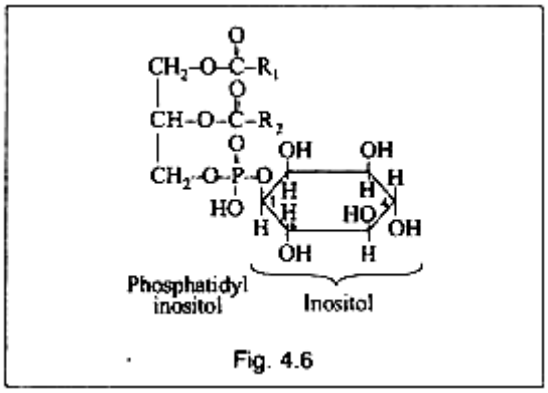


Fig. 4.6

6. Lysophospholipids:

(i) These are phosphoacylglycerols containing only one acyl radical in a position e.g., Lysolecithin.

(ii) Formation:

(a) By the action of phospholipase.

(b) By interaction of lecithin and cholesterol in presence of the enzyme lecithin cholesterol acyl transferase, so lysolecithin and cholesterol ester are formed

Lecithin + cholesterol
↓ LCAT
Lysolecithin + cholesterol ester.

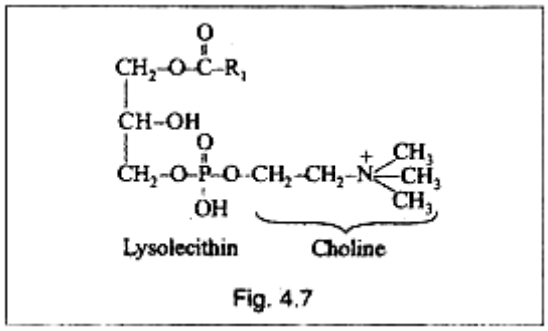


Fig. 4.7

7. Plasmalogens:

(i) These are the contents of brain and muscle.

(ii) Structurally, these resemble lecithin's and cephalins but give a positive reaction when tested for aldehydes with Schiff s reagent (fuchsin-sulphurous acid) after pretreatment of the phospholipid with mercuric chloride.

(iii) They possess an ether link in a position instead of ester link. The alkyl radical is an unsaturated alcohol.

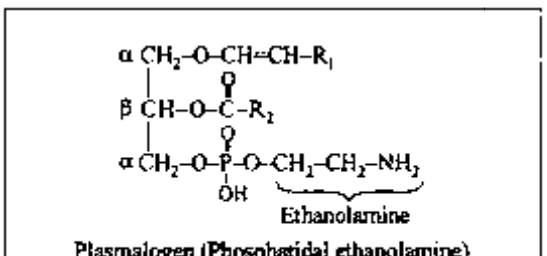


Fig. 4.8

8. Sphingomyelins:

(i) These are found in large quantities in brain and nerve tissue.

(ii) The concentrations of these phospholipids are increased in Niemann-Pick disease in the liver and spleen.

(iii) These contain sphingosine (18 carbon) (amino alcohol) fatty acid, phosphoric acid and choline. No glycerol is present.

(iv) In sphingosine molecule -NH_2 group binds a fatty acid by an amide linkage to produce ceramide. When phosphate group is attached to ceramide it is called ceramide phosphate.

(v) When choline is split off from sphingomyelin, ceramide phosphate is left.

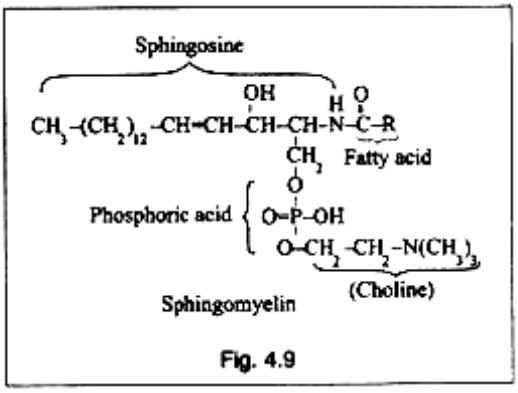


Fig. 4.9

CLINICAL ASPECT

- In Niemann-Pick disease excess amount of sphingomyelin are deposited in brain, liver, spleen.
- It is a lipid storage disease (lipidoses) and hereditary. It is caused by the deficiency of enzyme sphingomyelinase.
- The clinical findings are :
  - (a) Enlarged liver and spleen.
  - (b) Mental retardation.
  - (c) Nervous system is affected.
  - (d) Anemia and leukocytosis.

Action of Phospholipase:

(a) Phospholipase A_1 attacks the ester bond in position 1 of phospholipid.

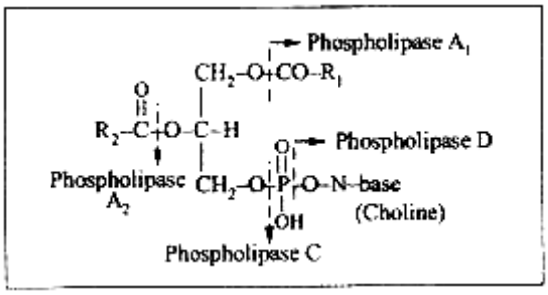
(b) Phospholipase A_2 attacks β position and form

Lysolecithin + one mol. fatty acid.

(c) Phospholipase B (lysophospholipase) attacks lysolecithin and hydrolyzes ester bond in α position and forms glyceryl phosphoryl choline + 1 mol. fatty acid.

(d) Phospholipase C hydrolyzes phosphate ester bond and produces α, β di-acyl glycerol + phosphoryl choline.

(e) Phospholipase D-splits off choline and phosphatidic acid is formed



B. Glycolipids:

These contain an amino alcohol (sphingosine or iso-sphingosine) attached with an amide linkage to a fatty acid and glycosidically to a carbohydrate moiety (sugars, amino sugar, sialic acid).

These are further classified into:

(i) Cerebrosides.

(ii) Gangliosides.

(i) Cerebrosides:

(a) Cerebrosides contain galactose, a high molecular weight fatty acid and sphingosine. Therefore, they may also be classified as sphingolipids.

(b) They are the chief constituent of myelin sheath.

(c) They may be differentiated by the type of fatty acid in the molecule.

These are:

Kerasin—Containing lignoceric acid [CH, — $(CH_2)_{22}$ — COOH].

Cerebron—Containing a hydroxylignoceric acid (cerebronic acid).

[CH_3—$(CH_2)_2$, — CH(OH)—COOH].

Nervon—Containing an unsaturated homologue of lignoceric acid called nervonic acid. [CH, — $(CH_2)_7$ — CH = CH — $(CH_2)_{13}$ — COOH].

Oxynervon—Containing hydroxy-nervonic acid [CH_3 — $(CH_2)_7$ — CH = CH — $(CH_2)_{12}$— CH(OH) — COOH].

(d) Stearic acid is a major component of the fatty acids of rat brain cerebrosides.

(e) Cerebrosides, specially cerebronic acid, increases in Gaucher's disease and the Kerasin characterized by glucose replacing galactose.

(f) The cerebrosides are in much higher concentration in medullated than in non-medullated nerve fibers.

CLINICAL ASPECT OF CEREBROSIDE

Gaucher's disease

- The cerebroside content of the reticuloendothelial cell (spleen) is very high.
- In cerebroside molecule, the kerasin is characterised by glucose replacing galactose.
- The disease is caused by the deficiency of enzyme glucocerebrosidase.

Symptoms

- Spleen is increased, signs of leucopenia.
- Liver is enlarged.
- Eyes show a yellow-brown wedge shaped elevation.

(ii) Gangliosides:

(a) These are glycolipids occurring in the brain.

(b) Gangliosides contain ceramide (sphingosine + fatty acids), glucose, galactose, N-acetylgalactosamine and sialic acid.

(c) Some gangliosides also contain di-hydro-sphingosine or gangliosine in place of sphingosine.

(d) Most of the gangliosides contain a glucose, two molecules of galactose, one N-acetylgalactosamine and up to three molecules of sialic acid.

Types of Ganglioside:

Tay Sach's disease (GM_2 gangliosidosis)

- This disease is characterised by increased accumulation of GM_2 ganglioside in brain and spleen.
- It is caused by deficiency of enzyme hexosaminidase A.

Symptom

- Mental retardation, blindness, muscular weakness.
- Red spot appears in the muscular region of eye in first year of life.

GM_1 ganglioside :

Gal → Gal NAC → Gal → Glucose → Ceramide
↓
NANA

GM_2 ganglioside :

Gal NAC → Gal → Glucose → Ceramide
↓
NANA

GM_3 ganglioside :

Gal → Glucose → Ceramide
↓
NANA

G = Ganglioside, M = Monosialo variety.

1, 2, 3 = Position in chromatography.

GM_1 gangliosidosis : This is due to deficiency of enzyme β-galactosidase.

C. Other compound lipids:

1. Lipoproteins:

(i) Triacylglycerol (45%), phospholipids (35%), cholesterol and cholesteryl esters (15%), free fatty acids (less than 5%) and also protein combine to form a hydrophilic lipoprotein complex.

(ii) Since pure fat is less dense than water, the proportion of lipid to protein in lipoproteins in plasma is by ultracentrifugation.

(iii) The density of lipoproteins increases as the protein content rises and the lipid content falls and the size of the particle becomes smaller.

(iv) Lipoproteins may be separated on the basis of their electrophoretic properties and may be identified more accurately by means of immuno-electrophoresis.

(v) Four major groups of lipoproteins have been identified which are important physiologically and in clinical diagnosis in some metabolic disorders of fat metabolism.

These are:

(a) Chylomicrons.

(b) Very low density lipoproteins (VLDL or pre-β-lipoproteins).

(c) Low density lipoproteins (LDL or β-lipoproteins).

(d) High density lipoproteins (HDL or α-lipoproteins).

(vi) Chylomicrons and VLDL: Predominant lipid is triacylglycerol (50%) and cholesterol (23%). The concentrations of these are increased in atherosclerosis and coronary thrombosis etc.

LDL:

Predominant lipid is cholesterol (46%) and phospholipids (23%). Increase in atherosclerosis and coronary thrombosis, etc.

HDL:

Predominant lipid is phospholipid (27%) and proteins (45%).

(vii) The protein moiety lipoprotein is known as an apoprotein which constitute nearly 60% of some HDL and 1% of chylomicrons. Many lipoproteins contain more than one type of apoprotein polypeptide.

(viii) The larger lipoproteins (such as chylomicrons and VLDL) consist of a lipid core of nonpolar triacylglycerol and cholesteryl ester surrounded by more polar phospholipid, cholesterol and Apo proteins.

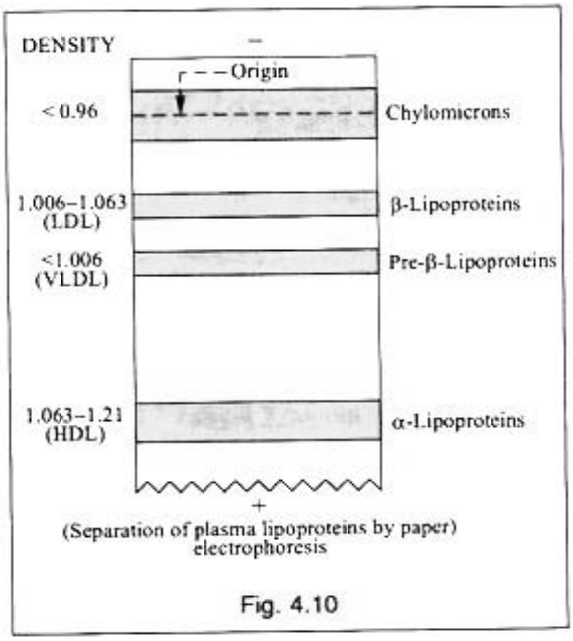


Fig. 4.10

Importance:

(i) To transport and deliver the lipids to tissues.

(ii) To maintain structural integrity of cell surface and subcellular particles like mitochondria and microsomes.

(iii) The β-lipoprotein fraction increases in severe diabetes mellitus, atherosclerosis etc. Hence determination of the relative concentrations of α-and β-lipoproteins and pre-β- lipoproteins are of diagnostic importance.

2. Amino lipids:

Phosphatidyl ethanolamine and serines are amino lipids and sphingomyelins and gangliosides contain substituted amino groups.

3. Sulpholipids (Sulphatides):

(i) These have been isolated from brain and other animal tissues.

(ii) These are sulphate derivatives of the galactosyl residue in cerebrosides.

3. Derived Lipids:

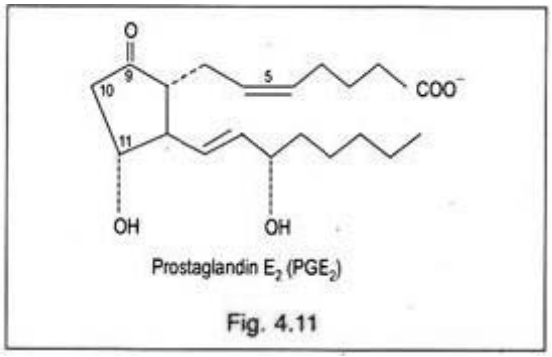
Prostaglandins (PG):

(a) They virtually exist in every mammalian tissue and act as local hormones.

(b) They have important physiologic and pharmacologic activities.

(c) They are synthesized in vivo by cyclization of the center of the carbon chain of 20-C polyunsaturated fatty acids (e.g., arachidonic acid) to form a cyclopentane ring.

(d) Three different eicosanoic fatty acids give rise to three groups of eicosanoids characterized by the number of double bonds in the side chains, e.g., PG_1, PG_2, PG_3. Variations in the substituent groups attached to the rings give rise to different types in each series of prostaglandins, as for example, "E" type of Prostaglandin has a keto group in position 9, whereas the "F" type has a hydroxyl group in this position.



Prostaglandin E_2 (PGE_2)

Fig. 4.11

Prostacyclin's (PGI):

(a) They are formed in vascular endothelium and continually formed in heart. They are also formed in kidneys.

(b) They are formed from cyclic endo-peroxide PGH_2 by the action of microsomal Prostacyclin synthetase.

(c) They inhibit platelet aggregation and gastric secretion from the pyloric mucosa.

(d) They decrease blood pressure and protect coronary arteries.

(e) They increase renal blood flow and stimulate renin production.

(f) They are inhibited by hyperlipemia, vit. E deficiency and radiation.

Thromboxane's:

(a) They contract smooth muscles on blood vessels, GI Tract, uterus, bronchioles.

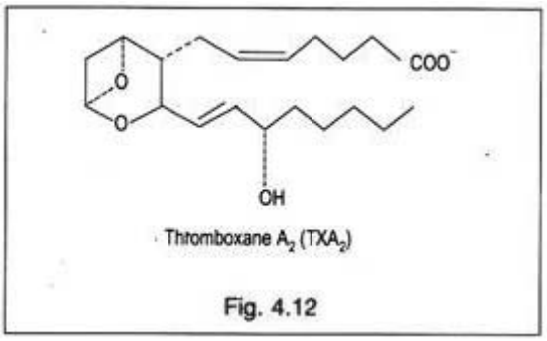
(b) They are discovered in platelets, and have the cyclopentane ring interrupted with an oxygen atom (Oxane ring).

(c) The substituent groups attached to the rings being varied give rise to different types in each series of thromboxane's labelled A, B, etc.

(d) They produce vasoconstriction and increase blood pressure.

(e) They cause release of serotonin and calcium ion (Ca^{++}) from platelet granules.

(f) Imidazole's inhibit their synthesis.



Thromboxane A_2 (TXA_2)

Fig. 4.12

Leukotriene's:

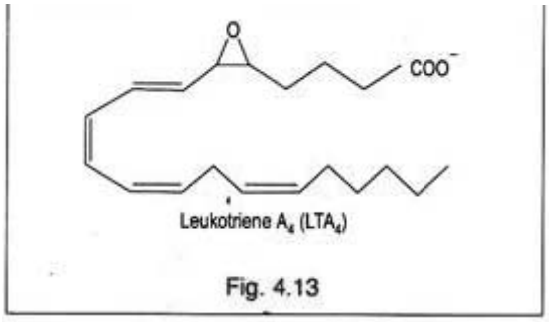
(a) They are the third group of eicosanoid derivatives formed via the lipoxygenase pathway rather than cyclization of the fatty acid chain.

(b) They are first described in leukocytes.

(c) They are characterized by the presence of three conjugated double bonds.

(d) They are stimulators of mucus secretion and are responsible for vasoconstriction of bronchial muscles.

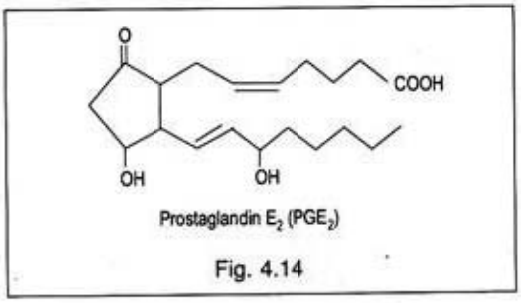
(e) They are inhibited by prolonged use of aspirin.



Leukotriene A_4 (LTA_4)

Fig. 4.13

The group of compounds known as prostaglandins are synthesized from arachidonic acid in the body. They have pharmacologic and biochemical activity.



Prostaglandin E_2 (PGE_2)

Fig. 4.14

Example : Prostaglandin E_2(PGE_2)

C. Many Other Fatty Acids:

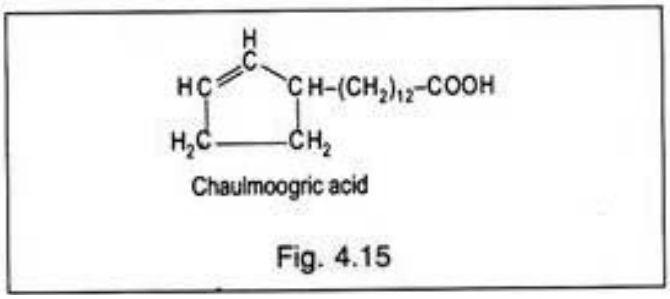
(i) These have been detected in biologic material.

Example:

Fish oil contain 5 and 6 unsaturated fatty acids having carbon atoms 22.

(ii) Various other structures with hydroxy groups (ricinoleic acid) or cyclic groups have been found in nature.

Example of cyclic groups is chaulmoogric acid which was used many years ago in the treatment of leprosy.



Chaulmoogric acid

Fig. 4.15

AMINO ACIDS

Amino acids are the building blocks of proteins. Among the thousands of amino acids available in nature, proteins contain only 20 different kinds of amino acids, all of them are L-alpha-amino acids. The same 20 standard amino acids make proteins in all the living cells, may it either be a virus, bacteria, yeast, plant or human cell. These 20 amino acids combine in different sequences and numbers to form various kinds of proteins.

The number of proteins that can be had from these 20 amino acids can be calculated from 20 factorial, i.e., 20 x 19 x 18 x 17 x 16 x …… x 2 x 1 = 2.4 x 10^{18}. In human beings alone there are more than 100 000 different types of proteins.

The general formulae for an amino acid can be written as 'R-CH-NH_2—COOH'. Depending upon the 'R' group present in the amino acid it is named accordingly. The 20 amino acids found in the proteins are known as primary or standard amino acids. In addition to these, some other amino acids are also found in proteins like 4-hydroxyproline, 5-hydroxylysine, 6-IV-methyllysine, gamma carboxyglutamic acid and desmosine, all of these are derivatives of standard amino acids.

Classification of Amino Acids:

I. Depending upon the Charge:

Amino acids can be broadly classified into three major groups:

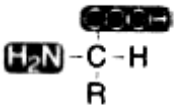
(1) Neutral

(2) Acidic and

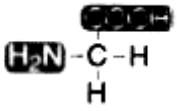
(3) Basic.

1. Neutral amino acids:

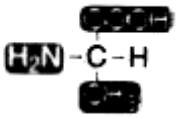
Those amino acids that do not contain any charge on the 'R' group.



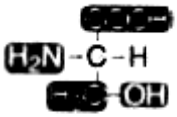
GENERAL FORMULA



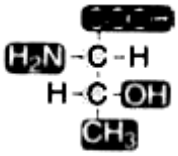
Glycine (Gly)
Mw = 75.08



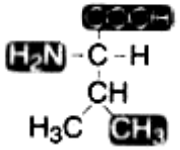
Alanine (Ala)



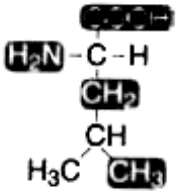
Serine (Ser)



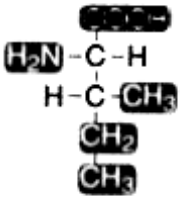
Threonine (Thr)



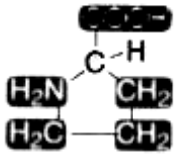
Valine (Val)



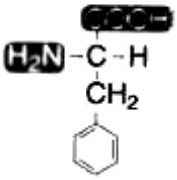
Leucine (Leu)



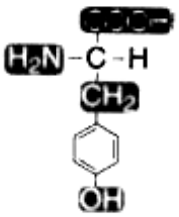
Isoleucine (Ile)



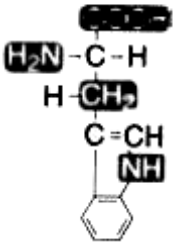
Proline (Pro)



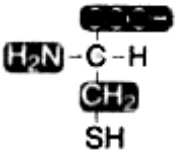
Phenylalanine (Phe)



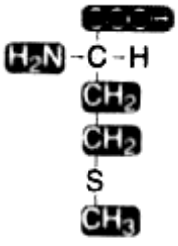
Tyrosine (Tyr)



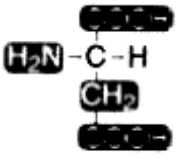
Tryptophan (Trp)
Mw = 206.27



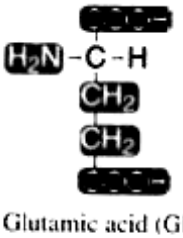
Cystine (Cys)



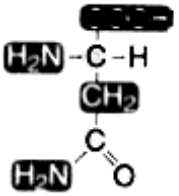
Methionine (Met)



Aspartic acid (Asp)



Glutamic acid (Glu)



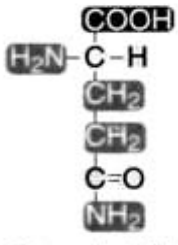
Asparagine (Asn)

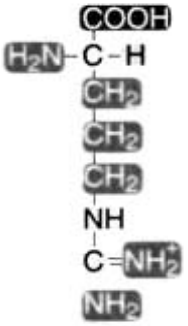
They are further classified into the following categories:

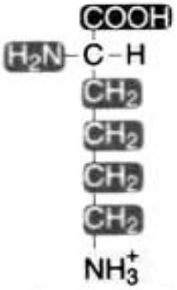
(a) Aliphatic:

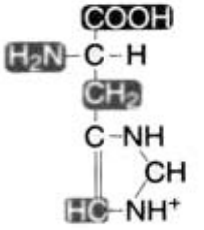
Those amino acids whose 'R' group contains a chain of carbon atoms—Gly, Ala, Ser, Thr, Val, Leu, lie, Asn, Gin.

(b) Aromatic:
Those amino acids whose 'R' group has a benzene ring—Phe, Tyr, Trp.
(c) Heterocyclic:
The "R" group has a heterocylic ring, i.e., any of the ring structures which contain different atoms—Pro, His.
(d) Sulphur containing:
Those amino acids which contain a sulphur atom-Cys, Met.
2. Acidic amino acids:
Those amino acids that contain a negative charge or an acidic group-Asp, Glu.
3. Basic amino acids:
Those amino acids that contain a positive charge or a basic group-Arg, Lys and His.


Glutamine (Gln)


Arginine (Arg)
Mw = 175.25


Lysine (Lys)


Histidine (His)

II. Depending upon the Solubility in Water:

The amino acids can also be grouped into two different categories, depending upon their solubility in water. They are—
1. Hydrophobic amino acids:
Amino acids insoluble in water are known as hydrophobic amino acids. They are—Ala, Val, Leu, lie, Pro, Met, Phe, Trp.
2. Hydrophilic amino acids:
Amino acids soluble in water are known as hydrophilic amino acids. They are—Gly, Ser, Thr, Cys, Tyr, Asp, Asn, Glu, Gin, Lys, Arg, His.
III. Depending upon their Nutritional Requirements:

The amino acids are classified into two groups.
They are:
1. Essential amino acids:
Are those which cannot be synthesized by the human body and hence they should be taken through the diet. There are 10 essential amino acids. Among these amino acids, arginine and histidine are known as semi-essential amino acids.

M – Methionine	**V** – Valine	**P** – Phenylalanine
A – Arginine	**I** – Isoleucine	**H** – Histidine
T – Threonine	**L** – Leucine	**Ly** – Lysine
T – Tryptophan		

2. Non-essential amino acids:
These acids are those that can be synthesized in the human body and are not required in the diet. These include gly, ala, ser, pro, tyr, cys, asp, asn, glu, gin.

Reactions of Amino Acids:

Physical Characters of Amino Acids:

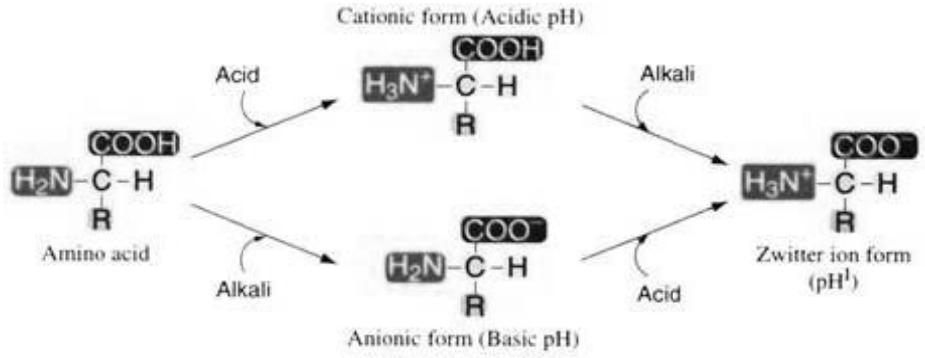
1. Zwitter ions:

Amino acids have an acidic group (—COOH group), i.e., a proton, donor. They also have a basic group (—NH_2 group), i.e., a proton, acceptor. A compound capable of both donating and accepting protons and thus able to act either as an acid or a base is known as amphoteric molecule. Amino acids have both anions and cations in solution and such compounds are called zwitter ions.



2. Isoelectric pH (pH^1):

The pH at which the positive charge on the amino acid (or any other molecule) is equal to the negative charges, is known as isoelectric pH. At this pH the net charge will be zero and hence it does not move either to positive (anode) or to negative (cathode) electrode, when subjected to an electric field. At pH^1 all the molecules exists in zwitter ion form.

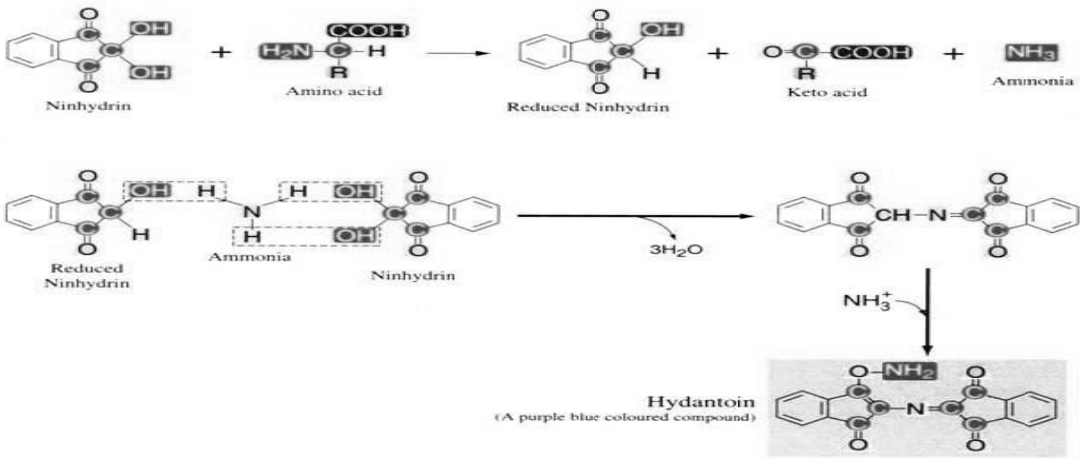


Chemical Properties:

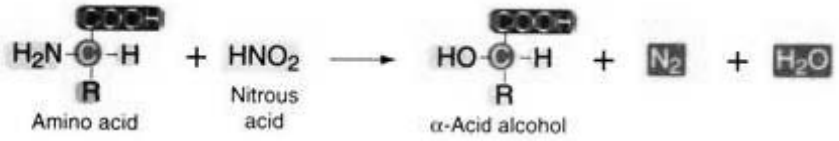
1. Reactions due to amino group:

(a) Ninhydrin test:

This test identifies or detects amino acids. If amino acids are heated with ninhydrin, they form a purple blue coloured compound, which is measured colorimetrically.



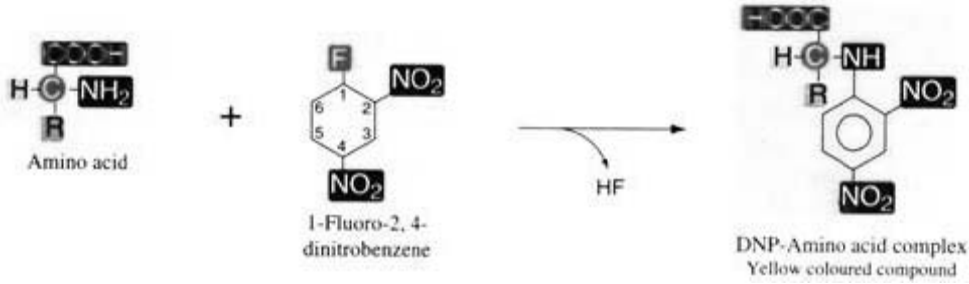
(b)Reaction with nitrous acid:
It is a method by which amino acids are measured depending upon the amount of nitrogen released.



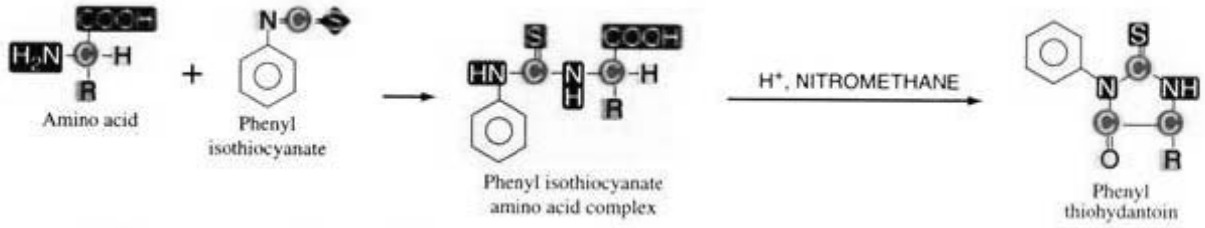
(c) Reaction with carbonyl compounds (RCHO):
The amino group in the amino acids reacts with carbonyl compounds forming a Schiff's base.



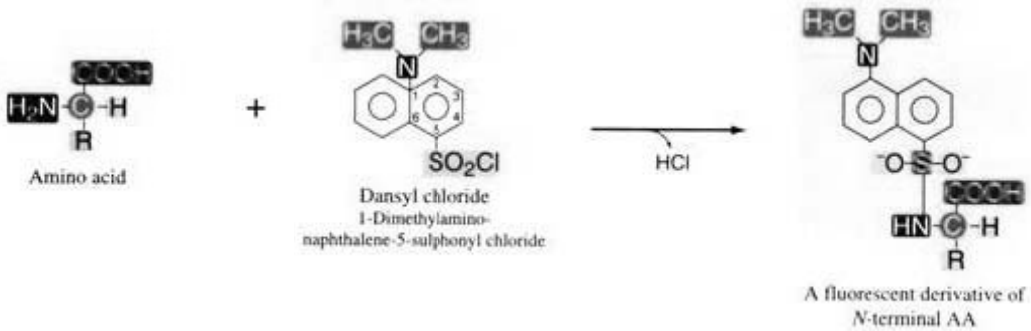
(d) Reaction with Sanger's reagent:
Amino acids react with Sanger's reagent, i.e., 1-fluoro-2, 4-dinitrobenzene, forming a yellow coloured complex. This reagent is used to detect the IV-terminal amino acid in the proteins.



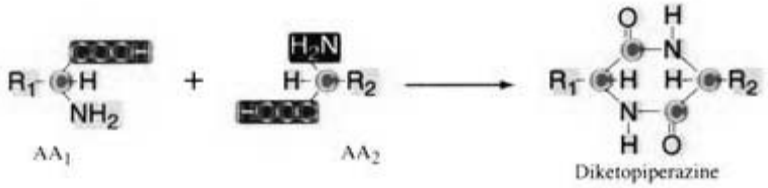
(e) Edmann's reaction:
Edmann's reagent is phenyl isothiocyanate, which is also used to detect the N-terminal amino acid in a protein. It forms a purple coloured derivative.



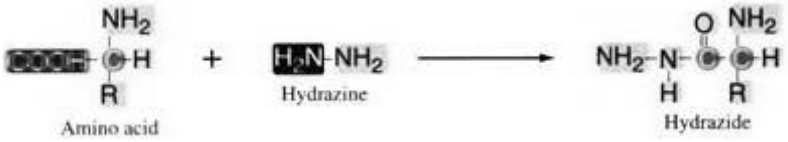
(f) Reaction with dansyl chloride:
Dansyl chloride, i.e., 1-dimethyl-amino-naphthalene-5-sulphonyl chloride forms a fluorescent derivate of the N-terminal amino acid of proteins. This is yet another reagent available for the detection of N-terminal amino acid.



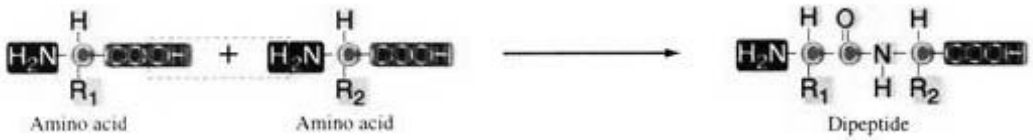
(g) Condensation of two amino acids to form diketopiperazine:
Two amino acids react with amino groups of each amino acid and the carboxylic groups of the other amino acid forming a diketopiperazine.



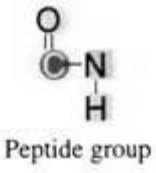
2. Reaction due to carboxylic:
(a) Reaction with hydrazine:
Hydrazine is used to detect the C-terminal amino acid in proteins. It forms a complex with the amino acid by reacting with the carboxylic group.



3. Reaction due to both amino and carboxylic group:
Due to the presence of both amino (basic) and carboxylic (acid) groups in amino acids, the amino group of one amino acid reacts with the carboxylic group of another amino acid to form a peptide bond.



Polymerization of amino acids in a similar manner gives a polypeptide chain.
Peptide bond:
The bond linking two amino acids is known as a peptide bond. It is formed due to reaction between an amino group of one amino acid and carboxylic group of another amino acid.
Peptide group:
The group forming the peptide bond is known as peptide group. It has a double bond character and hence is very rigid in nature.

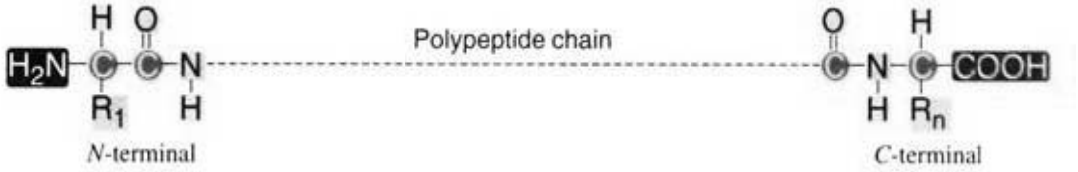


Polypeptide or peptide:
A chain made up of two or more amino acids, linked by a peptide bond is known as a polypeptide or just a peptide.
Difference between a peptide and a protein:
A peptide is that which has less than 50 amino acids or whose molecular weight is less than 5000 Daltons. A protein is that which has more than 50 amino acids or whose molecular weight is more

than 5000 Daltons. This differentiation is based upon the immunological property of the two units. Peptides are non-immunogenic, whereas proteins are immunogenic.

N-terminal and C-terminal of a protein:

The end of a protein or polypeptide where the amino group is free in known as N-terminal end and that amino acid whose amino group is free is known as N-terminal amino acid. Sanger's, Edmann's and Dansyl chloride are the reagents used to determine the N-terminal amino acids.



The end of the protein or polypeptide whose carboxylic group is free is known as C-terminal end and that amino acid whose carboxylic group is free in the protein is known as C-terminal amino acid. Hydrazine is used to detect the C-terminal amino acid. While representing a protein on paper, the N-terminal amino acid is written first (on the left) and the C-terminal amino acid is the last one (written at the right side of the paper).

Peptides of physiological importance:

(a) Glutathione:

It is a tripeptide made up of Glu, Cys and Gly. It is found in RBC and other tissues and functions to prevent oxidation of —SH groups of many enzymes.

(b) Bradykinin and kallidin:

These are small polypeptides containing 9 and 10 amino acids respectively. They are formed by partial hydrolysis of plasma protein due to snake poisoning (venom). They are powerful vasodepressors and inhibitors of heart function. Others are tyrocidin, gramicidin, glucagon, insulin, oxytocin, etc.

Derivatives of standard amino acids:

Some polypeptides chain unusual or rare amino acid residues which are derived by the post transcriptional modification from one of the 20 amino acids. The rare amino acids are not coded by DNA.

Some of them are as follows:

1. N-formylmethionine. It is the 1st amino acid of prokaryotic protein.
2. 4-hydroxyproline and 5-hydroxylysine found in collagen fibers in connective tissue.
3. carboxyglutamic acid found as a constituent of blood clotting factor prothrombin.
4. Cystine. The sulfhydryl groups (- SH) of two cysteine resides oxidized to form a cystine residue having disulfide bond (-S -S-). Cystine residues mostly found in extracellular proteins.
5. Acetylation: The N- termini when acetylated, makes the proteins more resistance to degradation.
6. Phosphorylation: Phsophoserine, phosphothreonine and phosphotyrosine are formed by the phosphorylation of their – OH group.

Non protein amino acids:

Over 150 modified amino acids are known that are not components of proteins.

A few examples are as follows:

(a) D-amino acids present in the peptides of bacterial cell wall are defensive in nature as they are not hydrolyzed by peptidases.
(b) Ornithine and citrulline, participate in Urea cycle.
(c) λ-aminobutyric acid (GABA) is a neurotransmitter derived from decarboxylation of glutamate.
{d) Dopamine is also a neurotransmitter derived from tyrosine.
(e) Thyroxin, a thyroid hormone, is a product of tyrosine.
(f) Histamine is a decarboxylation product of histidine. It is a vasodilator, causes allergic response, powerful stimulant of gastric secretion and constrict bronchial smooth muscles.
(g) Azaserine, valinomycin, atinomycin D, gramicidin S and tyrocidine are the useful components of antibiotics.

Properties:

Some of the major properties of amino acids are as follows:

1. Amino acids are colourless crystalline water soluble but largely insoluble in organic solvents. Most α-amino acid have melting points near 300°C whereas their non-ionic derivative usually melt around 100°C.

2. Glutamic acid was the first amino acid formed during the amino acid synthesis. From this all other amino acids are formed by reductive deamination and transamination.

3. Stereoisomerism:

Glycine is the simplest AA where the R-group is a hydrogen atom. Except glycine, all amino acids are asymmetrical (optically active) and exist in two stereo-isomeric forms i.e. L- isomer and D-isomer. Only the a-amino acids are found in proteins, while D-amino acids are rare in nature. Recently 2 free D-amino acids found in mammals, i.e. D-serine in the forebrain and D-aspertate in brain and in PNS.

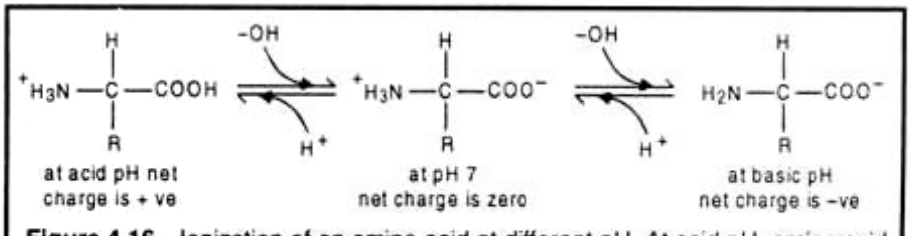


Figure 4.16 Ionization of an amino acid at different pH. At acid pH, amino acid is in cationic form and in neutral pH amino acid is in zwitter ionic form and at basic pH amino acid is in anionic form.

4. Ionization:

Amino acids have two ionizable groups i.e. a-amino and a-carboxyl groups. In addition to these, some amino acids have additional ionizable side-chain group (Asp, Glu, Arg, Lys,). In neutral solution (pH=7.0), amino acids are predominantly zwitterions or dipolar ions, where the α- amino group is protonated ($-NH_3^+$) and the a-carboxyl group is dissociated ($-COO^-$).

Therefore, a- amino acids are ampholytes (amphoteric electrolytes) i.e. can act as both acid (protein donor) and base (proton acceptor). In acid solution (e.g., pH1), only the a-amino group is ionized. In alkaline solution (e.g., pH1), only the a-carboxyl group is ionized. The pH at which an amino acid bears no net electric charge i.e. exist in form of Zwitter ion is called pi or isoelectric pH. pI can be calculated as the midpoint between the pK_1 and pK_2 values.

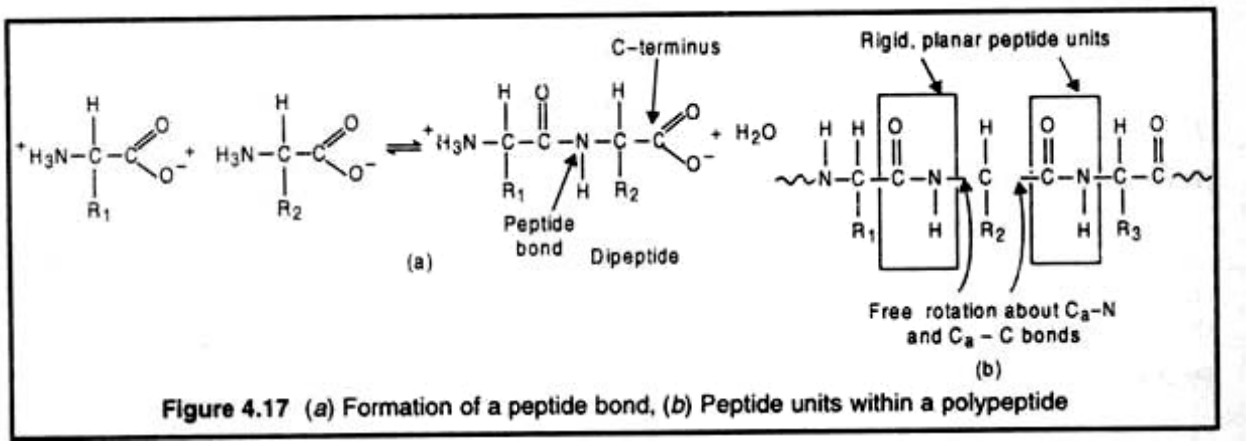


Figure 4.17 (*a*) Formation of a peptide bond, (*b*) Peptide units within a polypeptide

5. pK value :

All amino acids possess at least two weakly acidic functional groups. However, – COOH is several thousand times stronger acid than – NH^{3+}. The pK value is the pH at which these acidic groups are half dissociated. The pK ot- COOH group and – NH^{3+} group are respectively called pK_1 and pK_2. In all the 20 standard amino acids, the value of pK_1 varies from 1.8-2.9 and the pK_2 value varies from 8,

84 -10.78. Aromatic amino acids like tryptophan, tyrosine, histidine and phenylalanine absorb UV-light.

6. Peptide bond formation:

The peptide or amide bond (CO-NH) is a covalent linkage formed by removal of one water molecule between the a-amino group of one amino acid and the a-carboxyl group of another amino acid. After the formation of peptide bond, each amino acid is known as amino acid residue.

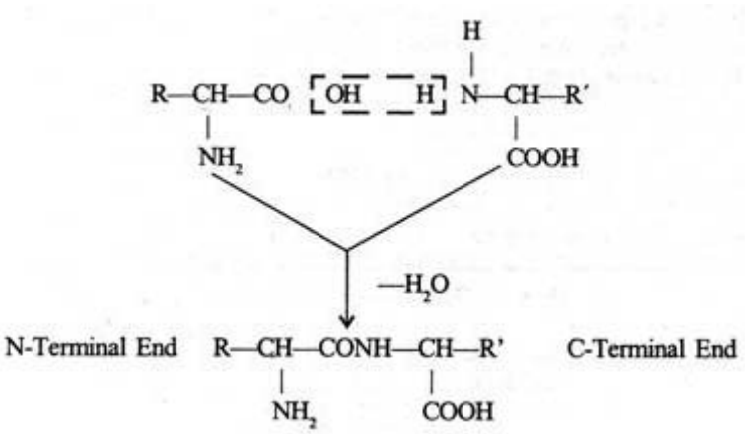
The peptide group is rigid and planer, while the peptide bond has partial double bond character with no rotational freedom. A peptide is a molecule that consists of two or more amino acid residues, e.g., dipeptide (2 amino acid residues), tripeptide (3 amino acid residue) etc. A polypeptide usually contains 20-4000 residues.

The backbone of the polypeptide includes the repeating sequence of carbon atoms and peptide bonds while the side chains of different amino acids responsible for functioning of the protein. A polypeptide with more than 100 residues is generally called a protein. A protein may consist of one or more polypeptide. A peptide is a polar molecule starting with the amino terminus (N-terminus) and ends with the carboxy terminus (C-terminus).

Proteins

Proteins are organic nitrogenous compounds in which a large number of amino acids are joined together by peptide linkages to form long polypeptide chains. Peptide-linkage (—CONH—) is formed when amino group (—NH_2) of one amino acid condenses with carboxylic group (—COOH) of another amino acid eliminating one molecule of water.

That end of the polypeptide chain where the —COOH group of the amino acid is not involved in peptide linkage is called as C-terminal end. The other end of the polypeptide chain with amino acid having free —NH_2 group is called as N-terminal end.



Although there may be hundreds of amino acids in a single polypeptide chain but fundamentally there are only about 20 different types of amino acids that constitute proteins in plants (there may be repetition of amino acids continuously or at intervals in the polypeptide chain).

Because of their very large size the proteins are often called as gigantic molecules or macromolecules of the cells. Their molecular weight may range from few thousands to over a million (10 lakhs.)

Structure of the Proteins:

The structure of the proteins can be studied under the following heads:

(1) Primary Structure of the Proteins:

Specific sequence or the arrangement of amino acids in the polypeptide, chain constitutes the primary structure of the proteins (Fig. 9.25).

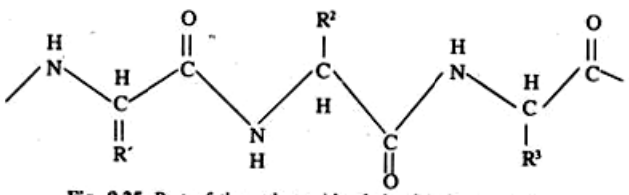


Fig. 9.25. Part of the polypeptide chain showing arrangement of amino acids.

(2) Secondary Structure of the Proteins :

Three types of helical structures are found:

(a) Alpha helix

(b) Beta pleated and

(c) Reverse turn.

1. Alpha helix:

α means the first and the structure described below was the first among the helical structures to be discovered, hence known as alpha (α) helix.

Polypeptide chain of the protein molecule is held in a coiled or helical shape by hydrogen bonds which are established in between the peptide linkages. The coiled or helical shape of polypeptide chain constitutes the α-helix or secondary structure of the protein (Fig. 9.26).

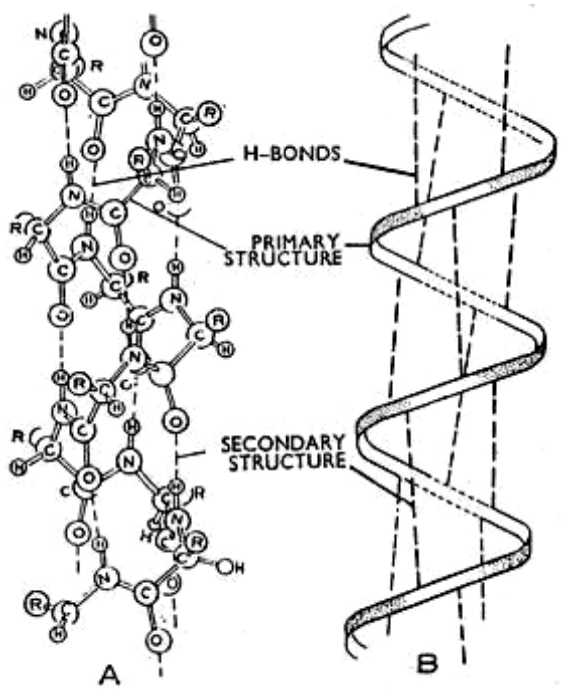


Fig. 9.26. α-helix structure of a protein (A) Molecular (B) Diagrammatic.

Although hydrogen bonds are very weak but when they are present in very large number all along the backbone of the polypeptide chain, they reinforce one another to stabilize the helical structure.

In a typical helical protein,

i. Each NH group (of peptide bond) is connected to a C=O group (of another peptide bond) by a H-bond at a distance equivalent to 3 amino acid residues.

ii. An α-helix or complete turn of a coil contains about 3.67 amino acid residues.

iii. The pitch of the helix is 5.4 Å (vertical distance along the axis from any point on the helix to a corresponding point directly above it is called as the pitch of the helix).

iv. Therefore, each amino acid is about 1.5 Å (5.4/3.6) distant from the next amino acid residue.

Besides a-helix structure, other types of secondary structures of proteins also occur. Among these, β-conformations (P-pleated sheets) are most common that are found in fibrous proteins called β-keratins. In β-pleated sheets, a number of polypeptide chains (which do not form helices) are cross linked with H-bonds in which amino acid to carboxyl terminal orientation may be in the same direction (parallel) or inverse (antiparallel).

Linus Pauling and Robert Corey are credited with doing pioneer work on peptide bond and organization of proteins. They even predicted the existence of secondary structures of proteins many years before the first complete structure of protein was elucidated.

The salient features of this structure are as under:

i. Here the polypeptide is twisted or coiled to form a right handed helical structure.

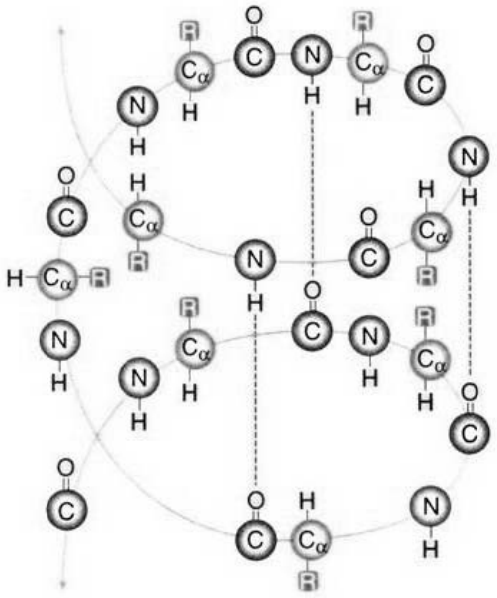
ii. The distance between each turn of the coil is 5.4 Å.

iii. There are 3.6 amino acids per turn.

iv. The 'R' groups are seen protruding out of the helix.

v. There are intra chain hydrogen bonding, wherein the hydrogen of —NH group combines with oxygen of -CO group of the 4th amino acid behind it. So every peptide group participates in hydrogen bonding.

vi. This type of structure is found in many proteins in combination with other structures. Pure a-helix structure is seen in hair protein, i.e., keratin.



2. Beta pleated:

β means the second and the structure described below was the second discovery after α helix.

The salient features of this structure are:

i. Here the chain is not helical but zigzag.

ii. The distance between each turn is 7 Å.

iii. Polypeptide chains are arranged side by side in the form of pleats.

iv. There is inter-chain hydrogen bonding between the chains and each peptide group participates in hydrogen bonding.

The chains are anti-parallel to each other.

3. Reverse turn:

Folds back on itself in reverse direction of the chain.

(3) Tertiary Structure of the Proteins:

The coiled (α-helix) polypeptide chain is further folded in various ways. This folding which is very specific for a particular protein constitutes its tertiary structure and is determined by its primary structure (Fig. 9.27).

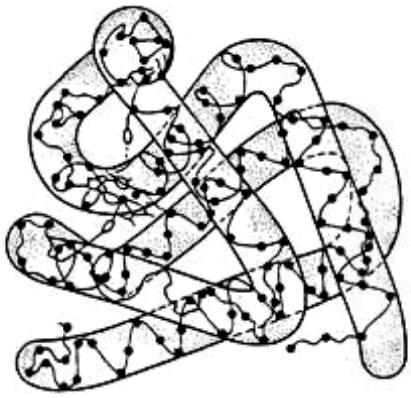


Fig. 9.27. Diagrammatic representation of the tertiary structure of a protein (sperm whale myoglobin).

(Tertiary structure of the proteins is essential for biologically active proteins i.e., the enzymes. They are rendered useless (or are denatured) if their tertiary structure is lost).

The tertiary structure of the protein is stabilized by the following forces which have also been shown in Fig. 9.28.

i. H-bonds (other than those established between the peptide linkages).

ii. Di-sulphide (S-S) bonds.

iii. Ionic bonds or salt linkages.

iv. Steric Effects i.e., the interaction of non-polar side chains caused by the mutual repulsion of the solvent.

v. Van der Waals forces.

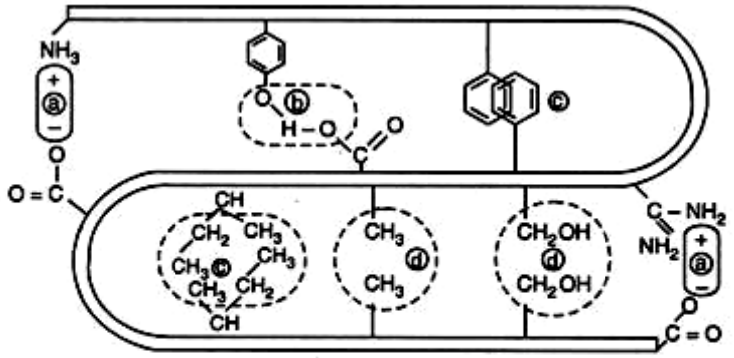


Fig. 9.28. Some types of non-covalent bonds which stabilize protein structure (*a*) electrostatic interaction (*b*) hydrogen bonding between tyrosine residues and carboxylate groups on side chains (*c*) interaction of non-polar side chains caused by the mutual repulsion of solvent (*d*) Van der Waals interactions.

1. Hydrogen bonds:

Formed between hydrogen and an electronegative atom like oxygen or nitrogen in the 'R' group of amino acids.

2. Ionic interactions:

Formed between acidic (glutamic and aspartic) and basic (arginine, lysine or histidine) amino acids.

3. Disulphide bonds:

This is a strong bond formed between the sulphahydryl groups of two cysteine amino acids. The resultant dimer structure formed is known as cystine (an amino acid found in proteins only and not in free form).

4. Hydrophobic interactions:

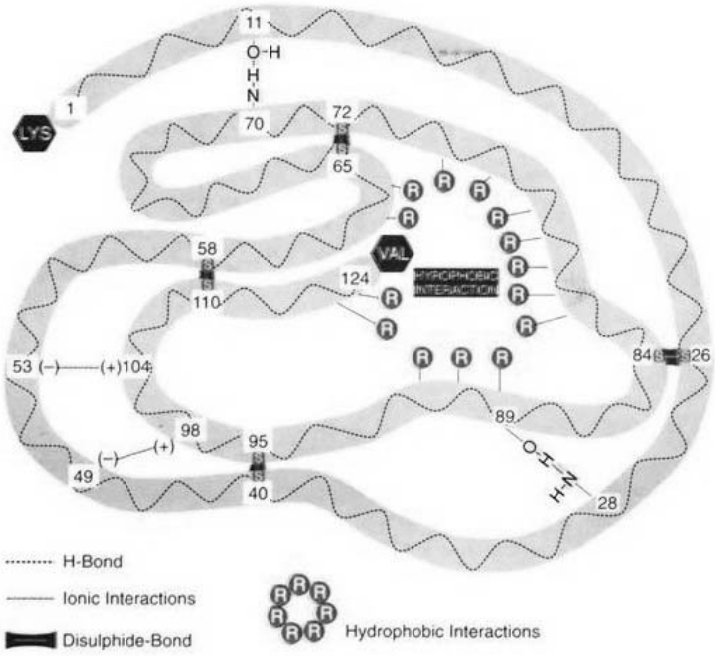
The 'R' groups of the hydrophobic amino acids aggregate together in the centre away from water, thereby developing a force of attraction between each "R" group and a force of repulsion from the water and these interactions are known as hydrophobic interactions.

(All the molecules exert a week force of attraction upon one another due to mutual interaction of their electrons and nuclei. There is a electrostatic attraction between the electrons of one molecule and the nuclei of the other while on other hand, there is electrostatic repulsion of nuclei and electrons of the molecule by the nuclei and electrons of the other molecule respectively. The resultant week force of attraction between the two molecules is known as the Van der Waals attraction or Van der Waals force. The energy of such forces is about 1 k.cal/mole).

(4) Quaternary Structure of the Proteins:

Sometimes more than one polypeptide chains are associated together to form a relatively more stable super molecule of protein. This constitutes the quaternary structure of the protein.

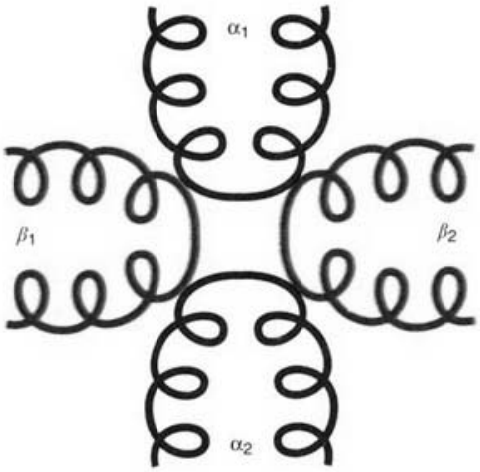
For example, in blood haemoglobin there are four polypeptide chains or subunits that constitute the protein.



Quaternary structure is maintained by various forces like di-sulphide-linkages, H-bonds etc., between the different polypeptide chains of the protein.

Oligomeric proteins:

Are those which have two or more polypeptide chains.



Quaternary structure refers to the type of arrangement of the polypeptides in an oligomeric protein. These polypeptides are held together by either hydrogen bonds, ionic bonds or Vander Waals' forces, e.g., Hemoglobin has four polypeptide chains which are arranged in a particular fashion that is referred to the quaternary structure of hemoglobin.

The Quaternary structure of hemoglobin describes that it is made up of four polypeptide chains; two of which are α (α_1 & α_2) and the other two are β (β_1 & β_2). The two alpha chains are opposite to each other and adjacent to each β-chain. The α chains and the β chains are linked together by salt bridges.

Structure function relationship in proteins:

Hemoglobin plays a vital role in transport of oxygen from the lungs to the peripheral tissues and transport of carbon dioxide from the tissue to the lungs.

There are three types of normal hemoglobin with the following polypeptides:

(1) Adult hemoglobin (Hb A) has 2α2β chains.
(2) Foetal hemoglobin (Hb F) has 2α2γ chains.
(3) Minor adult hemoglobin (Hb A_1) has 2α2δ chains.

The number of amino acids in α chains is 141 amino acids and the other chains, i.e., β, γ & δ chains have 146 amino acids. These chains are differentiated based upon the difference in the sequence of arrangement of the amino acids in the chains. The quaternary structure of hemoglobin creates a cavity in between the tetramer in which 2, 3, diphosphoglycerate (DPG or BPG) is present forming a salt bridge with the amino terminal of β-chain that stabilizes the hemoglobin thereby lowering the affinity to oxygen.

In the lungs, the partial pressure of oxygen is high which results in binding of O_2 to one of the chains of Hb thereby rupturing the salt bridges between the four subunits. Subsequent oxygen binding (sigmoid curve of Hb-O_9 association) is facilitated by rupture of the salt bridges altering secondary, tertiary and quaternary structures thus allowing rotation of one α/β subunit with respect to another α/β chain thereby compressing the tetramer and release of DPG. This results in increasing its affinity towards oxygen (the R state of Hb).

In the peripheral tissues, CO_2 binds with the a-amino group of the amino terminal with its conversion from positive to negative charge which favours salt bridge formation between the polypeptide chains with return to the deoxy state (T-state), i.e., release of oxygen from Hb. Release of O_2 from the Hb is also facilitated by binding of DPG to the tetramer.

When a person takes off on a flight, the aero plane slowly rises in altitude resulting in lowering of the O_2 tension due to which oxygenation of Hb is not possible. Thus the person feels hypoxic, but the physiological mechanism of the body starts decreasing the production of DPG, due to which Hb can bind the oxygen even at lower pressure of oxygen.

Therefore, when the aero plane reaches the maximum altitude and stays stable, the person feels comfortable. When Hb reaches the tissues DPG level increases enhancing release of oxygen. Similarly, the above process reverses, when a person dives deep into the sea. The high O_2 pressure results in increased production of DPG facilitating oxygenation of Hb in the lungs and deoxygenating in the peripheral tissues.

Classification of Proteins:

Proteins are classified based upon:

(1) Their solubility and

(2) Their structural complexity.

A. Classification Based upon Solubility:

On the basis of their solubility in water, proteins are classified into:

1. Fibrous proteins:

These are insoluble in water. They include the structural proteins. They have supportive function (e.g., collagen) and/or protective function (e.g., hair keratin and fibrin).

2. Globular proteins:

They are soluble in water. They include the functional proteins, e.g., enzymes, hemoglobin, etc.

B. Classification Based upon Structural Complexity:

On the basis of their structural complexity they are further divided into:

(1) Simple

(2) Conjugated and

(3) Derived proteins.

1. Simple proteins:

Proteins which are made up of amino acids only are known as simple proteins.

They are further sub-divided into:

(a) Albumins:

They are water soluble, heat coagulable and are precipitated on full saturation with ammonium sulphate, e.g., serum albumin, lactalbumin and ovalbumin.

(b) Globulins:

They are insoluble in water, but soluble in dilute salt solutions. They are heat coagulable and precipitate on half-saturation with ammonium sulphate, e.g., serum globulin and ovo-globulin.

(c) Glutelins:

They are insoluble in water and neutral solvents. Soluble in dilute acids and alkalies. They are coagulated by heat, e.g., glutelin of wheat.

(d) Prolamines:

Water insoluble but soluble in 70% alcohol, e.g., gliadin of wheat, proteins of corn, barley, etc.

(e) Histories:

Water soluble, basic in nature due to the presence of arginine and lysine, found in nucleus. They help in DNA packaging in the cell. They form the protein moiety of nucleoprotein.

(f) Protamine's:

Water soluble, basic in nature, not-heat coagulable. Found in sperm cells, hence component of sperm nucleoprotein.

(g) Globin's:

They are water soluble, non-heat coagulable. e.g., globin of haemoglobin.

(h) Albuminoids or scleroproteins:

Insoluble in all neutral solvents, dilute acids or alkalies, e.g., keratin of hair and proteins of bone and cartilage.

2. Conjugated proteins:

Proteins which are made up of amino acids and a non-amino acid/protein substance called the prosthetic group are known as conjugated proteins.

The various types of conjugated proteins are:

(a) Chromo proteins:

Here the non-protein part is a coloured compound in addition to the protein part. Ex. Haemoglobin has heme as the prosthetic group and cytochromes also have heme.

(b) Nucleoproteins:

These proteins are bound to nucleic acids, e.g., chromatin (histones + nucleic acids).

(c) Glycoproteins:

When a small amount of carbohydrate is attached to a protein it is known as glycoproteins, e.g., mucin of saliva. (Note: Glycoproteins have major amounts of protein and some amount of carbohydrates and proteoglycans contain major amounts of carbohydrates and little amount of proteins).

(d) Pbosphoprotein:

Phosphoric acid is present with the protein. Ex. Milk casein and egg yolk (vitellin).

(e) Lipoproteins:

Proteins in combination with lipids, e.g., LDL, HDL.

(f) Metalloproteins:

They contain metal ion in addition to the amino acids, e.g., hemoglobin (iron), ceruloplasmin (copper).

3. Derived proteins:

They are the proteins of low molecular weight produced from large molecular weight proteins by the action of heat, enzymes or chemical agents.

Proteins → Proteans → Proteoses → Peptones → Peptides → Amino acids

Depending upon the nature of the prosthetic group associated with them, conjugate proteins are classified as follows:-

1. Nucleoproteins (or Histones):

These are associated with nucleic acids.

2. Glycoproteins:

These are associated with some carbohydrate.

3. Chromoproteins:

These proteins are associated with some colouring matter e.g., chlorophylls, carotenoids, phycobillins etc.

4. Lipo-proteins:

These are associated with some lipid or fatty substances. (They are chiefly found in cy- to membranes).

5. Iron-porphyrin Proteins:

These are associated with iron-porphyrin compounds e.g., the cytochromes.

6. Simple Metal Containing Proteins:

These proteins are associated with some metal directly e.g., ferredoxin where Fe atoms are directly attached to the protein molecule.

7. Flavoproteins:

These are associated with some flavin compound e.g., FAD (Flavin Adenine-Dinucleotide).

Properties of Proteins:

1. Denaturation:

Partial or complete unfolding of the native (natural) conformation of the polypeptide chain is known as denaturation. This is caused by heat, acids, alkalies, alcohol, acetone, urea, beta- mercaptoethanol.

2. Coagulation:

When proteins are denatured by heat, they form insoluble aggregates known as coagulum. All the proteins are not heat coagulable, only a few like the albumins, globulins are heat coagulable.

3. Isoelectric pH (pH^1):

The pH at which a protein has equal number of positive and negative charges is known as isoelectric pH. When subjected to an electric field the proteins do not move either towards anode or cathode, hence this property is used to isolate proteins. The proteins become least soluble at pH^I and get precipitated. The pH^I of casein is 4.5 and at this pH the casein in milk curdles producing the curd.

4. Molecular Weights of Proteins:

The average molecular weight of an amino acid is taken to be 110. The total number of amino acids in a protein multiplied by 110 gives the approximate molecular weight of that protein. Different proteins have different amino acid composition and hence their molecular weights differ. The molecular weights of proteins range from 5000 to 10^9Daltons. Experimentally the molecular weight can be determined by methods like gel filtration, PAGE, ultra centrifugation or viscosity measurements.

Nucleic acids

In 1868 Meischer isolated a substance from the nucleus of pus cells. By digesting pus cells in HC1, he obtained a pure material and named it nuclein. Nuclein had strong acidic properties and contained considerable amount of phosphorus.

In 1889 Altmann coined the term nucleic acid. Later existence of two types of nucleic acid, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) was established.

The present knowledge about the structure of DNA is the result of research by great scientists like Chargaff, Stendel, Levene, Todd, Wilkins, Rosalind. Franklin, Astbury, Watson, Crick, Hargobind Khorana and many others.

All living organisms possess, nucleic acids. Nucleic acids are macromolecules of utmost biological importance. Nucleic acids possess all the information needed for an organism's cell structure, function, development and reproduction.

DNA:

DNA is mainly present in the chromosomes in the nucleus. A small amount of DNA is present in the mitochondria and chloroplasts. Mitochondria and chloroplasts are self- replicating bodies.
DNA molecule occupies central position among biological macromolecules. It is a storehouse of genetic information. Nucleotides sequences of DNA encode proteins and enzymes, which directly or indirectly control the synthesis of all cellular components.
DNA has an excellent mechanism for stable storage of genetic information. The enzymes that synthesize DNA, faithfully copy DNA molecules containing millions of bases. They perform this function with high degree of accuracy and speed. DNA is the only macromolecule for which repair mechanism exists if any damage is caused to it.

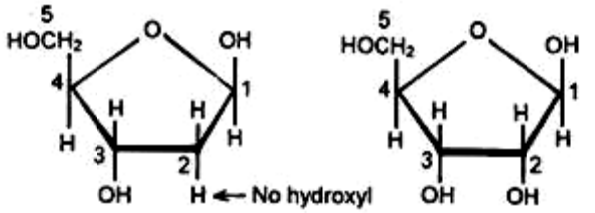
Structure of DNA:

DNA molecules are largest molecules of the cell, much larger than those of proteins. DNA is found in all animal and plant cells, prokaryotes and most of the viruses. In addition, it is also found in mitochondria and chloroplasts.

Chemical Composition of DNA:

DNA is made up of numerous monomer units called deoxyribonucleotides. Each deoxyribonucleotide consists of three components.
1. Pentose sugar – deoxyribose
2. A nitrogenous base – Purine or Pyrimidine
3. Phosphorus molecule.

Pentose sugar:
In DNA, the pentose sugar is deoxyribose. A similar pentose sugar ribose is present in RNA. The only difference between the two is the absence of hydroxyl group at position number two of the sugar ring in DNA. Therefore, it is called 2′-deoxyribose. This makes DNA more stable. Deoxyribose can be stained with Feulgen stain.



Deoxyribose
$C_5H_{10}O_4$

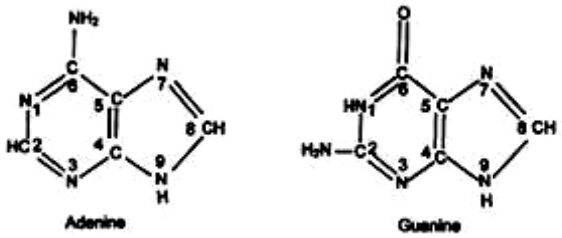
Ribose
$C_5H_{10}O_5$

Nitrogen Bases:

Nitrogen bases in DNA are of two kinds, Purine and Pyrimidine.

Purine:
Purines are dicyclic and have fused five and six member rings. Purines are of two kinds, Adenine (A) and Guanine (G).

Pyrimidines:
Pyrimidines are monocyclic and have a six member ring. Pyrimidines are of two kinds – Thymine (T) and Cytosine (C).
In RNA, thymine is replaced by uracil (U). The only difference between uracil and thymine is the presence of a methyl subunit at position C-5 in thymine.



Adenine Guanine

Fig. 3.2

In this way DNA consists of four bases A, G, C, T and RNA has A, G, C, U.

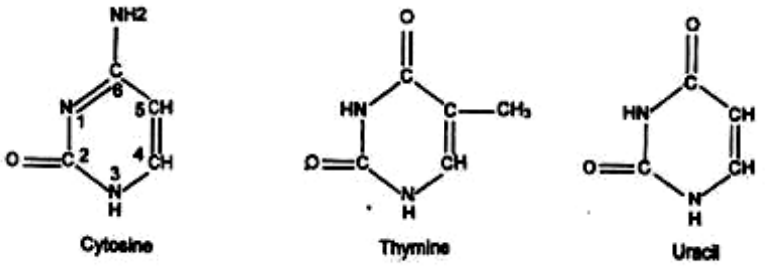


Fig. 3.3

The nitrogen base is linked to the sugar molecule by a glycosidic bond. The bond is formed between the first carbon atom of the sugar and nitrogen at position 1 in the case of pyrimidine and at position 9 in the case of purine.

Phosphate Group:

Phosphate group is attached to the 5′-carbon of deoxyribose sugar of one nucleotide and 3′-carbon of the deoxyribose sugar of next nucleotide. Phosphate group provides strong negative charge to the nucleic acid. The bond between phosphate and deoxyribose sugar is phosphodiester bond.

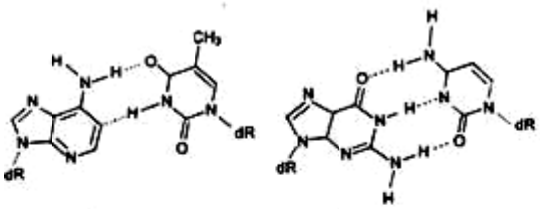


Fig. 3.4 (a)

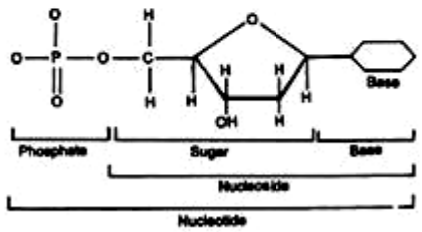


Fig. 3.4. (b)

Nucleosides and Nucleotides:

Within the structure of the nucleic acid, a nitrogen base is linked to sugar to form nucleoside. Nucleosides are called deoxyribouncleosides. Four kinds of nucleosides are formed in DNA.

Deoxyribose + Adenine → Deoxyadenosine

Deoxyribose + Guanine → Deoxyguanosine

Deoxyribose + Cytosine → Deoxycytidine

Deoxyribose + Thymine → Deoxythymidine

Purine nucleosides are suffixed by sine while pyramidine nucleosides are suffixed by dine.

Nucleotides:

Nucleotides are phosphoric acid esters of nucleosides with phosphorous at position C-5 of the sugar of one nucleoside and at C-3 of the next nucleoside. Nucleoside is joined to phosphorus by phosphodiester bands.

Base	Ribonucleoside	Deoxyribonucleoside
Adenine	Adenosine	Deoxyadenosine
Guanine	Guanosine	Deoxyguanosine
Cytosine	Cytidine	Deoxycitidine
Uracil	Uridine	—
Thymine	—	Thymidine (since only the deoxyriboside of thymine) occurs naturally)

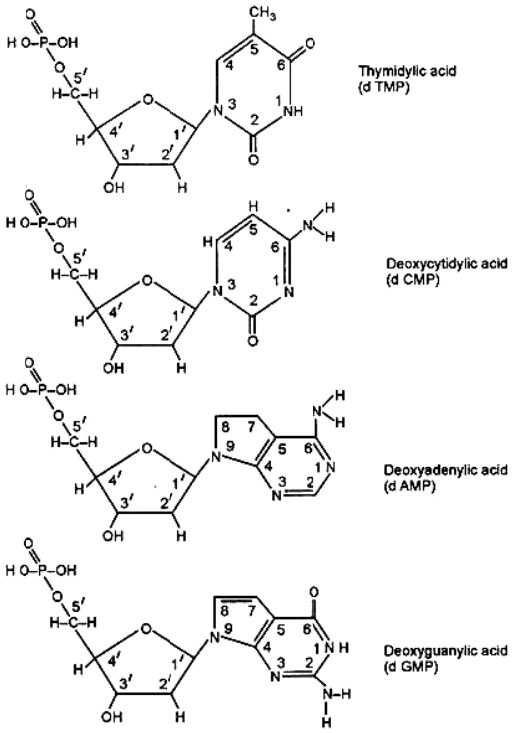


Fig. 3.5. The four deoxyribonucleotides found in DNA. The ring positions of bases are designated by plain numbers.

TABLE 3.2. The bases and nucleotides usually found in RNA and DNA

Base	RNA nucleotide	DNA nucleotide
Adenine (A)	Adenylic acid or Adenosine monophosphate (AMP)	Deoxyadenylic acid or deoxyadenosine monophosphate (dAMP)
Guanine (G)	Guanylic acid or guanosine monophosphate (GMP)	Deoxyguanylic acid or deoxyguanosine monophosphate (dGMP)
Cytosine (C)	Cytidylic acid of cytidine monophosphate (CMP)	Deoxycytidylic acid or deoxycytidine monophosphate (dCMP)
Thymine (T)	–	Thymidylic acid or Thymidine monophosphate
Uracil (U)	Uridylic acid or uridine monophosphate (UMP)	–

Polymerization:

Both strands of DNA are polynucleotide strands. Nucleotides undergo polymerization to form long chain of nucleotides. Nucleotides are repeating units. They are joined end to end to form chain or strands. Adjacent nucleotides are linked to each other – phosphodiester bonds between 5′-C of one nucleotide and 3′-C of the next nucleotide.

Each strand may possess thousands to millions of nucleotides within a single macromolecule. Each – DNA strand has free 5′-phosphate at one end and free 3′-hydroxyl at the other end.

Phosphodiester linkages provide polarity to DNA. Each strand has a polarity. One polynucleotide chain is written 3′ → 5′ direction upward or 5′ → 3′ direction downward. The other strand is antiparallel By convention the sequence of nucleotides is written in 5′ → 3′ direction from the left.

Nucleotides are building blocks of nucleic acids. Alternating series of sugar-phosphate- sugar molecules form the backbone of the each strand linked by phosphodiester bonds. During nucleic acid synthesis, the chain elongation takes place by addition of nucleotides one by one.

The 5′-end of the triphosphate of one nucleotide reacts with 3′-OH group of sugar of the next nucleotide. A phosphodiester bond is formed between first phosphate of incoming nucleotide with 3′-OH of the sugar of next nucleotide. The other two phosphate groups are released as the single pyrophosphate molecule.

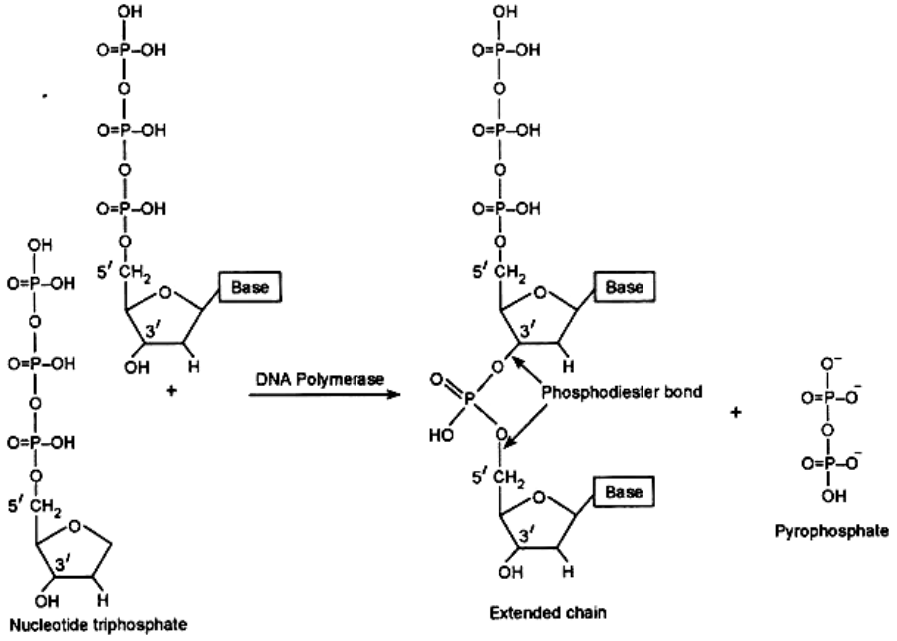


Fig. 3.6. Polymerisation reaction catalysed by DNA polymerase. Nucleotides are added to the 3′ end of the DNA chain since the DNA polymerase has the absolute requirement of a free 3′ OH in the chain to which it adds new bases.

Arthur Korenberg demonstrated that nucleotide building blocks for DNA are energy rich d ATP, *d*GTP, *d*CTP and *d*TTP.

Order of bases along polynucleotide chain is irregular. This unique irregularity along with long length is the basis for the enormous information stored in DNA. Thus arrangement of bases is specific for every gene.

Genetic Information in DNA:

The genetic information in DNA is conveyed by the sequence of four nucleotides. The genetic specificity exists in the linear sequence of four nucleotide building blocks. The four bases A, G, T and C can carry infinite number of genetic messages (4^N).

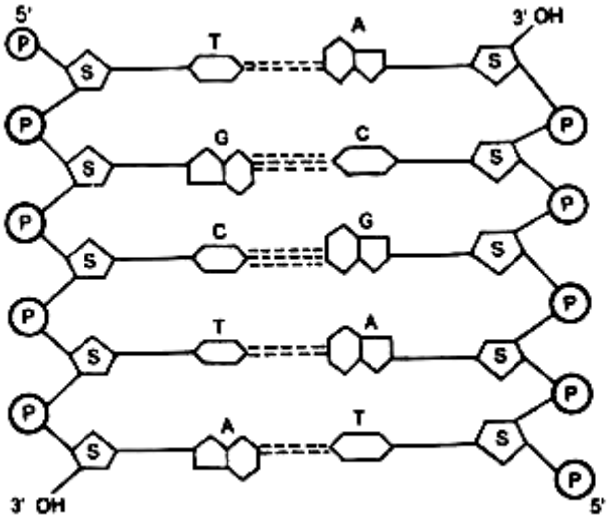


Fig. 3.6. Double polynucleotide strands of DNA.

Chargaff Equivalence Rule:

In 1950 Erwin Chargaff analysed and measured the base composition of DNA from different organisms by using paper chromatography. Exact ratios of of the four nucleotides vary from species to species. He discovered that in all DNAs the amount of purine was equal to the amount of pyrimidine. Thus A + G = C + T. Further, the amount of Adenine (A) was equal to the amount of thymine (T) and amount of cytosine (C) was equal to the amount of guanine (G).

However, A + T/G + C ratio varies from species to species. A + T/G + C ratio in human sperm is 1.62, in yeast it is 1.79 and in bacteriophages T_2 it is 1.86. In higher plants and animals generally A – T composition is high whereas in lower plants and animals G – C composition is high.

X-Ray crystallographic studies of DNA:

By X-ray crystallographic studies of DNA, Astbury gave three dimensional structure of DNA. Based on X-ray diffraction data provided by Wilkins, Rosalind Franklin and others, now famous pair of American scientist James Watson and English scientist Francis Crick proposed a model for DNA structure in 1953 in the journal Nature.

Watson and Crick were awarded Nobel Prize for this in 1962. They shared the prize with Wilkins who investigated X-ray diffraction photographs of DNA. Rosalind Franklin missed the Nobel prize because of her death in 1958.

This DNA model called double helix DNA model was a landmark research in the field of biology. It provided explanation for base composition, DNA replication and its biological properties.

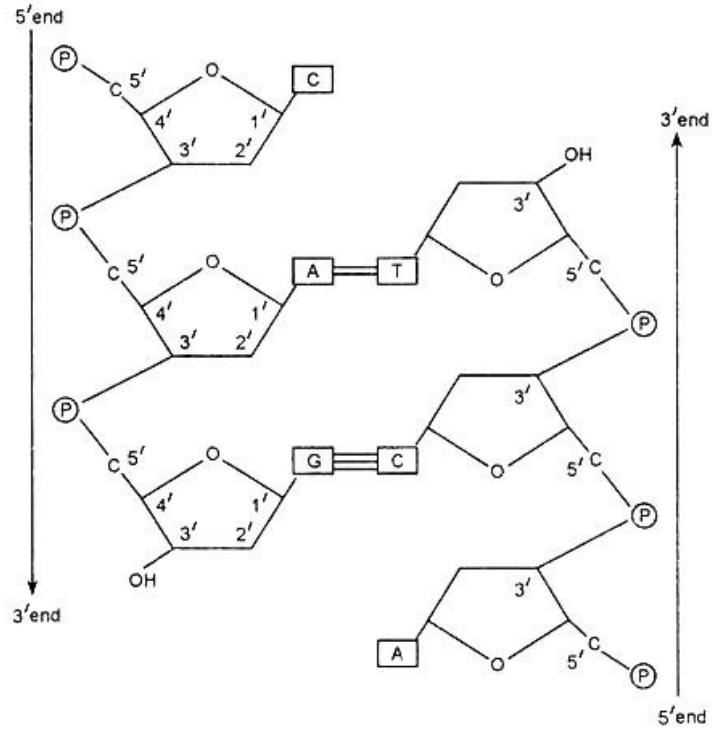


Fig. 3.7. Representation of the DNA double helix showing opposite polarities of the sugar-phosphate linkages in two strands.

Double helix structure of DNA:

Watson and Crick model of DNA has the following characteristic features:

DNA molecule consists of two polynucleotide strands twisted around each other to form a double helix. The two polynucleotide chains are held together by weak, non-covalent bonds between pairs of bases.

Each nucleotide of a polynucleotide chain consists of phosphate-sugar-base components. Each strand is made up of alternating sugar and phosphate molecules. Bases lie in pairs perpendicular to the axis of the helix. Base pairs are stacked above each other like steps of the staircase.

In double helix molecule of DNA, adenine always pairs with thymine and cytosine with guanine. According to Watson and Crick model hydrogen bonds between nitrogen bases bind

the two polynucleotide strands. Adenine is bonded to thymine by two hydrogen bonds A = T and guanine is bonded to cytosine by triple hydrogen bonds C = G.
These hydrogen bonds are weak and can easily break. This enables the two strands to separate easily. Hydrogen bonds between complementary bases contribute to thermodynamic stability of the helix and specificity of base pairing.

(1) A DNA molecule is made up of two complementary polynucleotide chains that are oriented in opposite polarity $\begin{matrix} 5' \rightarrow 3' \\ 3' \leftarrow 5' \end{matrix}$ to each other (Fig. 3.7, 3.8).

(2) Adenine (A) of one chain pairs with thymine (T) of the other, while guanine (G) of one chain pairs with cytosine (C) of the other. Thus both polynucleotide chains are complementary to each other. Thus DNA double-helix gives a quantitative relationship in which (A + G) = (C + T) or A + G/C + T = 1.

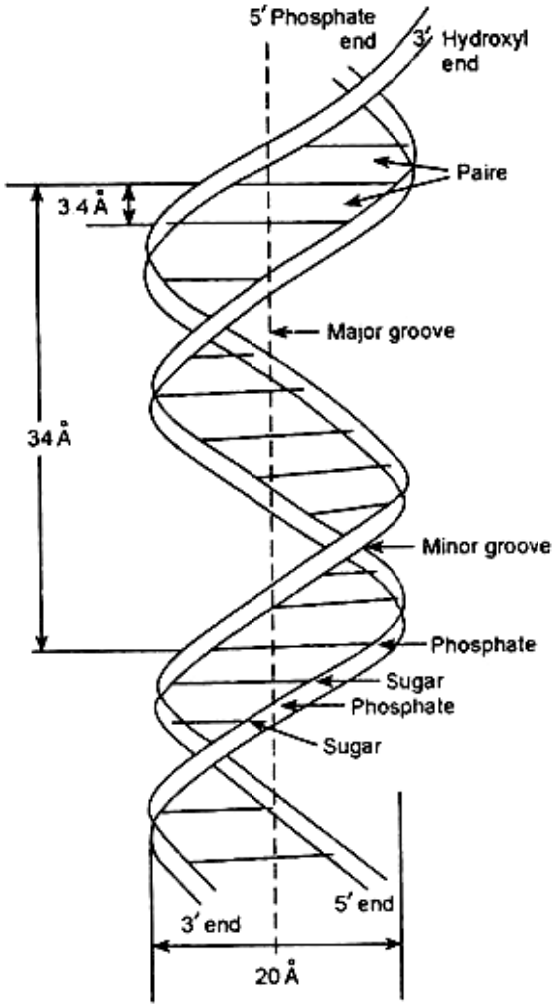


Fig. 3.8. The Watson Crick model of the DNA molecule. The phosphate sugar backbone is shown as long ribbon. Distance between two nucleotides is 3.4 Å and a complete turn of the double helix (pitch) takes 34 Å. The dashed line indicates the imaginary axis.

Two strands of double helix have complementary sequences:
As the adenine of one strand always pairs with thymine of the other strand and cytosine of one strand always pairs with guanine of the other strand, bases of one strand are complementary to the bases of the other strand. The sequence of bases of one strand dictates the base sequence of the other strand. This is known as Watson-Crick base pairing rule.

To have a constant diameter of DNA double helix, the adenine-thymine and cytosine- guanine pairs are the only ones that can fit the physical dimensions of the helical model. Because purine-purine pair (dicyclic) will be too thick and pyrimidine-pyrimidine pair (monocyclic) too thin.

```
5′   A T C T G A G A A G C 3′ one strand
     ‖ ‖ ||| ‖ ||| ‖ ||| ‖ ‖ ||| |||
3′   T A G A C T C T T C G 5′ complementary strand
```

Following are the other important properties revealed by X-ray diffraction data:

1. The diameter of the helix is 20A or 2 nm. It means the distance between two polynucleotide strands is 20A (1 nm = 10 Angstrom units).
2. The distance between two adjacent base pairs is 3.4 A or 0.34 nm.
3. One complete turn of the helix i.e., 360° takes 34 A or 3.4 nm length of DNA. In this way there are 10 base pairs in one complete turn.
4. Two strands or polynucleotide chains of a helix run in opposite directions, therefore they are antiparallel. One strand has 5′ → 3′ polarity while the other has 3′ → 5′ polarity.

In this way 10 base pairs make one complete turn of 360°. Helical coiling of two strands around the common axis is right handed. Such a DNA is called B-DNA.

The twisting or coiling makes alternating minor and major grooves. The major groove is rich in chemical information and allows regulatory proteins to bind to specific sequences on DNA.

Types of DNA:

DNA is not a rigid molecule. It can exist in different forms depending upon different conditions.

B-Form DNA (B-DNA):

Most of the double helix DNA in the cell has right handed coiling (clockwise) and is called B-form DNA. It has 10 base pairs per turn. Base pairs lie perpendicular to the axis of the helix. It is most stable configuration. When humidity is high (92%) and concentration of ions is low, DNA exists in B-Form. The B-Form of DNA is stable but can change to A, C and D form depending upon the concentration of excess salts and sequence of nucleotides.

A-Form DNA (A-DNA):

A form DNA is also right handed helix. It exists at 75% humidity in the presence of Na^+, K^+ ions. There are eleven base pairs per turn, which tilt from the axis of helix by 20.2°. It can quickly change to D-form.

C-Form DNA (C-DNA):

C-form of DNA is found at 66% relative humidity in the presence of lithium (Li^+) ions. Number of base pairs is 9.33 per turn. The base pairs show a negative tilt of 7.8°.

D-Form DNA (D-DNA):

It is rarely found. There are eight bases per turn. Base pairs show a negative tilt of 16.7°.

Z-Form DNA (Z-DNA):

It is a left handed (sinistral) coiling double helix. The sugar- phosphate backbones are zigzag instead of regular helix. That is why it is called Z-Form. It has 12 base pairs per turn.

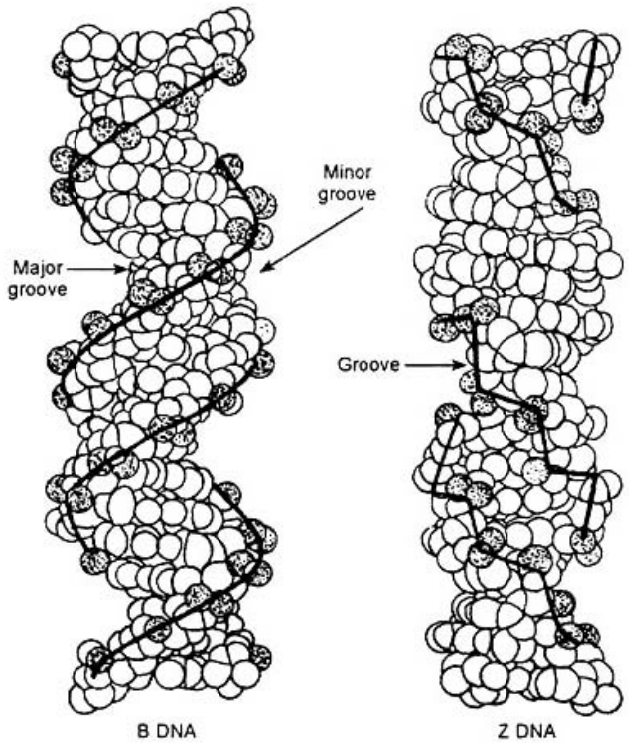


Fig. 3.10. B and Z forms of DNA double helices

The Z-form DNA makes one complete turn at 45A as compared to 34 A in B-DNA.

It has 18 A diameter therefore it is thin. Z-form DNA is assumed to play a role in gene regulation. Z-form is found in large number of living organisms including mammals, protozoans and plants.

TABLE 3.4. Chief distinguishing Characteristics of B-DNA and Z-DNA

Characteristic	B-DNA	Z-DNA
1. Helix	Right-handed	Left-handed
2. Sugar-phosphate backbone	Regular	Zig-Zag
3. Repeating unit	Mononucleotide	Dinucleotide
4. Helix pitch (turn)	34 Å	45 Å
5. Base pairs per turn of helix	10.4	12 (6 dinucleotides)
6. Axis rises per base pair	3.4 Å	3.7 Å
7. Diameter of helix	20 Å	18 Å
8. Angle of turn per base pair	36°	60°

Single Stranded DNA (ssDNA):

All organisms contain double stranded DNA (ds DNA) except a few viruses such as bacteriophage φ x 174, Provirus phage fd which contains single strand DNA (ds DNA). This single strand DNA is a circular molecule. It becomes double stranded only at the time of replication.

Denaturation and Renaturation:

Because the structure of DNA double helix is kept intact by weak hydrogen bonds, it is possible to separate the two strands. The separation of two strands is called melting or denaturation of DNA. The two hydrogen bonds of A = T can be broken more easily than the three bonds of C = G which require higher temperature. Therefore the temperature at which the two DNA strands become separate depends upon AT/CG ratio. Therefore G : C base pairs contribute more to the stability of DNA than A: T pair.

Similarly, at higher salt concentration of the solution, DNA denatures at higher temperature. This is because two DNA strands contain phosphate groups, which carry a negative charge. These negative charges of two strands repel each other causing their separation. At high salt concentration, the negative charges are shielded by cations, thus providing stability to the helix. Therefore, at low ionic strength, the helix is less stable.

When the temperature is brought down, the complementary strands again form hydrogen bonds to restore original double helix. This process is called renaturation or annealing. It helps to estimate the size of DNA molecule. Time taken to anneal depends upon the length of DNA molecule.

The size of a genome can be estimated by denaturation of DNA and allowing it to re-aneal. The speed of reassociation can be measured in cot units which is concentration x time.

Functions of DNA:

DNA is the genetic material. DNA molecules possess all the information needed for an organism's cell structure, function, development and reproduction.

It performs the following very important functions:

1. Inheritance:

DNA which is the genetic material carries genetic information from cell to cell and to the next generation. This function is performed by DNA replication. It forms exact copies of itself. During DNA replication the two strands separate and each strand functions as a template for the new strand.
In this way one molecule of DNA gives rise to two daughter molecules of DNA both identical to the parent molecule. Each daughter molecule has two strands, one parental (template) and the other new complementary strand.

2. Synthesis of proteins and enzymes:

DNA is transcribed into mRNA and other RNAs. The information contained in the sequence of bases (triplet codons) is translated into proteins and enzymes.

3. DNA molecule is capable of variation:

Variations are caused by mutation and recombination. These variations lead to change in structure and functions of the organisms and lead to evolution of new species.

RNA:

RNA is a polymer of ribo-nucleoside-phosphates. Dr. Severo Ochoa discovered the RNA and got 1959 Nobel Prize for Medicine. The sequence of the 77 nucleotides of yeast RNA was found by Robert W. Holley in 1964, winning Holley the 1968 Nobel Prize for Medicine. In 1976, Walter Fiers and his team at the University of Ghent determined the complete nucleotide sequence of bacteriophage MS2-RNA.
Its backbone is comprised of alternating ribose and phosphate groups. Ribose is a five carbon sugar with carbons numbered 1' through 5′. A base is attached to the Y position, generally adenine (A), cytosine (C), guanine (G) or uracil (U). Adenine and guanine are purines, cytosine and uracil are pyrimidine's. A phosphate group is attached to the 3′ position of one ribose and the 5′ position of the next. Most cellular RNA is single stranded, although some viruses have double stranded RNA (Fig. 5.1).

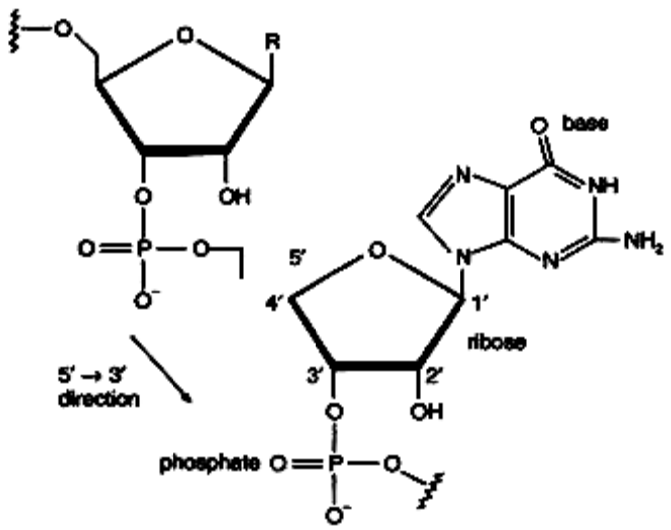


Fig. 5.1. Chemical structure of RNA.

Several other bases are occasionally found in RNAs including: thymine, pseudouridine and methylated cytosine and guanine. However, there are also numerous modified bases and sugars found in RNA that serve many different roles. Pseudouridine (Ψ), in which the linkage between uracil and ribose is changed from a C-N bond to a C-C bond, and ribothymidine (T), are found in various places (most notably in the TΨC loop of tRNA). Thus, it is not technically correct to say that uracil is found in RNA in place of thymine. Another notable modified base is hypoxanthine (a deaminated Guanine base whose nucleoside is called Inosine).
Inosine plays a key role in the Wobble Hypothesis of the Genetic Code. There are nearly 100 other naturally occurring modified nucleosides, of which pseudouridine and nucleosides with 2′-0-

methylribose are by far the most common. The specific roles of many of these modifications in RNA are not fully understood. However, it is notable that in ribosomal RNA, many of the post-translational modifications occur in highly functional regions, such as the peptidyl transferase center and the subunit interface, implying that they are important for normal function. The single RNA strand is folded upon itself, either entirely or in certain regions.

In the folded region a majority of the bases are complementary and are joined by hydrogen bonds. This helps in the stability of the molecule. In the unfolded region the bases have no complements. Because of this RNA does not have the purine pyrimidine equality that is found in DNA.

Inside of cells, there are three major types of RNA: messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). There are a number of other types of RNA present in smaller quantities as well, including small nuclear RNA (snRNA), small nucleolar RNA (snoRNA) and the 4.5S signal recognition particle (SRP) RNA. Novel species of RNA continue to be identified. RNA serves a multitude of roles in living cells.

These include: serving as a temporary copy of genes that is used as a template for protein synthesis (mRNA), functioning as adaptor molecules that decode the genetic code (tRNA) and catalyzing the synthesis of proteins (rRNA). There is much evidence implicating RNA structure in biological regulation and catalysis. Interestingly, RNA is the only biological polymer that serves as both a catalyst (like proteins) and as information storage (like DNA).

For this reason, it has be postulated RNA, or an RNA-like molecule, was the basis of life early in evolution. RNA is a nucleic acid polymer consisting of nucleotide monomers that plays several important roles in the processes that translate genetic information from deoxyribonucleic acid (DNA) into protein products; RNA acts as a messenger between DNA and the protein synthesis complexes known as ribosomes, forms vital portions of ribosomes, and acts as an essential carrier molecule for amino acids to be used in protein synthesis.

Synthesis of RNA:

There are three types of RNA; messenger RNA (mRNA) or template RNA, ribosomal RNA (rRNA) and soluble RNA (sRNA) or transfer RNA (tRNA). Ribosomal and transfer RNA comprise about 98% of all RNA. All three forms of RNA are made on a DNA template. Transfer RNA and messenger RNA are synthesized on DNA templates of the chromosomes, while ribosomal RNA is derived from nucleolar DNA.

The three types of RNA are synthesized during different stages in early development. Most of the RNA synthesized during cleavage is mRNA. Synthesis of tRNA occurs at the end of cleavage, and rRNA synthesis begins during gastrulation.

Synthesis of RNA is usually catalyzed by an enzyme RNA polymerase using DNA as a template, a process known as transcription. Initiation of transcription begins with the binding of the enzyme to a promoter sequence in the DNA (usually found “upstream” of a gene).

The DNA double helix is unwound by the helicase activity of the enzyme. The enzyme then progresses along the template strand in the 3′ to 5′ direction, synthesizing a complementary RNA molecule with elongation occurring in the 5′ to 3′ direction.

The DNA sequence also dictates where termination of RNA synthesis will occur. RNAs are often modified by enzymes after transcription. For example, a poly (A) tail and a 5′ cap are added to eukaryotic pre-mRNA and introns are removed by the splice some.

There are also a number of RNA-dependent RNA polymerases that use RNA as their template for synthesis of a new strand of RNA. For instance, a number of RNA viruses (such as poliovirus) use this type of enzyme to replicate their genetic material.

TABLE 3.5 Differences between DNA and RNA

Characteristic	DNA	RNA
1. Pentose sugar	2′-deoxyribose	Ribose
2. Bases	Adenine (A), Guanine (G), Thymine (T) and Cytosine (C)	Adenine (A), Guanine (G) Uracil (U) and Cytosine (C)
3. Strands	Generally double stranded ; single-stranded DNA is found in some viruses, *e.g.*, ϕX174 viruses,	Generally single-stranded; double-stranded RNA is found in some viruses, *e.g.*, reovirus
4. Native from	Double-stranded DNA usually in B-form	Double stranded RNA usually in A-form
5. Origin	Replication of pre-existing DNA	Usually synthesized on a DNA template; only genetic RNA may replicate to yield RNA
6. Function	As genetic material	(i) Functions as genetic material in some viruses (ii) Generally, nongenetic function, as tRNA, mRNA, rRNA, chromosomal RNA, primer RNA.

Types of RNA:

A. Messenger RNA (mRNA):

Messenger RNA (mRNA) is only 5-10% of total RNA present in the cell. In 1961, Francis Jacob and Jacques Monod for the first time proposed the name mRNA. This RNA carries genetic information from DNA to the ribosome. Since mRNA is transcribed on DNA (genes), its base sequence is complementary to that of the segment of DNA on which it is transcribed. Usually each gene transcribes its own mRNA. Therefore, there are approximately as many types of mRNA molecules as there are genes.

The structure of prokaryotic and eukaryotic mRNA is as follows:

1. 5′ Cap:

At the 5′ end of the mRNA molecule in most eukaryote cells and animal virus molecules is found a ‘cap’. This cap is formed by the methylation of any of the four nucleotides. The cap helps mRNA to bind to the ribosomes. Without the cap mRNA molecules bind very poorly to the ribosomes. The bacterial mRNA does not have 5’cap. But they have specific ribosome binding site about six nucleotide long, which occurs at several places in the mRNA molecules. These are located at four nucleotides upstream from AUC.

2. Non-coding Regions:

As the name indicates these regions do not code for protein. There are two non-coding regions. First non-coding region (NCI) is followed by a 5’cap and is 10 to 100 nucleotides in length. This region is rich in A and U residues. The second non- coding region (NC2) is followed by termination codon and is 50-150 nucleotides long and contains an AAUAAA residues.

3. Initiation Codon:

Initiation codon is AUG in both prokaryotes and eukaryotes. Bacterial ribosomes bind directly to the AUG region of the mRNA to start the protein synthesis, whereas this is not there in the case of eukaryotes.

4. The Coding Region:
It consists of about 1,500 nucleotides. This region is responsible for coding protein with several ribosomes. The combination of mRNA strand with several ribosomes is called polyribosomes.

5. The Termination Codon:
The termination codon is required to give the signal to stop protein synthesis.

6. The poly (A) Sequence:
The non-coding region II is followed by poly (A) sequence in the eukaryotic mRNA. The prokaryotic mRNAs lack poly (A). The poly (A) sequences of 200-250 nucleotides are present at 3'OH end of mRNA. Poly (A) sequences are added when mRNA is present inside the nucleus. The function of poly (A) sequence in translation is unknown (Fig. 5.3).

Difference between prokaryotic and eukaryotic mRNA:

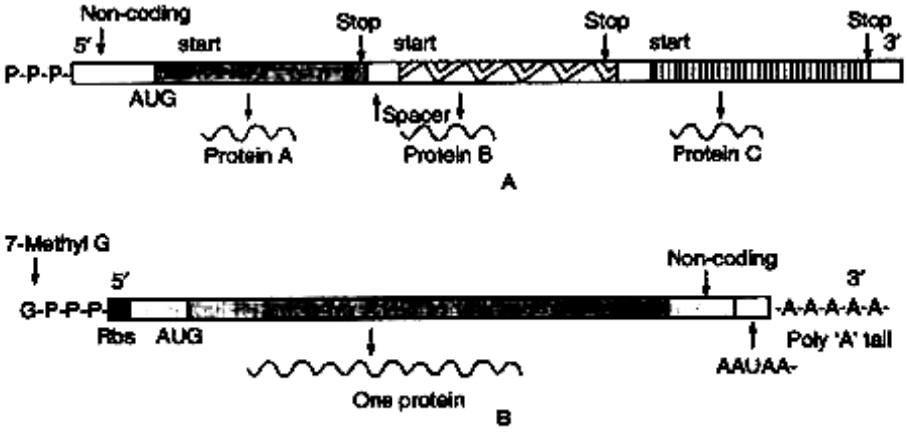


Fig. 5.3. Structure of prokaryotic (A) and eukaryotic (B) mRNA showing gene product. This requires processing of mRNA in eukaryotes before translation.

Prokaryotic mRNA:

1. Translation begins when the mRNA is still being transcribed on DNA.

2. Prokaryote mRNA are very short lived. It constantly under goes breakdown to its constituent ribonucleotides by ribonucleases.

3. In Prokaryotic mRNA are polycistronic.

4. The mRNA undergo very little processing after being transcribed.

5. Prokaryotic mRNA do not have poly (A) tail.

Eukaryotic mRNA:

1. Translation begins when the transcription is Completed.

2. Eukaryotic nRNAs are long lived. Thus are metabolically stable.

3. In Eukaryotic mRna are monocistronic .

4. The mRNA undergoes several processing after being transcribed such as polyadenylation capping and methylation.

5. Eukaryotic mRNA have poly (A) tail.

B. Ribosomal RNA (rRNA):

Ribosomal RNA is extremely abundant and makes up to 80% of RNA found in a typical eukaryotic cytoplasm. Ribosomal RNA consists of a single strand twisted upon itself in some regions. It has

helical regions connected by intervening single strand regions. The helical regions may show presence or absence of positive interaction. In the helical region most of the base pairs are complementary, and are joined by hydrogen bonds. In the unfolded single strand regions the bases have no complements.
In the cytoplasm, ribosomal RNA and protein combine to form a nucleoprotein called a ribosome. Ribosomal RNA (rRNA) is a component of the ribosomes. The base sequence of rRNA is complementary to that of the region of DNA where it is synthesized. Ribosomal RNA is formed from only a small section of the DNA molecule, and hence there is no definite base relationship between rRNA and DNA as a whole.

Primarily there are two types of ribosomes, one is 70s (prokaryotes) and the other is 80s (eukaryotes). The 70S ribosome of prokaryotes consists of a 30S subunit and a 50S subunit. The 30S subunit contains 16S rRNA, while the 50S subunit contains 23S and 5S rRNA. The 80S eukaryote ribosome consists of a 40S and a 60S subunit.

In vertebrates the 40S subunit contains 18S rRNA, while the 60S subunit contains 28-29S, 5.8S and 5S rRNA. In plants and invertebrates, the 40S subunit contains 16-18S RNA, while the 60S subunit contains 25S and 58 and 5.8S rRNA.

C. Transfer or tRNA:

The tRNA molecules are key to the translation process of the mRNA sequence into the amino acid sequence of proteins (at least one type of tRNA for every amino acid). To be precise, the amino-acyl-tRNA-synthase proteins are the ‘true’ translators of the genetic code into an amino acid sequence. These synthetases acetylate tRNA molecules with the proper amino acid that corresponds to the anti-codon in the structure of the tRNA molecule.

The anti-codon later recognizes the codon, the triple base sequence which ‘codes’ for the amino acid along the mRNA strand. A failure of properly acetylating the tRNA with the right amino acid results in an amino acid mutation even though the DNA sequence has not been changed. tRNA molecules are small nucleic acids of 60- 95 nucleotides, mostly 76, with a molecular weight 18-20kD, with the secondary structure resembling a clover leaf. Here are a few common features shared by all tRNA molecules found in various organisms.

(1) 5′ terminus is always phosphorylated.

(2) 7 bp stem, may have non-Watson & Crick pairing (like GU) acceptor or amino acid stem at 3′ terminus in which last three nucleotides are CCA-3′-OH. These are added after transcription and amino acylation occurs at 3′-OH group of last base ‘A’.

(3) 3-4 bp stem and loop contains the base dihydrouridine (D) [D- arm.]

(4) 5 bp stem and loop containing anti-codon triplet [anti-codon arm].

(5) 5 bp stem and loop contains sequence T C, standing for [T- arm] pseudouridine.

(6) Variable arm (between anti-codon and T-arm) length, 3-21 nucleotides.

(7) Contains numerous modified bases (up to 25%) which are all post-transcriptionally modified.

The three dimensional structure of tRNA resembles an L-shaped molecule with the D-arm and anti-codon loop building one stretch and the T-arm and acceptor stem building the other stretch being deposed by ~90 to one another (interstem angle of 82 by X-ray refinement and 92 in an electron microscopy study). The molecule is about 6 nm in each direction with the anti-codon to acceptor 3′-term ends being 7.6 nm apart. The diameter of both arms is about 2.0 to 2.5 nm.

The structural complexity of tRNA is reminiscent of that of a protein with 71 out of 76 bases participating in stacking interaction (of which 42 in double helical stem structures). 9 bp interactions are cross linking the tertiary structure, i.e., they interact with bases from a different stem and loop

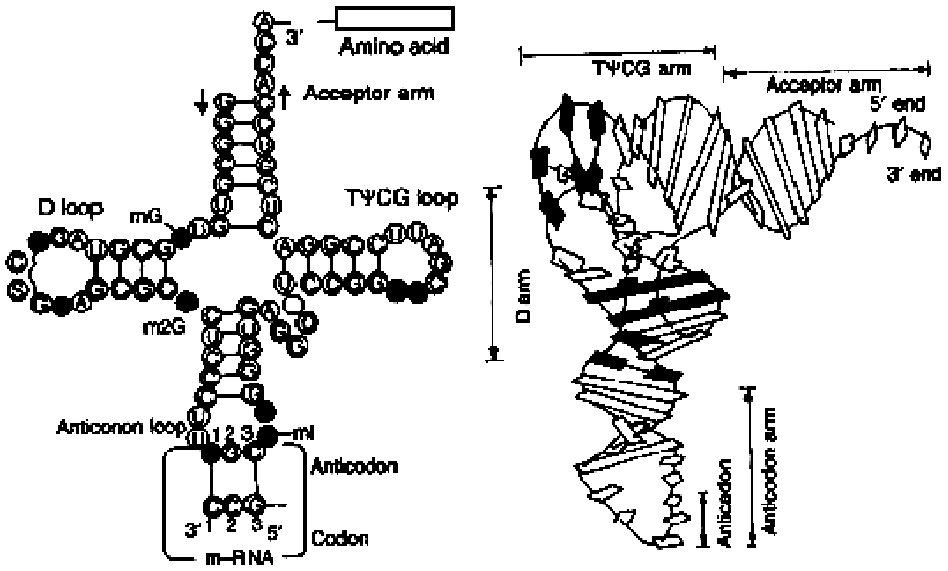


Fig. 5.4. A 3D structure of tRNA: Clover leaf model

Table 5.3. Difference between the three types of RNAs

	Ribosomal RNA (rRNA)	Messenger RNA (mRNA)	Transfer RNA (tRNA)
Percentage of total RNA of cell	~ 80%	5 to 10%	10 to 15%
Sedimentation coefficient	28S, 18S, 5.8S, 5S 23S, 16S, 5S	8S	3.8S
Number of nucleotides	5S RNA: 120 nucleotides. 16-18S RNA: 1,600 to 2,500 nucleotides. 23-28S RNA : 3,200 to 5,500 nucleotides	*E. coli:* 900 to 1,500 nucleotides	73 to 93 nucleotides
Unusual bases	Small amount of methylated bases (*E. coli*: 1 per 100-150 nucleotides).	Small amount of unusual bases.	High content of unusual bases. (*E. coli*: 1 per 3040 nucleotides).
Site of synthesis	Derived from nucleolar DNA.	Synthesized in nucleus on DNA template.	Synthesized in nucleus on DNA template.
Beginning of synthesis	Synthesis begins at gastrulation, and increases as development proceeds.	Some mRNAs are found in the ovum. New mRNA is synthesized during early cleavage.	tRNA synthesis occurs at the end of cleavage stages.
Base relationship with DNA	No obvious base relationship to DNA. rRNA is formed from only small sections of of DNA.	mRNA shows base relationship with DNA. It is formed from all sections of DNA.	rRNA also shows base relationship with DNA. It is formed from all sections of DNA.
Function	Unpaired bases may bind mRNA and tRNA to ribosomes.	Conveys genetic information from DNA to the ribosomes, where it takes part in protein synthesis.	Adaptor for attaching amino acids to mRNA template.

region. All of these 9 bp are non-Watson-Crick associations and are highly conserved which makes it likely to predict similar structures for all tRNA molecules (in fact, only few tRNA molecules have been crystallized and their structure determined) (Fig. 5.4).

New Types of RNA:

Besides mRNA, tRNA and rRNA, the three classically known RNA molecules, new types of RNA molecules are discovered in recent years, which are involved in various activities directly or they modulate the activity.

Some of the new types of RNA which are involved in various activities directly are as follows:

1. Non-Coding RNA:

RNA genes (sometimes referred to as non-coding RNA or small RNA) are genes that encode RNA that is not translated into a protein. The most prominent examples of RNA genes are transfer RNA (tRNA) and ribosomal RNA (rRNA), both of which are involved in the process of translation.

2. Small Nuclear RNA (snRNA):

About a dozen genes for snRNAs have been described, each present in multiple copies in the genome. Small nuclear RNAs combine with certain U-proteins to form snRNPs. These executive molecules have roles in editing other classes of RNA. The "U" designation was given to the snRNAs because they were found to be rich in uridylic acid.

An important example is the small ribonuclear proteins (snRNPs) that are components of the spliceosomes.

3. Small Nucleolar RNA (snoRNA):

In eukaryotic cells, rRNA and snRNA are extensively modified and processed in the nucleolus. Much of this activity is affected by snoRNAs. It appears that the coding for snoRNAs lies in introns and other intergenic regions (non-protein coding). Small nucleolar RNA can be considered to be a subgroup of the snRNAs but should not be confused with the snRNAs mediating mRNA splicing, i.e. the spliceosomal RNAs.

4. Short Interfering RNAs (siRNA):

Short interfering RNAs and miRNAs were discovered in different works, but their biogenesis and assembly into RNA-protein complexes and their function in down regulating gene expression are closely related. Short interfering RNAs and mi RNAs share common RNAse III processing enzyme,

5. Micro-RNAs (miRNAs):

Micro-RNAs are a class of small, non-coding RNAs that regulate gene expression in a sequence specific manner as required in embryonic development. Micro-RNAs have been found throughout diverse eukaryotes genomes including plants. They can inhibit protein expression by shutting off translation or by targeting mRNA for degradation. Micro-RNAs were first discovered in 2001 in the widely studied worm Caenorhabdtis elegans.

6. Ribozymes:

Ribozymes are catalytic RNA enzymes that act to alter covalent structure in other classes of RNAs and certain molecules. They occur in ribosomes, nucleus and chloroplasts of eukaryotic organisms. Some viruses including several bacteriophages also have ribozymes. An optimum concentration of metal ions such as Mg^{++} and K^{+} is associated with their effective functioning. Ribozymes generally act as molecular scissors cutting precursor RNA molecules at specific sites. Surprisingly, they also serve as molecular staplers, which ligate or join two RNA molecules together.

7. XISTRNA:

XIST stands for X-inactive-specific transcript. It is a large (~ 17kb) RNA coded by a gene on the X chromosome (8 exons are involved with human xist). xistRNA accumulates in female somatic cells

along the X chromosome containing the active xist gene and proceeds to inactivate nearly all the 100s of genes on that X chromosome..

8. Double-Stranded RNA (dsRNA):

Double-stranded RNA (dsRNA) is RNA with two complementary strands, similar to the DNA found in all 'higher' cells. dsRNA forms the genetic material of some viruses. In eukaryotes, it acts as a trigger to initiate the process of RNA interference and is present as an intermediate step in the formation of siRNAs (small interfering RNAs). siRNAs are often confused with miRNAs; siRNAs are double-stranded, whereas miRNAs are single-stranded.

Although initially single stranded, there are regions of intra-molecular association causing hairpin structures in pre-miRNAs. Very recently, dsRNA has been found to induce gene expression at transcriptional level, a phenomenon named "small RNA induced gene activation (RNAa)". Such dsRNA is called "small activating RNA (saRNA)".

9. RNA Secondary Structures:

The functional form of single stranded RNA molecules (like proteins) frequently requires a specific tertiary structure. The scaffold for this structure is provided by secondary structural elements which are hydrogen bonds within the molecule. This leads to several recognizable "domains" of secondary structure like hairpin loops, bulges and internal loops.

The secondary structure of RNA molecules can be predicted computationally by calculating the minimum free energies (MFE) structure for all different combinations of hydrogen bonding's and domains. There has been a significant amount of research directed at the RNA structure prediction problem.

Significance of nucleic acids:

Deoxyribonucleic acids and ribonucleic acids are the key centres which control all the metabolic activities of cell and in turn the whole organism.

(1) If there occurs any deficiency in the DNA amount, nucleus loses its capacity to support adenosine triphosphate (ATP) synthesis.

(2) Nucleus also becomes inefficient to incorporate amino acids into proteins.

(3) Besides, DNA is the main genetic material constituting genes and chromosomes which carry hereditary information from generation to generation. DNA helps in the RNA synthesis in the cell. If the loops of amphibian oocytic chromosome (lamp brush) are exposed to actinomycin (which has the property to fuse with DNA and thereby causing decrease in DNA amount), RNA synthesis is inhibited.

(4) Recently, McConnell and Cameron (1968) have produced the evidence that RNA amount increases the intelligence and learning capacity of men.

---

16. Photosynthesis: Light reactions, organization of light absorbing system, mechanism of electron and proton transport, Carbon concentrating mechanisms.

Photosynthesis literally means 'synthesis with the help of light'. It is the process that gives life to all living beings. The plants convert light energy into life energy. It is the only biological process that makes use of sun's light energy for driving the life machinery. Hence, photosynthesis is regarded as 'leader' of all processes both biological and abiological. It is the most fundamental of all biochemical reactions by which plants synthesize organic compounds in the chloroplast from carbon dioxide and water with the help of sunlight. It is an oxidation–reduction reaction between water and carbon dioxide.

Significance of photosynthesis:

Photosynthesis is a source of all our food and fuel. It is the only biological process that acts as the driving vital force for the whole animal kingdom and for the non-photosynthetic organism. It drives all other processes of biological and abiological world. It is responsible for the growth and sustenance of our biosphere. It provides organic substances, which are used in the production of fats, proteins, nucleoproteins, pigments, enzymes, vitamins, cellulose, organic acids, etc. Some of them become structural parts of the organisms. It makes use of simple raw materials such as CO_2, H_2O and inexhaustible light energy for the synthesis of energetic organic compounds. It is significant because it provides energy in terms of fossil fuels like coal and petrol obtained from plants, which lived millions and millions of years ago. Plants, from great trees to microscopic algae, are engaged in converting light energy into chemical energy, while man with all his knowledge in chemistry and physics cannot imitate them.

Site of photosynthesis:

Chloroplasts are the actual sites for photosynthesis. All green parts of a plant are involved in photosynthesis. Leaves are the most important organs of photosynthesis. In xerophytes like *Opuntia*, the stem is green and it performs photosynthesis. Over half a million chloroplasts are present in one square millimeter of a leaf. It measures about 4 to 6 micron. A typical chloroplast of higher plants is discoid shaped. It is a double membrane bound organelle containing chlorophyll, carotenoid, xanthophyll, cytochrome, DNA, RNA, manganese, etc. Chloroplasts are generally considerably larger than mitochondria. The space enclosed by the envelope is filled with matrix called stroma.

In the stroma, many grana are embedded. In each granum, several disc shaped lamellae are found. These disc shaped structures are called thylakoids. They resemble a stack of coins. This structure is known granum. Generally a chloroplast contains 40 to 60 grana. The photosynthetic pigments are found in grana. The stroma contains circular DNA, RNA and enzymes for starch synthesis.

Photochemical and biosynthetic phases:

The pigments involved in photosynthesis are called photosynthetic pigments. They are chlorophyll ‘a’, chlorophyll ‘b’, carotenoids, xanthophyll and phycobilins. Magnesium is an essential component for the formation of chlorophyll. Chlorophyll ‘a’ is a universal pigment present in the plants in which water is one of the raw materials for photosynthesis. Chlorophylls are highly efficient in absorbing solar energy and they are directly linked to photosynthetic electron transport. Photosynthetic pigments other than chlorophyll ‘a’ are generally called accessory pigments e.g. Chlorophyll ‘b’, carotenoids and xanthophyll, whereas chlorophyll ‘a’ is regarded as primary pigment. Photosynthetic pigments occur in the granum. They constitute the pigment system called photosystem. About 250 to 400 pigment molecules are present in a photosystem. Two types of photosystems are found in the granum. Photosystem I (PS I) has less accessory pigments and more

Chlorophyll ‘a’, while photosystem II (PS II) has more accessory pigments and less chlorophyll ‘a’. The primary function of photosystems is to trap light energy and converts it to chemical energy. The energy absorbed by accessory pigments is transferred to the chlorophyll ‘a’. The grana lamella where the photosynthetic pigments are aggregated to perform photosynthetic activities is called active center.

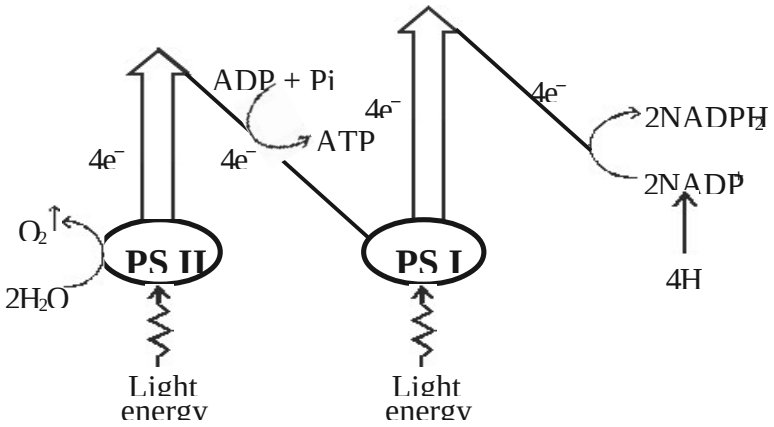
Mechanism of photosynthesis:

The overall reaction of photosynthesis can be written as follows.

$$CO_2 + 2H_2O \xrightarrow[Chlorophyll]{Solar\ energy} (CH_2O)_n + H_2O + O_2$$

The reactions of photosynthesis can be grouped into two – light reactions and dark reactions. The reactions involving pigments, solar energy and water that produce ATP and $NADPH_2$, are called light reactions. The photosynthetic reactions in which CO_2 is reduced to carbohydrates making use of ATP and $NADPH_2$ generated by light reactions are collectively called dark reactions.

The overall process of photosynthesis is illustrated in the following Fig.1 and Fig.2.



Electron transport system:

The light driven reactions of photosynthesis are referred to as electron transport chain. When PS II absorbs photons of light, it is excited and the electrons are transported through electron transport chain of plastoquinone, cytochrome b_6, cytochrome f and plastocyanin. The electrons released from PS II phosphorylate ADP to ATP. This process of ATP formation from ADP in the presence of light in chloroplast is called photophosphorylation.

Now, the PS II is in oxidised state. It creates a potential to split water molecules to protons, electrons and oxygen. This light dependent splitting of water molecules is called photolysis of water. Manganese, calcium and chloride ions play prominent roles in the photolysis of water. The electrons thus released are used in the reduction of PS II. Similar to PS II, PS I is excited by absorbing photons of light and gets oxidised. This oxidised state of the PS I draws electrons from PS II and gets reduced. The electrons released to PS I are transported through electron transport chain of ferredoxin reducing substrate, ferredoxin and ferredoxin NADP reductase to reduce $NADP^+$ to $NADPH_2$.

Cyclic and noncyclic photophosphorylation:

In chloroplasts, phosphorylation occurs in two ways – noncyclic photophosphorylation and cyclic photophosphorylation.

- **Noncyclic photophosphorylation**

When the molecules in the PS I are excited the electrons are released. So, an electron deficiency or a hole is made in the PS I. This electron is now transferred to ferredoxin to reduce $NADP^+$. When the molecules in the PS II get excited, electrons are released. They are transferred to fill the hole in PS I through plastoquinone, cytochrome b_6, cytochrome f and plastocyanin. When the electron is transported between plastoquinone and cytochrome f, ADP is phosphorylated to ATP.

The 'hole' in the PS I has been filled by the electron from PS II. Then the electrons are transferred from PS I to NADP for reduction. Therefore, this electron transport is called noncyclic electron transport and the accompanying phosphorylation as noncyclic photophosphorylation. The noncyclic electron transport takes place in the form of 'Z'. Hence, it is also called Z-scheme. ***Fig. 3.***

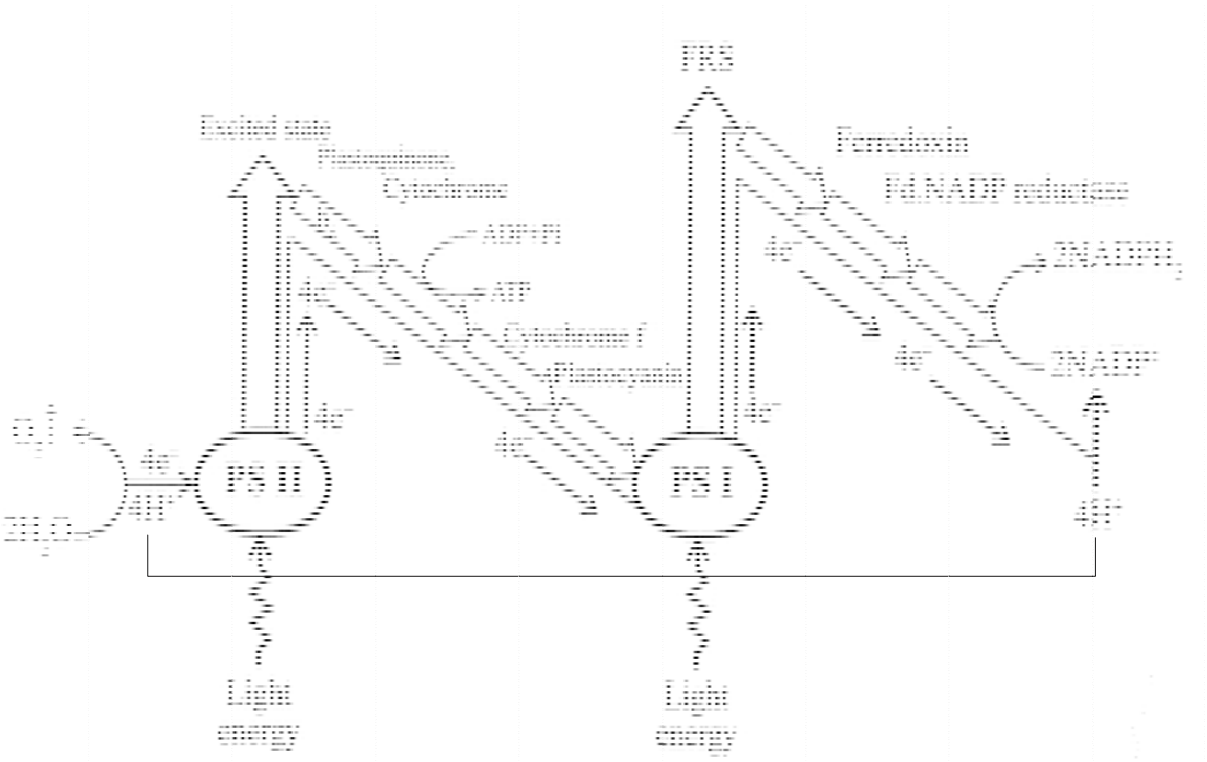
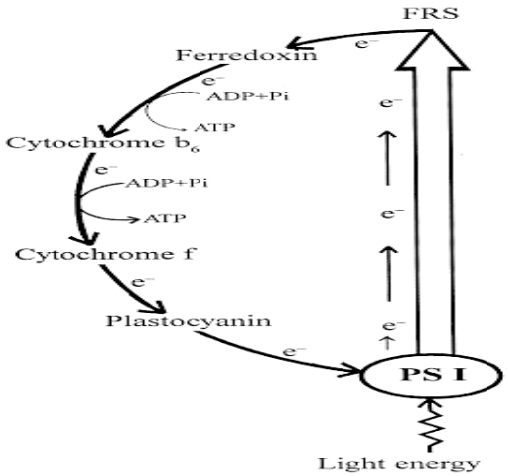


Fig. 3. Non-cyclic photophosphorylation

❖ **Cyclic photophosphorylation**



Under the conditions of (i) PS I only remains active (ii) photolysis of water does not take place (iii) requirement of ATP is more and (iv) no availability of NADP+ the cyclic photophosphorylation takes place. When the molecule in the PS I is excited, the electrons are released. The electrons are captured by ferredoxin through ferredoxin reducing substrate (FRS). Due to non-availability of NADP+, electrons from ferredoxin fall back to the molecules of PS I through the electron carrier's cytochrome b_6, cytochrome f and plastocyanin. These electron carriers facilitate the downhill transport of electrons from FRS to PS I.

Fig. 4. Cyclic photophosphorylation

Difference between cyclic and noncyclic electron transport and photophosphorylation

Cyclic photophosphorylation	Noncyclic photophosphorylation
1. It is associated with PS I	1. It is associated with both PS I and PS II.
2. The electron expelled from chlorophyll molecule is cycled back	2. The electrons are not cycled back but compensated by the electrons From photolysis of water.
3. Photolysis of water and evolution of oxygen do not take place.	3. Photolysis of water and evolution of oxygen take place.
4. Photophosphorylation takes place at two places.	4. Photophosphorylation takes place only at one place.
5. NADPis not reduced.	5. NADP+ is reduced to $NADPH_2$.

C_3 Cycle:

The enzymatic reduction of CO_2 by these reactions is also known as carbon fixation. These reactions that result in CO_2 fixation take place in a cyclic way and were discovered by Melvin Calvin. Hence, the cycle is called **Calvin cycle.**

Fixation of carbon dioxide in plants during photosynthesis occurs in three stages – fixation, reduction and regeneration of RuBP.

- **Fixation**

The acceptor molecule of CO_2 is a 5C compound called ribulose-1,5bisphosphate (RuBP). Fixation of a molecule of CO_2 to RuBP is catalyzed by the enzyme RuBP carboxylase. The resulting 6C compound is highly unstable and gets cleaved to form two molecules of 3C compounds called phosphoglyceric acid (PGA).

$$RuBP + CO_2 \xrightarrow{RuBP\,Carboxylase} 2\,moles\,of\,PGA$$

- **Reduction**

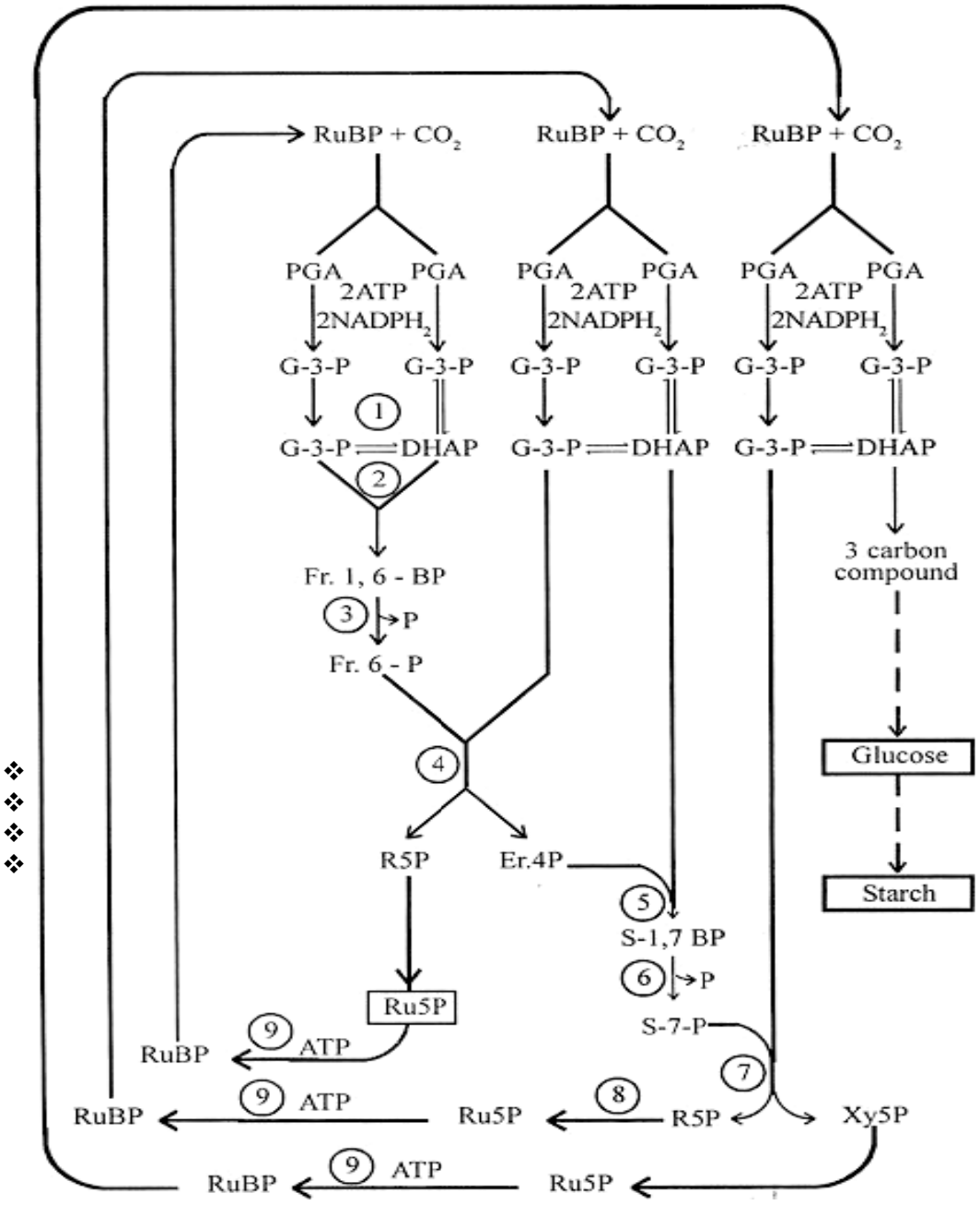
The two molecules of PGA are further reduced to glyceraldehyde-3phosphates in two steps. First, two PGA molecules are converted to 1, 3 -bisphosphoglyceric acids by the enzyme PGA kinase. This reaction consumes two molecules of ATP in the ratio of one ATP for each molecule of 1,3-bisphosphoglyceric acid formed.

In the second step, the two molecules of 1,3-bisphosphoglyceric acid are reduced to glyceraldehyde-3-phosphates by the enzyme glyceraldehyde3-phosphate dehydrogenase with the help of the light generated reducing

Fig. 5. Calvin cycle power $NADPH_2$. So, two molecules of $NADPH_2$ will be consumed during this reaction. To reduce one molecule of CO_2 up to reduction two ATP and two $NADPH_2$ are consumed.

$$2\,moles\,of\,PGA + 2\,ATP \xrightarrow{PGA\,kinase} 2\,moles\,1,3-bisphospho-glyceric\,acid$$

$$2\,moles\,1,3-bisphospho\,glyceric\,acid + 2NADPH_2 \xrightarrow{G-3-P\,dehydrogenase} 2\,moles\,G-3-P$$



❖ **Regeneration of RuBP**

The glyceraldehyde 3-phosphate molecules are converted to RuBP through a series of reactions, which generate 4C, 6C and 7C phosphorylated compounds as intermediates. For better and easy understanding of these reactions, a simplified scheme of Calvin cycle considering three CO_2 molecules fixation reactions is shown below.

The reactions of regeneration of RuBP are as follows.

1. Some of the Glyceraldehyde 3-phosphate molecules are converted to dihydroxyacetone phosphates.
2. Glyceraldehyde 3-phosphate combines with dihydroxyacetone phosphate to form fructose1,6-bisphosphate.
3. Fructose 1,6-bisphosphate undergoes dephosphorylation to form fructose 6-phosphate.
4. Fructose 6-phosphate combines with glyceraldehyde 3-phosphate obtained from the fixation of second molecule of CO_2 to form Ribose 5-phosphate (R5P) and Erythrose 4-phosphate (Er4P).
5. Erythrose 4-phosphate combines with DHAP obtained from the second CO_2 fixation, to form sedoheptulose 1,7-bisphosphate.
6. Sedoheptulose 1,7-bisphosphate undergoes dephosphorylation to form sedoheptulose 7-phosphate.
7. Sedoheptulose 7-phosphate combines with glyceraldehyde 3-phosphate obtained by the third CO_2 fixation, to form two molecules of 5C compounds – ribose 5-phosphate and xylulose 5-phosphate (Xy5P).
8. Ribose 5-phosphate and xylulose 5-phosphate molecules are transformed to ribulose 5-phosphate (Ru5P).
9. Ru5P molecules are then phosphorylated by ATP to form RuBP molecules, which again enter into the cycle of CO_2 fixation.

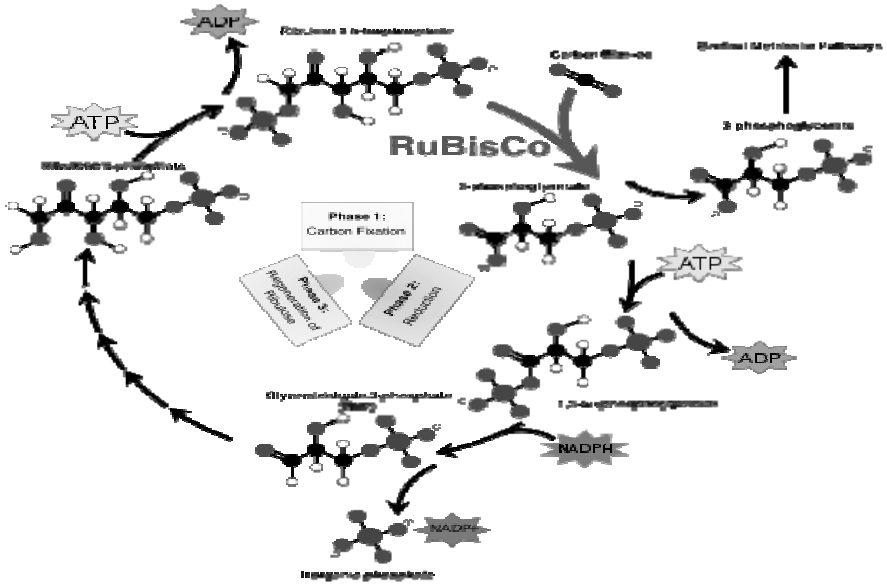


Fig.6. C_3 cycle

$$RuBP + CO_2 \xrightarrow{2ATP + 2NADPH_2} 2\,moles\,of\,G-3-P$$

$$Ru5P \xrightarrow{ATP} RuBP$$

In the above illustration, three CO_2 molecules are fixed and the net gain is a 3C called DHAP. These triose phosphate molecules combine to form hexose phosphates, which are used to form sucrose. For every carbon fixation 3ATP and 2 $NADPH_2$ are consumed.

C_4 Carbon Fixation

C_4 carbon fixation or **the Hatch–Slack pathway** is a photosynthetic process in some plants. It is the first step in extracting carbon from carbon dioxide to be able to use it in sugar and other biomolecules. It is one of three known processes for carbon fixation. "*C_4*" refers to the four-carbon molecule that is the first product of this type of carbon fixation.

C_4 fixation is an elaboration of the more common C_3 carbon fixation and is believed to have evolved more recently. C_4 overcomes the tendency of the enzyme RuBisCO to wastefully fix oxygen rather than carbon dioxide in the process of photorespiration. This is achieved by ensuring that RuBisCO works in an environment where there is a lot of carbon dioxide and very little oxygen. CO2 is shuttled via malate or aspartate from mesophyll cells to bundle-sheath cells. In these bundle-sheath cells CO2 is released by decarboxylation of the malate. C_4plants use PEP carboxylase to capture more CO2 in the mesophyll cells. PEP (phosphoenolpyruvate, three carbons) binds to CO2 to make oxaloacetic acid (OAA). OAA then makes malate (four carbons). Malate enters bundle sheath cells and releases the CO2. These additional steps, however, require more energy in the form of ATP. Using this extra energy, C_4 plants are able to more efficiently fix carbon in drought, high temperatures, and limitations of nitrogen or CO2. Since the more common C_3 pathway does not require this extra energy, it is more efficient in the other conditions.

The C_4 pathway was elucidated by Marshall Davidson Hatch and C. R. Slack, in Australia, in 1966; it is sometimes called the Hatch-Slack pathway.

In C_3 plants, the first step in the light-independent reactions of photosynthesis involves the fixation of CO2 by the enzyme RuBisCO into 3-phosphoglycerate. However, due to the dual carboxylase and oxygenase activity of RuBisCo, some part of the substrate is oxidized rather than carboxylated, resulting in loss of substrate and consumption of energy, in what is known as photorespiration.

In order to bypass the photorespiration pathway, C_4 plants have developed a mechanism to efficiently deliver CO2 to the RuBisCO enzyme. They use their specific leaf anatomy where chloroplasts exist not only in the mesophyll cells in the outer part of their leaves but in the bundle sheath cells as well. Instead of direct fixation to RuBisCO in the Calvin cycle, CO2 is incorporated into a four-carbon organic acid, which has the ability to regenerate CO2 in the chloroplasts of the bundle sheath cells. Bundle sheath cells can then use this CO2 to generate carbohydrates by the conventional C_3 pathway.

The first step in the pathway is the conversion of pyruvate to phosphoenolpyruvate (PEP), by the enzyme pyruvate orthophosphate dikinase. This reaction requires inorganic phosphate and ATP plus pyruvate, producing phosphoenolpyruvate, AMP, and inorganic pyrophosphate (PP_i).

The next step is the fixation of CO2 into oxaloacetate by the enzyme PEP carboxylase. Both of these steps occur in the mesophyll cells:

$$\text{pyruvate} + P_i + ATP \rightarrow PEP + AMP + PP_i$$

$$PEP + CO2 \rightarrow \text{oxaloacetate}$$

PEP carboxylase has a lower Km for HCO^-_3 — and, hence, higher affinity — than RuBisCO. Furthermore, O_2 is a very poor substrate for this enzyme. Thus, at relatively low concentrations of CO2, most CO2 will be fixed by this pathway.

The product is usually converted to malate, a simple organic compound, which is transported to the bundle-sheath cells surrounding a nearby vein. Here, it is decarboxylated to produce CO2 and pyruvate. The CO2 now enters the Calvin cycle and the pyruvate is transported back to the mesophyll cell.

Since every CO2 molecule has to be fixed twice, first by four-carbon organic acid and second by RuBisCO, the C_4 pathway uses more energy than the C_3 pathway. The C_3 pathway requires 18 molecules of ATP for the synthesis of one molecule of glucose, whereas the C_4 pathway requires 30 molecules of ATP. This energy debt is more than paid for by avoiding losing more than half of photosynthetic carbon in photorespiration as occurs in some tropical plant making it an adaptive mechanism for minimizing the loss.

There are several variants of this pathway:

1. The four-carbon acid transported from mesophyll cells may be malate, as above, or aspartate.
2. The three-carbon acid transported back from bundle-sheath cells may be pyruvate, as above, or alanine.
3. The enzyme that catalyses decarboxylation in bundle-sheath cells differs. In maize and sugarcane, the enzyme is NADP-malic enzyme; in millet, it is NAD-malic enzyme; and, in *Panicum maximum*, it is PEP carboxykinase.

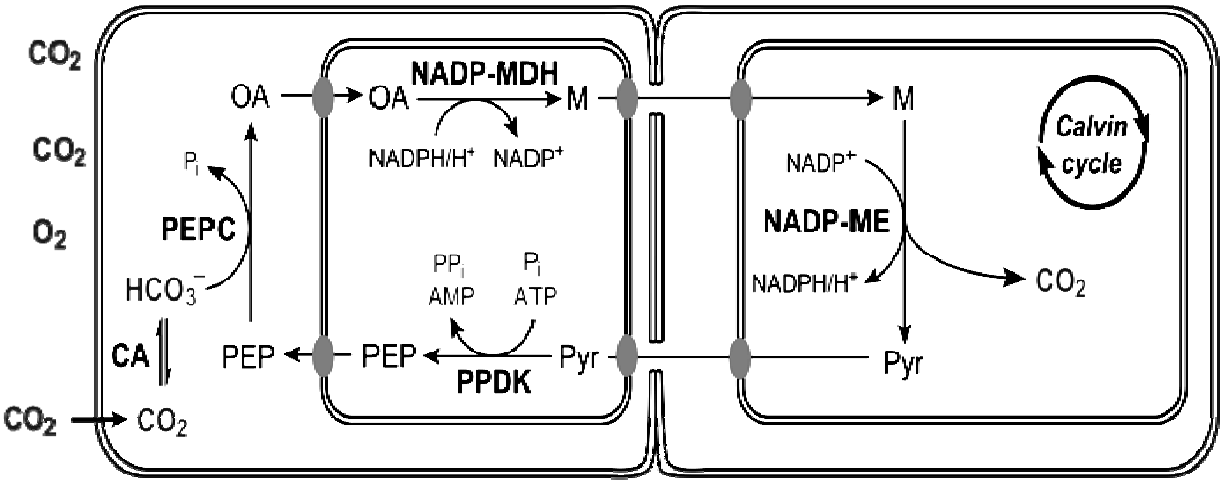


Fig 7. NADP-ME type C_4 pathway

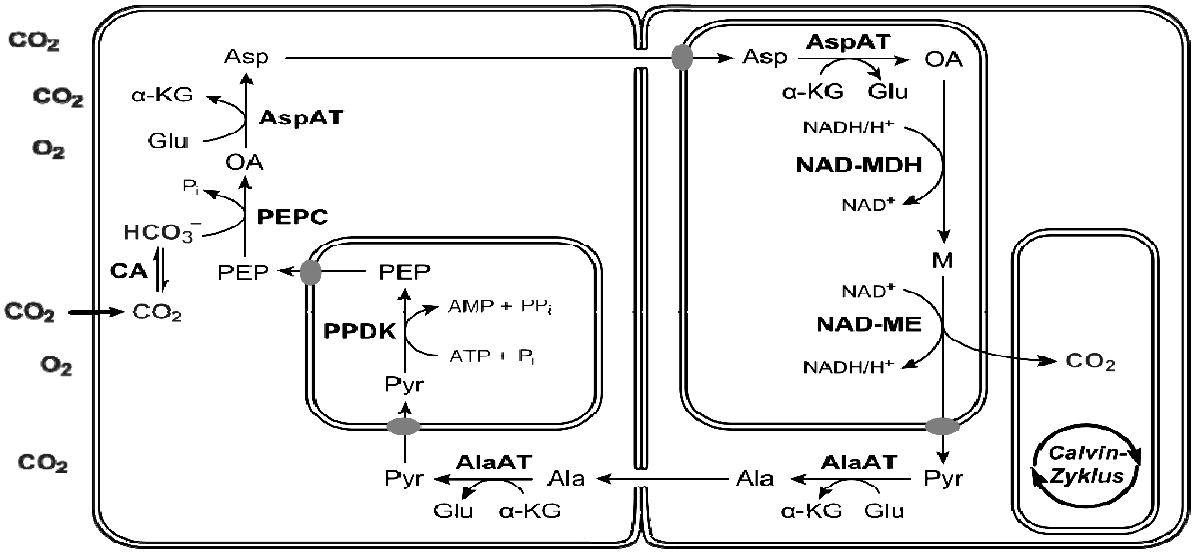


Fig 8. NAD-ME type C_4 pathway

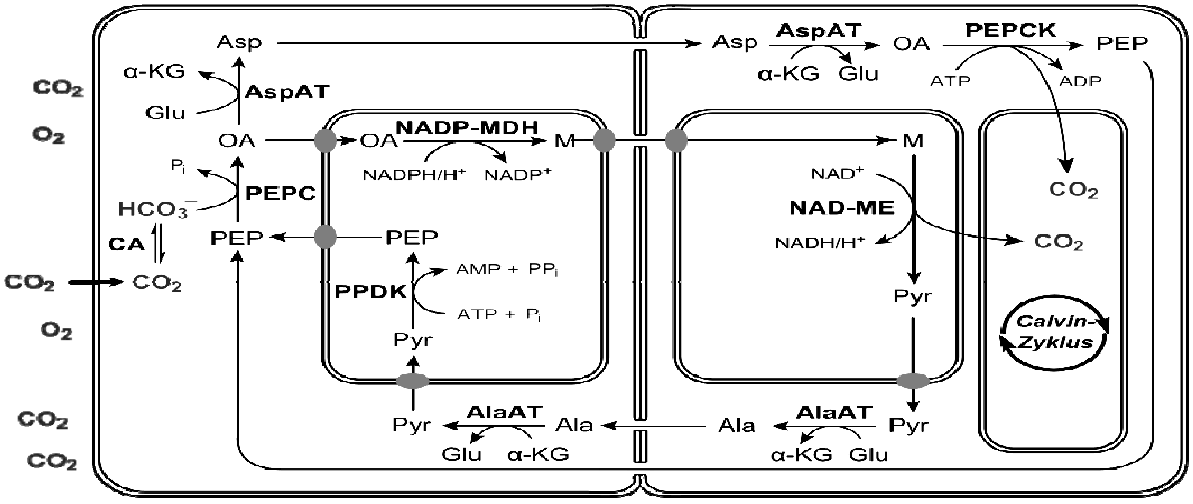


Fig 9. PEPCK type C4 pathway

Kranz anatomy:

The C_4 plants often possess a characteristic leaf anatomy called *kranz anatomy*, from the German word for wreath. Their vascular bundles are surrounded by two rings of cells; the inner ring, called bundle sheath cells, contains starch-rich chloroplasts lacking grana, which differ from those in mesophyll cells present as the outer ring. Hence, the chloroplasts are called dimorphic. The primary function of kranz anatomy is to provide a site in which CO_2 can be concentrated around RuBisCO, thereby avoiding photorespiration. In order to maintain

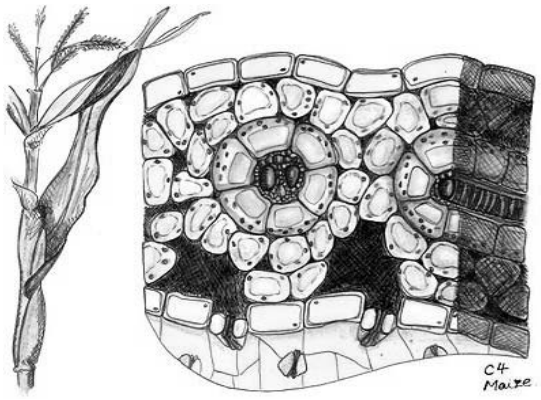


Fig 10. Cross section of a maize leaf, a C4 plant. Kranz anatomy

a significantly higher CO_2concentration in the bundle sheath compared to the mesophyll, the boundary layer of the kranz has a low conductance to CO_2, a property that may be enhanced by the presence of suberin. The carbon concentration mechanism in C_4 plants distinguishes their isotopic signature from other photosynthetic organisms.

Hatch-Slack pathway involves two carboxylation reactions. One takes place in chloroplasts of mesophyll cells and another in chloroplasts of bundle sheath cells.

1. The first step involves the carboxylation of phosphor-enol pyruvic acid in the chloroplasts of mesophyll cells to form a 4C compound, oxaloacetic acid. This reaction is catalyzed by the enzyme phosphor-enol pyruvate carboxylase

$$\underset{\text{pyruvic acid}}{\text{Phosphoenol}} + CO_2 + H_2O \xrightarrow{PEP\ Carboxylase} \underset{\text{Phosphoric acid}}{Oxaloacetic\ acid + H_3PO_4}$$

2. Oxaloacetic acid is converted into aspartic acid by the enzyme transaminase or it may be reduced to malic acid by $NADP^+$ specific malate dehydrogenase.

$$Oxaloacetic\ acid + NH_3 \xrightarrow{Transa\,min\,ase} Aspartic\ acid$$

$$Oxaloacetic\ acid + NADPH_2 \xrightarrow[malate\ dehydrogenase]{NADP^+\ specific} Malic\ acid + NADP^+$$

3. Malic acid or aspartic acid formed in chloroplast of mesophyll cells is transferred to the chloroplasts of bundle sheath where it is decarboxylated to form CO_2 and pyruvic acid in the presence of $NADP^+$ specific malic enzyme.

$$Malic\ acid + NADP^+ \xrightarrow[malic\ enzyme]{NADP^+\ specific} CO_2 + Pyruvic\ acid + NADPH_2$$

4. Now, second carboxylation occurs in chloroplasts of bundle sheath cells. Ribulose bisphosphate accepts CO_2 produced in step (3) in the presence of RuBP carboxylase and yields 3-phosphoglyceric acid. Some of the 3-phosphoglyceric acid molecules are utilized to produce sucrose and starch, while remaining PGA molecules are used for the regeneration of RuBP.

$$RuBP + CO_2 \xrightarrow{RuBP\ carboxylase} 2\,moles[3-phosphoglyceric\ acid]$$

5. The pyruvic acid produced in step (3) is transferred to the chloroplasts of mesophyll cells where it is phosphorylated to regenerate phosphor-enol pyruvic acid .This reaction is catalyzed by pyruvate kinase in the presence of Mg^{2+}.

$$\underset{\text{acid}}{\text{Pyruvic}} + ATP + Pi \xrightarrow[kinase]{Mg^{2+}\ /\ Pyruvic} \underset{\text{pyruvic acid}}{\text{Phosphoenol}} + AMP + \underset{\text{pyro phosphate}}{PPi}$$

$$AMP + ATP \underset{\text{Adenylate kinase}}{\rightleftharpoons} 2ADP$$

The AMP is phosphorylated by ATP in the presence of adenylate kinase to form 2 molecules of ADP. C_4 plants are photo synthetically more efficient than C_3 plants, because the net requirement of

ATP and $NADPH_2$ for the fixation of one molecule of CO2 is considerably lower in C4 plants than in C3 plants.

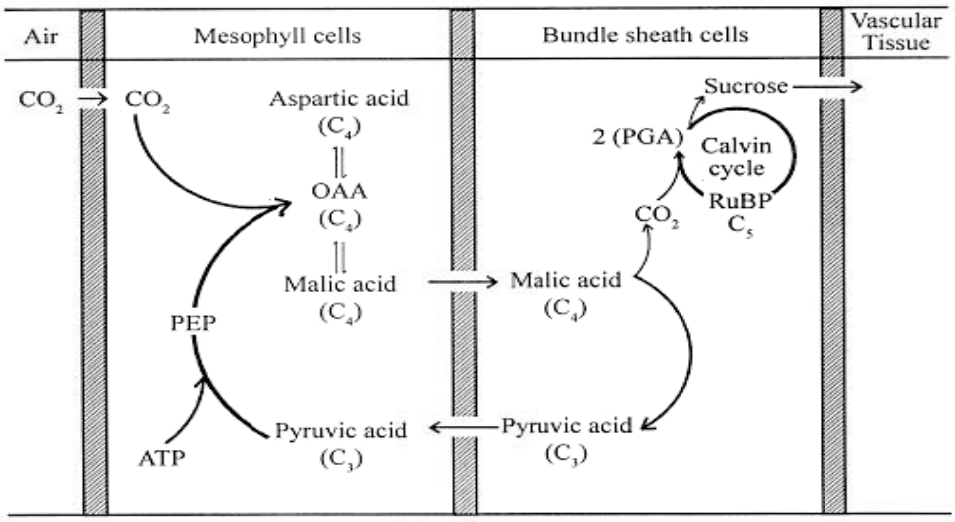


Fig 11. Hatch-Slack pathway of CO_2 fixation

Difference between C_3 and C_4 photosynthetic pathways

	C_3 pathway	**C_4 pathway**
1.	Photosynthesis occurs in meso - phyll cells.	Photosynthesis occurs in meso - phyll and bundle sheath cells.
2.	The CO_2 molecule acceptor is RuBP.	The CO_2 acceptor molecule is phosphoenol pyruvate.
3.	The first stable product is a 3C compound called 3 – PGA.	The first stable product is a 4C compound called OAA.
4.	Photorespiration rate is high and leads to loss of fixed CO_2. It decreases CO_2 fixation rate.	Photorespiration is negligible and it is almost absent. Hence, it increases CO_2 fixation rate.
5.	Optimum temperature is 20 to 25°C.	Optimum temperature is 30 to 45°C.
6.	Examples of C_3 plants are rice, wheat and potato.	Examples of C_4 plants are maize, sugarcane,Tribulus and Amaranthus

CAM Pathway:

Crassulacean acid metabolism, also known as CAM photosynthesis, is a carbon fixation pathway that evolved in some plants as an adaptation to arid conditions. In a plant using full CAM, the stomata in the leaves remain shut during the day to reduce evapotranspiration, but open at night to collect carbon dioxide (CO2). The CO2 is stored as the four-carbon acid malate in vacuoles at night, and then in the daytime, the malate is transported to chloroplasts where it is converted back to CO2, which is then

used during photosynthesis. The pre-collected CO2 is concentrated around the enzyme RuBisCO, increasing photosynthetic efficiency. The mechanism was first discovered in plants of the family Crassulaceae.

CAM was first suspected by de Saussure in 1804 in his Recherches Chimiques sur la Vegetation, confirmed and refined by Aubert, E. in 1892 in his Recherches physiologiques sur les plantes grasses and expounded upon by Richards, H. M. 1915 in Acidity and Gas Interchange in Cacti, Carnegie Institution. The term CAM may have been coined by Ranson and Thomas in 1940, but they were not the first to discover this cycle. It was observed by the botanists Ranson and Thomas, in the succulent family Crassulaceae (which includes jade plants and Sedum). Its name refers to acid metabolism in Crassulaceae, not the metabolism of "crassulacean acid"

Overview-A two part cycle:

During the night

During the night, a plant employing CAM has its stomata open, allowing CO2 to enter and be fixed as organic acids that are stored in vacuoles. During the day the stomata are closed (thus preventing water loss), and the carbon is released to the Calvin cycle so that photosynthesis may take place.

The carbon dioxide is fixed in the cytoplasm of mesophyll cells by a PEP reaction similar to that of C4 pathway. But, unlike the C4 mechanism, the resulting organic acids are stored in vacuoles for later use; that is, they are not immediately passed on to the Calvin cycle. The latter cannot operate during the night because the light reactions that provide it with ATP and NADPH cannot take place.

During the day

During the day, the CO2-storing organic acids are released from the vacuoles of the mesophyll cells and enter the stroma of the chloroplasts where an enzyme releases the CO2, which then enters into the Calvin cycle.

Benefits

The most important benefit of CAM to the plant is the ability to leave most leaf stomata closed during the day. Plants employing CAM are most common in arid environments, where water comes at a

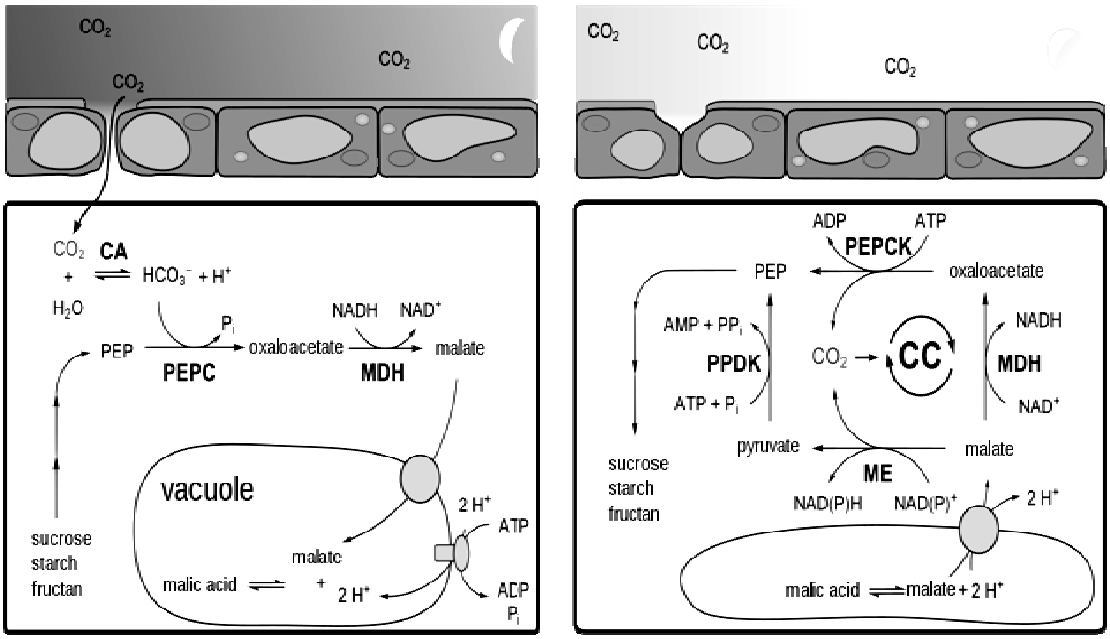


Fig 13. Overview of CAM

premium. Being able to keep stomata closed during the hottest and driest part of the day reduces the loss of water through evapotranspiration, allowing such plants to grow in environments that would otherwise be far too dry. Plants using only C3 carbon fixation, for example, lose 97% of the water they uptake through the roots to transpiration - a high cost avoided by plants able to employ CAM.

Comparison with C_4 metabolism

The C_4 pathway bears resemblance to CAM; both act to concentrate CO_2 around RuBisCO, thereby increasing its efficiency. CAM concentrates it temporally, providing CO_2 during the day, and not at night, when respiration is the dominant reaction. C_4 plants, in contrast, concentrate CO_2 spatially, with a RuBisCO reaction center in a "bundle sheath cell" being inundated with CO_2. Due to the inactivity required by the CAM mechanism, C_4 carbon fixation has a greater efficiency in terms of PGA synthesis.

Photorespiration or C_2 cycle:

In animals and bacteria, only one kind of respiration known as dark respiration occurs. This is not affected by the presence or absence of light. But in certain green plants, there are two distinct types of respiration – photorespiration and dark respiration. Respiration that occurs in photosynthetic tissues in the presence of light and results in increased rate of carbon dioxide evolution is called photorespiration or light respiration.

Photorespiration involves three organelles – chloroplasts, peroxisomes and mitochondria. Oxidation of RuBP in the presence of high oxygen is the first reaction of photorespiration. This reaction is catalyzed by Rubisco* enzyme called carboxylase. It leads to the formation of 2C compound – phosphoglycolic acid and 3C compound PGA. When PGA is used up in the Calvin cycle, the phosphoglycolic acid is dephosphorylated to form glycolic acid in the chloroplasts.

From the chloroplast, the glycolic acid diffuses into the peroxisome where it is oxidised to glyoxalic acid and hydrogen peroxide. In peroxisome from glyoxalic acid, glycine is formed.

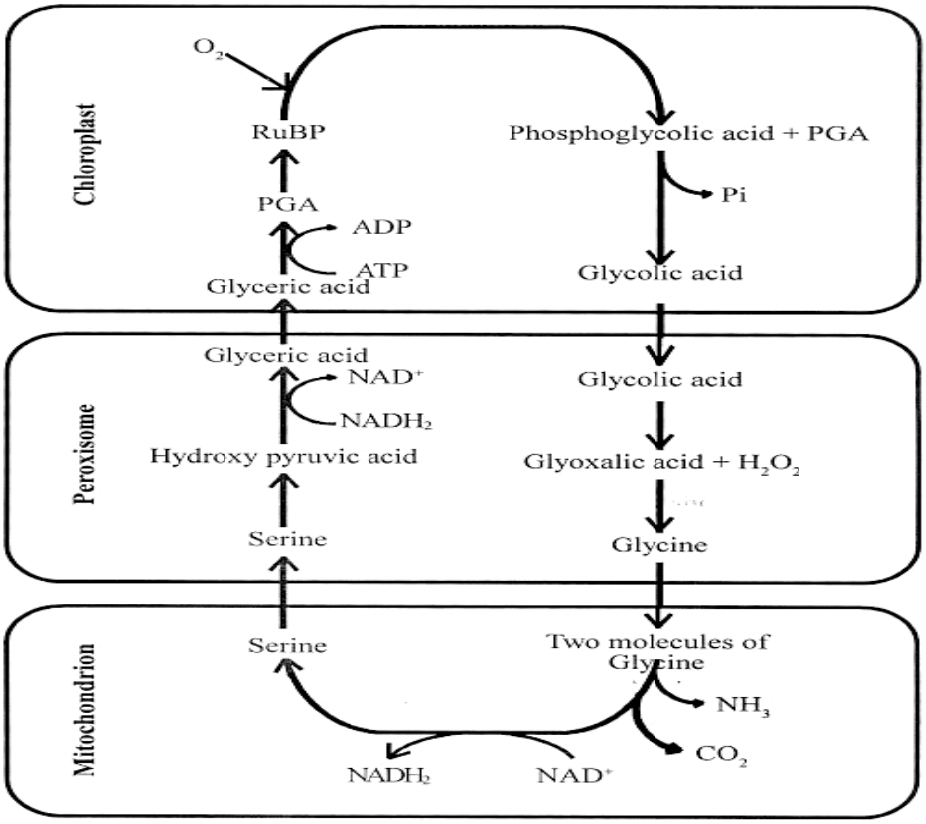


Fig.14. Photorespiratory pathway

Glycine molecules enter into mitochondria where two molecules of glycine combine to give a molecule of serine, NH_3 and CO_2. During this process, NAD^+ is reduced to $NADH_2$.

The amino acid serine is taken to peroxisome where, it is converted into hydroxyl pyruvic acid. Hydroxy pyruvic acid is reduced by $NADH_2$ to form glyceric acid.

The glyceric acid leaves peroxisome and enters chloroplast, where it is phosphorylated to PGA, which enters into Calvin cycle. During the photo respiratory pathway, one CO_2 molecule released in mitochondria is to be re-fixed.

Photorespiration is also known as photosynthetic carbon oxidation cycle or C_2 cycle. Under the conditions of high light and limited CO_2 supply, photorespiration protects the plants from photo oxidative damage. This means that, if enough CO_2 is not available to utilize light energy, excess energy causes damage to plant. However, photorespiration utilizes part of the light energy and saves the plant from photo oxidative damage. Increased O_2 level increases photorespiration whereas increased CO_2 level decreases photorespiration and increases photosynthesis.

Difference between photorespiration and dark respiration

	Photorespiration	Dark respiration
1.	It takes place only in photo synthetic cells in the presence of light.	It takes place in all living cells in the mitochondria.
2.	It is light dependent	It takes place in the presence and in the absence of light.
3.	It is the function of chloroplast, peroxisomes and mitochondria.	It is the function of mitochondria alone.

17. Energy yielding metabolisms: Paths of energy synthesis through Glycolysis, Citric acid cycle, plant mitochondrial electron transport chain, alternative oxidase, PPP cycle, regulation of respiratory pathways, Lipid metabolism: fatty acid biosynthesis and oxidation.

In respiration carbohydrate is broken down, as a result the potential energy is transformed into kinetic form. Though it is a destructive or katabolic process, yet respiration is extremely beneficial, because it releases the necessary energy for performing the life functions.

Plants take in free oxygen from the air which enters through the stomata and lenticels and diffuses through the continuous intercellular space system to reach the living cells. It oxidises sugar, particularly glucose, into carbon dioxide and water vapour, thereby liberating considerable energy.

The reaction may be represented thus:

$C_6H_{12}O_6 + 6O_6 = 6CO_2 + 6H_2O$ + energy (674 cal.)

Though sugar is the main oxidisable material used during the process, other materials and in extreme cases, even protoplasm may be broken down for the liberation of energy. Carbon dioxide and water vapour go out through the stomata and lenticels.

At night when stomata remain closed gaseous exchange takes place through the lenticels. Thus respiration is essentially a process of biological oxidation in which oxygen absorbed from the air breaks down the food (sugar) into carbon dioxide and water, thereby releasing the stored energy.

During, day time when the rate of photosynthesis is higher than the rate of respiration and when both the processes are going on simultaneously, all the oxygen liberated in photosynthesis is not consumed in respiration, whereas all the CO_2 liberated in respiration is completely consumed in photosynthesis and some more CO_2 must enter the plant for the higher rate of photosynthesis.

As a result, during day time, the exchange of gases between the plant and the atmosphere is that O_2 is liberated and CO_2 is consumed. Thus the effects of respiration are completely masked during day time. During night, however, there is undoubtedly no photosynthesis, and only exchange of gases between the plant and the atmosphere is the effect of the process of respiration—O_2 consumed and CO_2 liberated.

$$\text{Pyruvate} + \text{NAD+} + \text{Co-A} \xrightarrow[\text{dehydrogenase}]{\text{pyruvate}} \text{Acetyl Co-A} + \text{NADH} + H^- + CO_2^-$$

Acetyl Co-A can enter into mitochondria while pyruvic acid cannot.

Definition:

The term respiration (L. respirare = to breath) was first used to describe the breathing i.e. exchange of gases between the organism and the environment.

Subsequently the term respiration was used in a wider sense including breathing, transport of gases as well as the oxidation of food leading to liberation of CO_2 and energy.

It is now agreed that respiration has two fundamental processes-external respiration and internal respiration. The external respiration or breathing is a physical process of exchange of respiratory gases ($O_2$and CO_2) between the organisms and the surroundings. Lavoisier (1789) first studied external respiration in animals. He observed that animals take in oxygen and give out $CO_2 + H_2O$. Higher animal possesses special organs for gas exchange but plants have stomata and lenticels for this purpose. This is also facilitated by air spaces present in the parenchyma.

Internal respiration, on the other hand, is a chemical process of food oxidation that occurs within the cell to liberate free energy, CO_2 and H_2O. Internal respiration takes place in the cytosol and mitochondria of eukaryotic cells. Hence, it is also known as cellular or tissue respiration.

Types of Respiration:

Depending on the availability of oxygen, Sachs (1890) classified cellular respiration into two major types: aerobic and anaerobic.

(i) Aerobic Respiration:

It uses oxygen and completely oxidizes the organic food to carbon dioxide and water. It, therefore, releases the entire energy available in glucose. It occurs in most plants and animals. The organisms which carry on this type of respiration are called aerobes.

$$C_6H_{12}O_6 + 6O_2 \xrightarrow{\text{Enzymes}} 6CO_2 + 6H_2O + 686 \text{ kcal/2870 kJ}$$

686 kcal or 2870 kJ of energy is liberated per mole of glucose. The value was previously calculated to be 673 Kcal. One Kcal is equal to 1000 calories. It is that amount of energy (as heat) which can raise the temperature of one litre of water 1^o C.

(ii) Anaerobic Respiration:

It does not use molecular oxygen and incompletely oxidizes the organic food with or without production of carbon dioxide. It, therefore, releases a small amount of energy. It occurs in yeasts, certain bacteria and some parasitic worms (e.g. Ascaris, Taenia). The organisms which carry on anaerobic respiration are termed anaerobes. Anaerobes may be either facultative or obligate.

(a) Facultative anaerobes:

Organisms which normally require oxygen but which can live anaerobically when grown on suitable media e.g., Butyric acid bacteria, Lactic acid bacteria, Bacillus phosphoresces.

(b) Obligate anaerobes:

They live in the absence of oxygen or in the presence of negligible concentration of this gas. The common products of anaerobic respiration are CO_2 ethyl alcohol and lactic acid.

$$C_6H_{12}O_6 \xrightarrow{\text{Enzymes}} \underset{\text{ethyl alcohol}}{2CO_2 + 2C_2H_5OH} + 59\ \text{kcal} / 247\ \text{kJ}$$

$$C_6H_{12}O_6 \xrightarrow{\text{Enzymes}} \underset{\text{lactic acid}}{2C_3H_6O_3} + 36\ \text{kcal} / 150\ \text{kJ}$$

Anaerobic respiration is the only mode of respiration in some micro-organisms. In higher organisms it occurs as a temporary measure.

Anaerobic respiration cannot continue for long in higher organisms because:

(i) It yields little energy;

(ii) More substrate is decomposed so that little is left for growth and repair;

(iii) Some of the end products and intermediates of anaerobic respiration are toxic in higher concentration.

Protoplasmic and Floating Respiration:

Respiration which utilizes proteins as substrate is called protoplasmic respiration, whereas that uses carbohydrates or fats is termed floating respiration. Protoplasmic respiration cannot be continued for long as it depletes protoplasm of structural and functional proteins as well as liberates toxic ammonia.

Gaseous Exchange:

Lavosier observed that in respiration of animals, oxygen is taken in from the air. In return they give out carbon dioxide and water. There is a regular exchange of $O_2 \rightarrow CO_2$ in most of the organisms. Some organisms, especially micro-organisms, do not require oxygen for their respiration. Some of them give out CO_2 while a few do not do so. In these organisms there is no gaseous exchange.

Gaseous exchange occurs not only between the organisms and its environment but also between every cell and its surrounding environment. An animal cell exchanges gases with the extracellular fluid while a plant cell does so with the air present in intercellular spaces. An organism shows exchange of gases in a liquid or gaseous environment depending upon the habitat.

Mechanism of Respiration:

There are two major phases of respiration:

(i) Glycolysis, and

(ii) Krebs cycle.

During process of respiration, carbohydrates are converted into pyruvic acid through a series of enzymatic reactions. This series of reactions is known as glycolysis which takes place in cytosol.

Now, pyruvic acid enters mitochondria, where several enzymes catalyse the reactions, and pyruvic acid finally converts into CO_2 and water. This series of enzymatic reactions is known as Krebs cycle (after name of its discoverer Sir Hans Adolf Krebs (1900-1981), awarded Nobel Prize in 1953), or tricarboxylic acid (TCA) or citric acid cycle.

1.Glycolysis:

Glycolysis is a term used to describe the sequential series of reactions present in a wide variety of tissues that starts with a hexose sugar (usually glucose) and ends with pyruvic acid. This term has originated from Greek words, glycos = sugar and lysis = splitting.

The scheme of glycolysis was discovered by three German Scientists, Gustav Embden, Otto Meyerhof and J. Parnas, and therefore, referred as EMP pathway, after the abbreviation of their last names.

Glycolysis is the first stage in the breakdown of glucose and is common to all organisms. This means, glycolysis is common to both aerobic and anaerobic modes of respiration. In anaerobic organisms, this is only process in respiration. Glycolysis occurs in cytoplasm of cells. During this process, glucose undergoes partial oxidation to form two molecules of pyruvic acid.

In plants, glucose is derived from sucrose, which is the end product of photosynthetic carbon reactions (also known as dark reactions) or from storage carbohydrates.

Sucrose is converted into glucose and fructose by the enzyme invertase. Now, these two monosaccharides (i.e., glucose and fructose) enter glycolysis or EMP pathway.

The main steps of glycolytic pathway are as follows:

Glycolysis is carried out in following different steps:

a. Phosphorylation of Sugar (i.e., First Phosphorylation):

Glucose and fructose are phosphorylated to give rise to glucose-6-phosphate and fructose-6-phosphate, respectively, by the activity of enzyme hexokinase, in presence of ATR The phosphorylated form of glucose then isomerises to produce fructose-6-phosphate. Isomerisation takes place with the help of enzyme phosphohexose isomerase.

Further steps of metabolism of glucose and fructose are quite similar.

Equations are as follows:

$$\text{Glucose (6C)} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{hexokinase}} \text{Glucose-6-phosphate} + \text{ADP}$$

Now isomerisation occurs :

$$\text{Glucose-6-phosphate} \underset{\text{isomerase } Mg^{2+}}{\overset{\text{phosphohexose}}{\rightleftharpoons}} \text{Fructose-6-phosphate}$$

b. Phosphorylation of Fructose-6-Phosphate (i.e., Second Phosphorylation):

Now, fructose-6-phosphate is phosphorylated and fructose-1, 6-bisphosphate produced by the action of enzyme phosphofructokinase in presence of ATP.

$$\text{Fructose-6-phosphate} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{phosphofructokinase}} \text{Fructose-1, 6-bisphosphate} + \text{ADP}$$

c. Splitting:

Now, fructose- 1, 6-bisphosphate splits into two molecules of triose phosphate, i.e., 3-phosphoglyceraldehyde (PGAL) and dihydroxyacetone phosphate (Di HAP), which are interconvertible.

$$\text{Fructose-1, 6-bisphosphate} \overset{\text{aldolase}}{\rightleftharpoons} \text{3-phosphoglyceraldehyde (PGAL)} + \text{dihydroxyacetone phosphate (Di HAP).}$$

d. Oxidative Dehydrogenation:

After formation of 3-phosphoglycerldehyde (PGAL), the glycolytic pathway enters the energy conserving phase. Here, it is oxidized to a carboxylic acid, i.e., 1,3-bisphosphoglycerate, and NAD is reduced to NADH.

$$\text{3-phosphoglyceraldehyde} + \text{NAD} \underset{\text{dehydrogenase}}{\overset{\text{glyceraldehyde phosphate}}{\rightleftharpoons}} \text{1, 3-bisphosphoglycerate} + \text{NADH} + \text{H}$$

e. Formation of ATP:

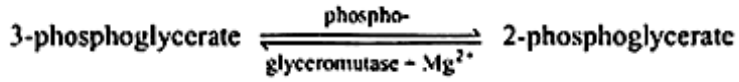
In next step of glycolysis, 3-phosphoglycerate is formed from 1, 3-bisphosphoglycerate by enzymatic activity of phosphoglycerate kinase, and ATP is generated during this process. Direct synthesis of ATP from intermediate metabolites is called substrate level phosphorylsation.

$$\text{1, 3-bisphosphoglycerate} + \text{ADP} \underset{\text{kinase} + Mg^{2+}}{\overset{\text{phosphoglycerate}}{\rightleftharpoons}} \text{3-phosphoglycerate} + \text{ATP}$$

This type of formation of ATP, where a phosphate group is directly transferred from a substrate to ADP to form ATP, is different from the ATP produced by ATP synthesis during oxidative phosphorylation in mitochondria or in chloroplasts (During photophosphorylation in photosynthesis).

f. Isomerisation:

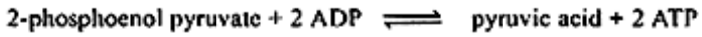
In next step 3-phosphoglycerate converts into its isomer 2-phosphoglycerate by catalytic activity of enzyme phosphoglyceromutase.



g. Dehydration:

In subsequent step 2-phosphoglycerate converts into phosphoenol pyruvate (PEP) in the presence of enzyme pyruvate kinase and liberates ATP.





h. Generation and Utilisation of ATP during Glycolysis:

During glycolytic pathway, the molecules of ATP are produced as follows:

(i) Direct transfer of phosphate to ATP.

(ii) Oxidation of NADH produced during glycolytic pathway to NAD^+.

i. In the end of glycolysis net gain of ATP:

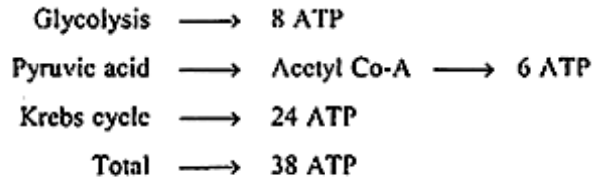
(i) During glycolysis two triose phosphate molecules are formed from one glucose molecule, and 4 ATP molecules are produced.

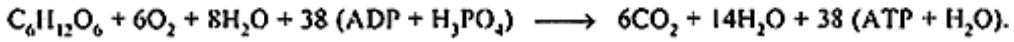
(ii) Out of 4 ATP molecules, 2 ATP molecules are utilised in first few steps in converting glucose to fructose-1, 6 bisphosphate.

(iii) Moreover, three ATP molecules are produced from oxidation of each of two molecules of NADH produced during catabolism of glucose.

(iv) In all, a net gain of 8 molecules occurs during process of glycolysis.

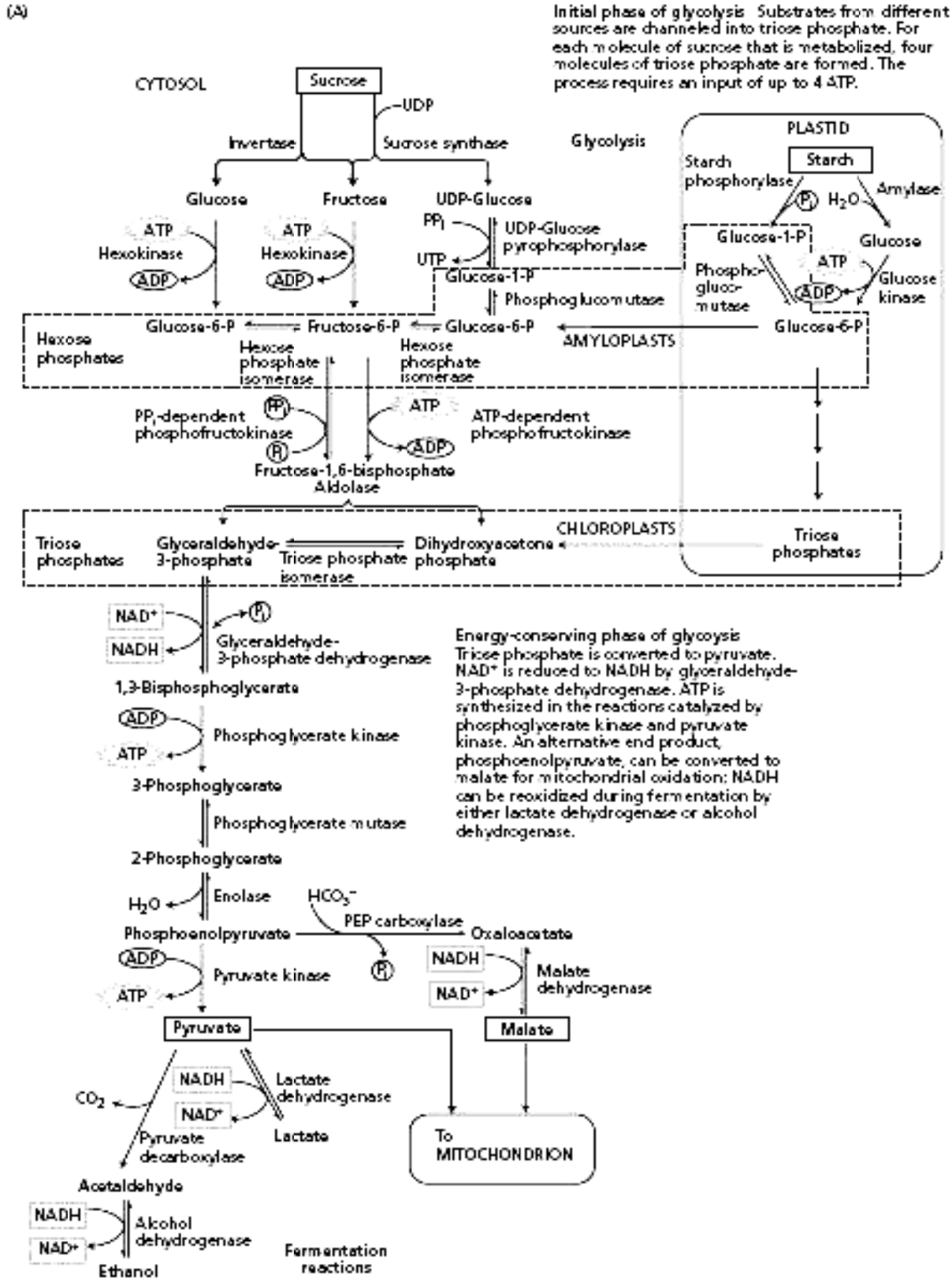
(v) However, in anaerobic respiration, NADH + H^ is not converted to ATP, and therefore, only 2 ATP molecules are produced.





Regulation of Glycolysis:

- When ATP is needed, glycolysis is activated
- When ATP levels are sufficient, glycolysis activity decreases
- Control points:
  1. Hexokinase
  2. Phosphofrucktokinase-1
  3. Pyruvate kinase

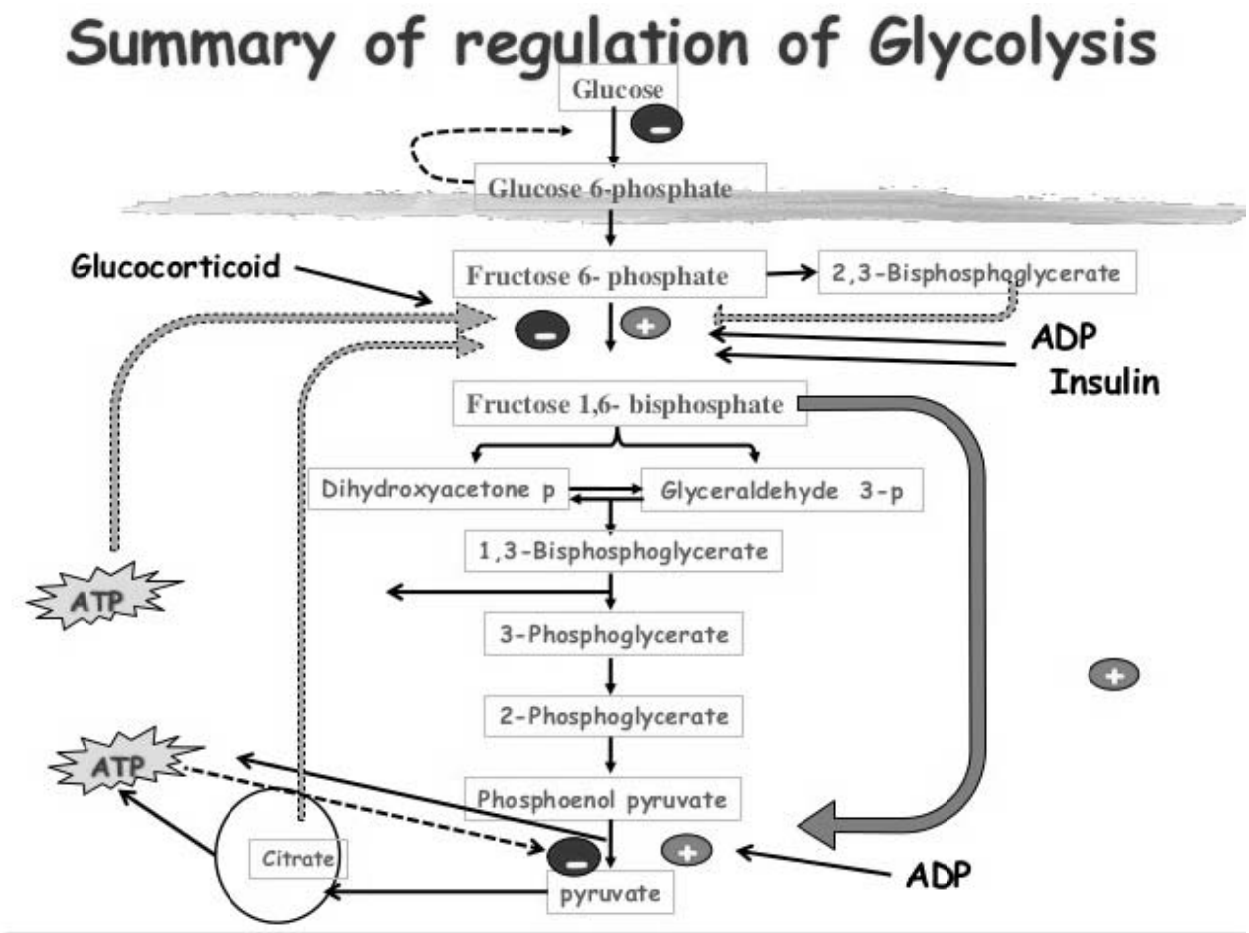

(A)
Initial phase of glycolysis Substrates from different sources are channeled into triose phosphate. For each molecule of sucrose that is metabolized, four molecules of triose phosphate are formed. The process requires an input of up to 4 ATP.
CYTOSOL
Sucrose
UDP
Invertase
Sucrose synthase
Glycolysis
PLASTID
Starch
Starch phosphorylase
Amylase
Glucose
Fructose
UDP-Glucose
ATP
Hexokinase
ADP
PPi
UTP
UDP-Glucose pyrophosphorylase
Glucose-1-P
Phosphoglucomutase
Glucose-6-P
Fructose-6-P
Hexose phosphates
Hexose phosphate isomerase
AMYLOPLASTS
Phospho-gluco-mutase
Glucose kinase
PPi-dependent phosphofructokinase
ATP-dependent phosphofructokinase
Fructose-1,6-bisphosphate
Aldolase
CHLOROPLASTS
Triose phosphates
Glyceraldehyde-3-phosphate
Triose phosphate isomerase
Dihydroxyacetone phosphate
NAD+
NADH
Glyceraldehyde-3-phosphate dehydrogenase
1,3-Bisphosphoglycerate
Phosphoglycerate kinase
3-Phosphoglycerate
Phosphoglycerate mutase
2-Phosphoglycerate
Enolase
H2O
HCO3−
PEP carboxylase
Phosphoenolpyruvate
Oxaloacetate
Pyruvate kinase
Malate dehydrogenase
Pyruvate
Malate
CO2
Lactate dehydrogenase
Lactate
Pyruvate decarboxylase
To MITOCHONDRION
Acetaldehyde
Alcohol dehydrogenase
Ethanol
Fermentation reactions
Energy-conserving phase of glycoysis Triose phosphate is converted to pyruvate. NAD+ is reduced to NADH by glyceraldehyde-3-phosphate dehydrogenase. ATP is synthesized in the reactions catalyzed by phosphoglycerate kinase and pyruvate kinase. An alternative end product, phosphoenolpyruvate, can be converted to malate for mitochondrial oxidation; NADH can be reoxidized during fermentation by either lactate dehydrogenase or alcohol dehydrogenase.

Regulation of Glycolysis takes place at three irreversible reactions

1. Hexokinase (feed back inhibition) or Glucokinase (insulin)

2. Regulation of Phosphofructokinase (Allosteric Activator/Inhibitor)

3. Regulation of Pyruvate kinase (Allosteric Activator/ inhibitor)



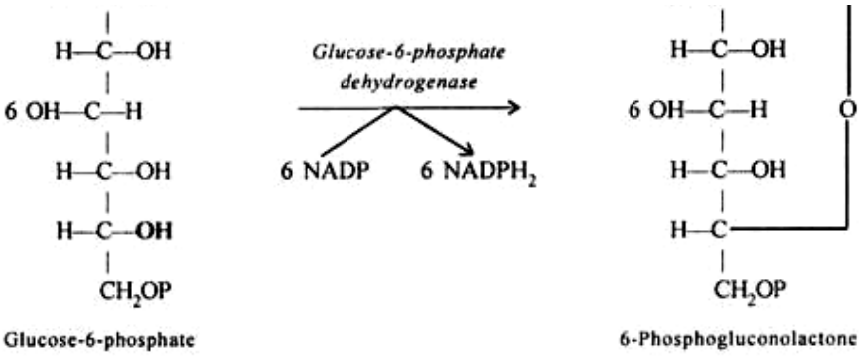
Pentose Phosphate Pathway (Warburg-Dicken's Pathway):

It involves the oxidation of Glucose-6-Phosphate to 6-Phosphogluconic acid which in turn is converted into pentose phosphates. In this pathway glucose-6-phosphate is directly oxidised without entering glycolysis, hence it is also known as Direct Oxidation Pathway or Hexose Monophosphate Shunt.

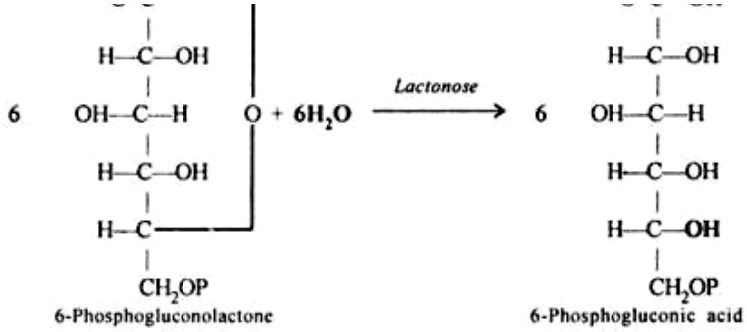
Reactions of Pentose Phosphate Pathway:

Starting from 6-molecules of glucose-6-phosphate, the various reactions of this pathway (Fig. 16.18) can be summarised as follows:

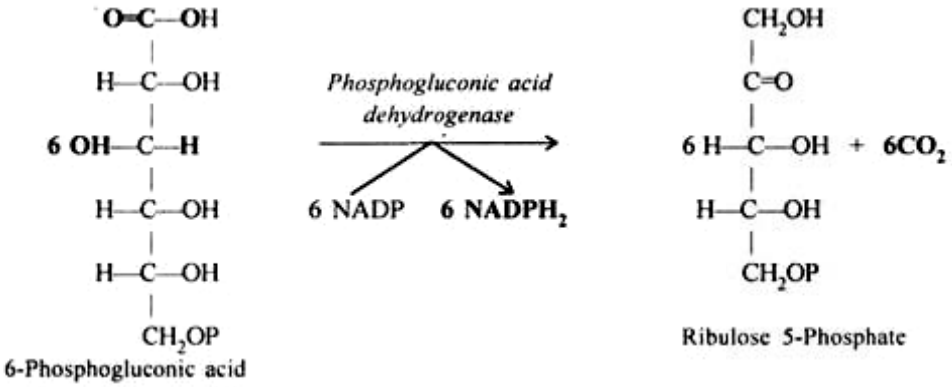
(1) 6 molecules of glucose-6-phosphate in the presence of coenzyme NADP are converted (oxidised) into 6 molecules of 6-phosphogluconolactone by the enzyme glucose-6-phosphate dehydrogenase. 6 molecules of NADP are reduced in the reaction which is reversible.



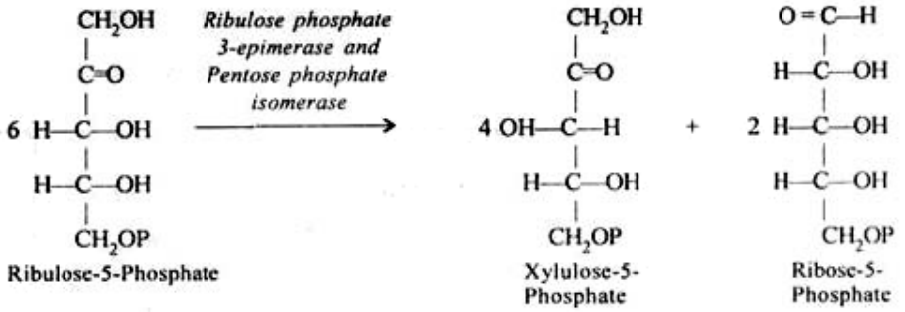
(2) 6-Phosphogluconolactone is hydrolysed by the enzyme Lactonase to produced 6 molecule of 6-phosphogluconic acid.



(3) 6-Phosphogluconic acid is oxidatively decarboxylated by the enzyme 6-Phosphogluconic acid dehydrogenase. 6 molecules of NADP are reduced, 6 molecules of CO_2 are released and 6 mols, of Ribulose-5-Phosphate are produced.

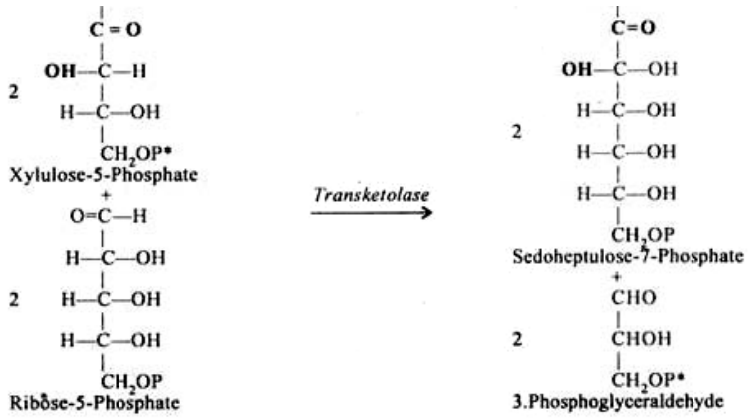


(4) 6 mols. of Ribulose-5-P isomerise into 4 mols. of XyIuIose-5-Phosphate and 2 mols. of Ribose-5-Phosphate in the presence of Ribulose phosphate-3-epimerase and Pentose phosphate isomerase

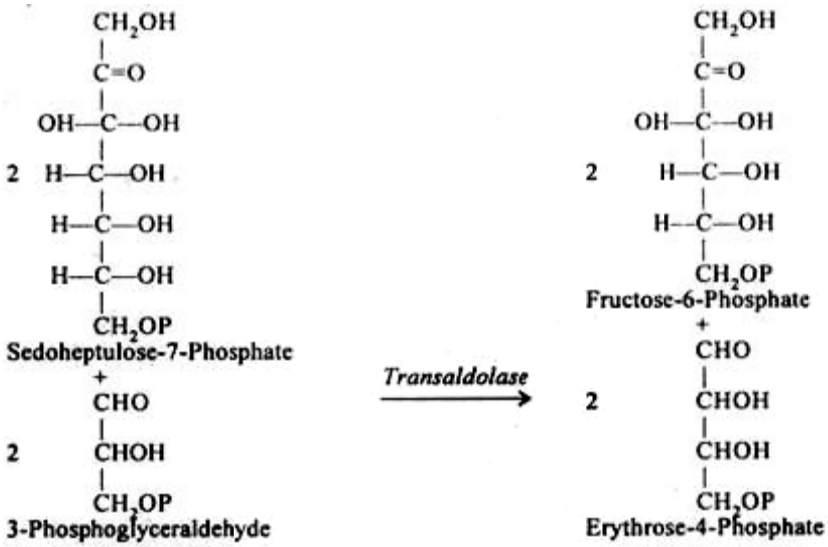


respectively.

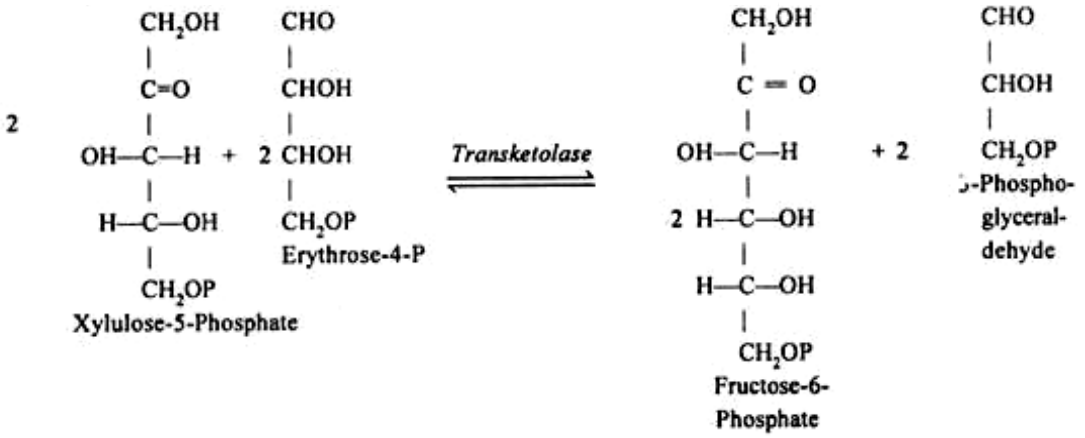
(5) 2 mols. of xylulose-5-Phosphate and 2 mols. of Ribose-5-phosphate combine in the presence of Transketolase to form 2 mols. of Sedoheptulose-7-Phosphate and 2 mols. of 3-Phosphoglyceraldehyde.



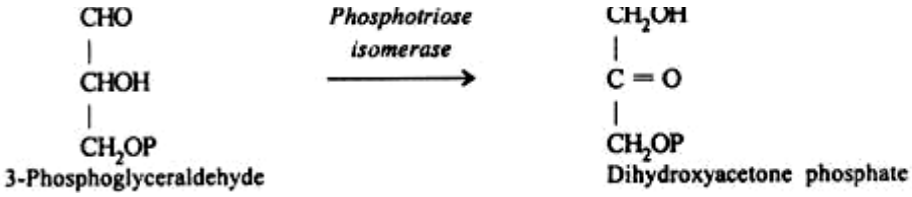
(6) 2 mols. of Sedoheptulose-7-Phosphate and 2 mols. of 3-Phosphoglyceraldehyde combine in the presence of Transaldolase to form 2 mols. of Fructose-6-Phosphate and 2 mols. of Erythrose-4-Phosphate (4-carbon atoms sugar).



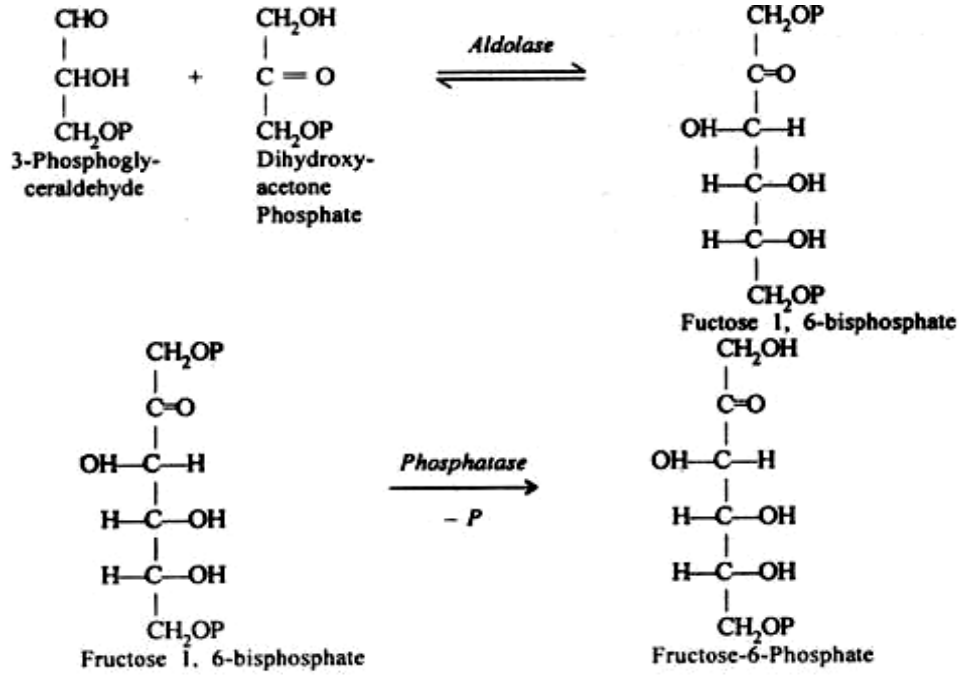
(7) 2 mols. of Erythose-4-Phosphate react with remaining two mols. of xylulose-5-Phosphate (see reaction No. 4 and 5) in the presence of Transketolase to form 2 mols. of Fructose- 6-Phosphate and 2 mols of 3-Phosphoglyceraldehyde.



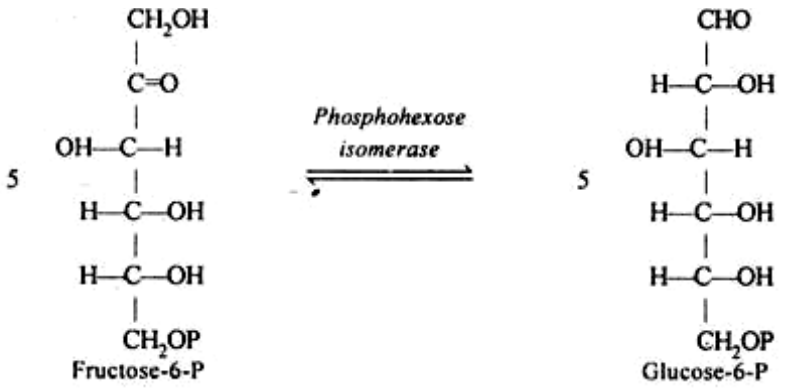
(8) One mol. of 3-phosphoglyceraldehyde isomerises into dihydroxyacetone phosphate. The enzyme is Phosphotriose isomerase.



(9) Remaining one mole, of 3-Phosphoglyceraldehyde unites with Dihydroxyacetone phosphate in presence of Aldolase to form one mol. of Fructose 1, 6-bisphosphate. The latter, in the presence of Phosphatase forms one mol. of Fructose 6-Phosphate.



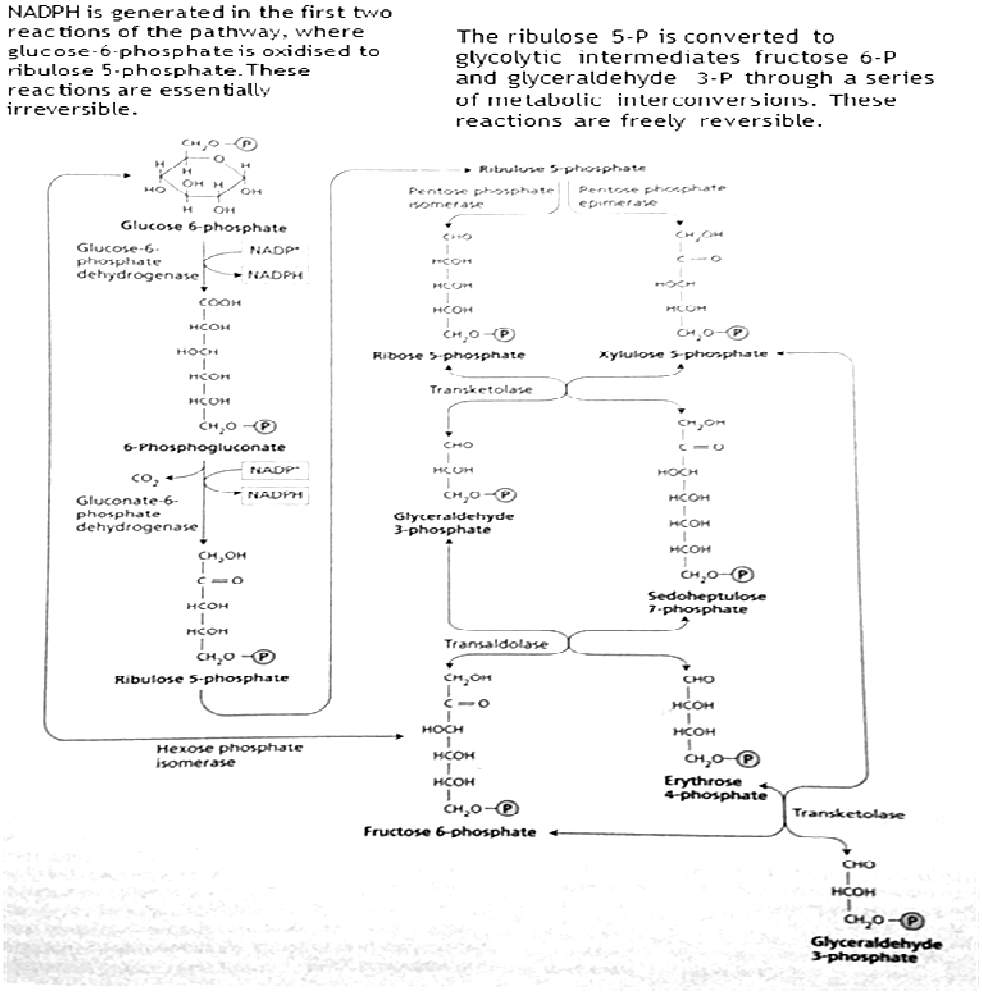
(10) 5 molecules of Fructose-6-phosphate produced in reactions 6, 7 and 9, isomerise into 5 mols. of Glucose-6-P in presence of Phosphohexose isomerase.



To summarise, 6 mols. of Glucose-6-P which enter into this pathway after oxidation produce 6 mols. of CO_2 (Reaction No. 3. CO_2 comes from C-No. 1 of the glucose molecule) and 12 mols. of reduced coenzymes $NADPH_2$ (reaction 1 and 3) while 5 mols of Glucose-6-Phosphate are regenerated (Reaction No. 10).

6 (Glucose-6-P) + 12 $NADP^+$ → 5 (Glucose-6-P) + 12 (NADPH + H^+) + 6 CO_2

All the enzymes of pentose phosphate pathway are present in cytosol.



Significance of Pentose-Phosphate-Pathway:

(i) It provides alternative route for carbohydrate breakdown.

(ii) It generates NADPH molecules which are used as reductants in biosynthetic processes under conditions when NADPH molecules are not generated by photosynthesis. It is therefore, especially important in non-photosynthetic tissues such as in differentiating tissues, germinating seeds and during periods of darkness. Production of NADPH is not linked to ATP generation in pentose phosphate pathway.

(iii) It provides Ribose sugars for the synthesis of nucleic acids.

(iv) It plays important role in fixation of CO_2 in photosynthesis through Ribulose-5-Phosphate. (Ribulose 1, 5-bisphosphate derived from Ribulose-5-Phosphate is the primary acceptor of CO_2 in photosynthesis).

(v) It provides Erythrose-4-phosphate which is required for the synthesis of shikimic acid. The latter is precursor of aromatic ring compounds.

2. Oxidative Decarboxylation Pyruvic Acid:

Now, pyruvic acid generated in cytoplasm through glycolysis is transferred to mitochondria. This is initiation of second phase of respiration. As soon as, pyruvic acid enters the mitochondria, one of the three carbon atoms of pyruvic acid is oxidised to carbon dioxide in a reaction called oxidative decarboxylation.

Here, pyruvate is first decarboxylated, and thereafter oxidised by enzyme pyruvate dehydrogenase. This enzyme is made up of a decarboxylase, lipoic acid, TPP, transacetylase and Mg^{+2}.

Acetyl Co-A acts as substrate entrant for Krebs cycle.

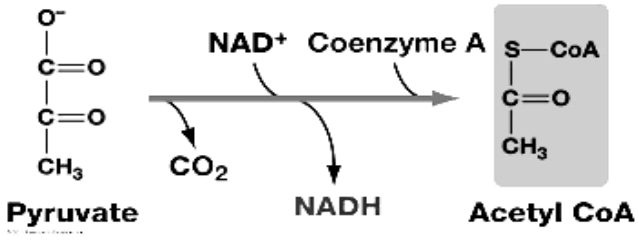
The equation is as follows:

$$\text{Pyruvate} + \text{NAD+} + \text{Co-A} \xrightarrow[\text{dehydrogenase}]{\text{pyruvate}} \text{Acetyl Co-A} + \text{NADH} + H^{-} + CO_2^{-}$$

Acetyl Co-A can enter into mitochondria while pyruvic acid cannot.

Pyruvate oxidation, which follows glycolysis, is essentially the conversion of **pyruvate** molecules to **carbon dioxide**, **acetyl coenzyme A**, and **NADH**. The last step of glycolysis produces two **pyruvate** molecules in the cytosol, which are then brought to the mitochondrial matrix via active transport.

Acetyl Co-A can enter into mitochondria while pyruvate acid cannot.



3. Krebs Cycle:

Sir Hans Adolf Krebs, discovered role of pyruvate in conversion of glucose hydrogens into fumarate. He discovered, in 1937, tricarboxylic acid cycle (i.e., TCA cycle), also known as Citric acid cycle or Krebs cycle. Citric acid cycle occurs in matrix of mitochondria. This cycle involves two decarboxylations and four dehydrogenations.

Various steps of these reactions are as follows:

The starting point of Krebs cycle is entrance of acetyl Co-A into a reaction to form citric acid. Krebs elucidated this cycle, and explained how pyruvate is broken down to $CO_2$and H_2O. For this pioneer work Krebs was awarded Nobel Prize in 1953.

In the first reaction of Krebs cycle, one molecule of acetyl Co-A combines with 4-carbon oxaloacetic acid (OAA); with the result 6-carbon citric acid is produced, and Co-A is released. This reaction is catalysed by enzyme citrate synthase.

$$\underset{(4C)}{\text{Acetyl Co-A + oxaloacetic acid}} \xrightarrow[\text{enzyme}]{\text{citrate synthase}} \underset{(6C)}{\text{Citric acid + Co-A}}$$

Now, citrate (citric acid) is isomerised to isocitrate (isocitric acid).

$$\text{Citric acid} \xrightarrow[\text{enzyme}]{\text{aconitase}} \text{Cis-aconitic acid + } H_2O$$

$$\text{Cis-aconitic acid + } H_2O \xrightarrow[\text{enzyme}]{\text{aconitase}} \underset{\text{(Isocitrate)}}{\text{Isocitric acid}}$$

Cis-aconitic acid is converted into isocitric acid with the addition of water in the presence of iron containing enzyme aconitase.

$$\underset{\text{(Isocitrate)}}{\text{Isocitric acid + NAD}^+} \xrightarrow[\text{dehydrogenase}]{\text{isocitrate}} \underset{\text{(oxalosuccinate)}}{\text{Oxalosuccinic acid + NADH + H}^+}$$

$$\underset{\text{(oxalosuccinate)}}{\text{Oxalosuccinic acid}} \xrightarrow[\text{decarboxylase}]{\text{oxalosuccinic}} \text{α-Ketoglutaric acid + } CO_2$$

$$\text{α-ketoglutaric acid + Co-A + NAD}^+ \xrightarrow[\text{dehydrogenase}]{\text{α- ketoglutaric}} \text{Succinyl Co-A + NADH + H}^+ + CO_2$$

$$\text{Succinyl Co-A + } H_2O + \text{GDP + iP} \xrightarrow[\text{thiokinase}]{\text{succinic}} \text{Succinic acid + Co-A + GTP}$$

$$\text{GTP + ADP} \longrightarrow \text{GDP + ATP (substrate phosphorylation)}$$

$$\text{Succinic acid + FAD} \xrightarrow[\text{dehydrogenase}]{\text{Succinic}} \text{Fumaric acid + } FADH_2$$

$$\text{Fumaric acid + } H_2O \xrightarrow[\text{enzyme}]{\text{Fumarase}} \text{Malic acid}$$

$$\text{Malic acid + NAD}^+ \xrightarrow[\text{dehydrogenase}]{\text{Malic}} \text{Oxaloacetic acid (OAA) + NADH + H}^+$$

During citric acid cycle (Krebs cycle) 3 molecules of NAD^+ and one molecule of FAD (Flavin Adenine Dinucleotide) are reduced to produce NADH and $FADH_2$, respectively.

During citric acid cycle NADH and FADH, are produced. Now, they are linked with electron transport system (ETS) and produce ATP by oxidative phosphorylation.

This may be summarised in following equation:

$$\text{Pyruvic acid + 4NAD}^+ + \text{FAD + 2}H_2O + \text{ADP + iP}$$

$$\downarrow \text{ mitochondrial matrix}$$

$$3CO_2 + \text{4NADH + 4H}^+ + FADH_2 + \text{ATP}$$

In the end of Krebs cycle, glucose molecule is completely oxidised. From one glucose molecule, two pyruvic acid molecules are formed. After oxidation of one pyruvic acid molecule, three CO_2 molecules are released. Thus, in all 6 molecules of CO_2 are released.

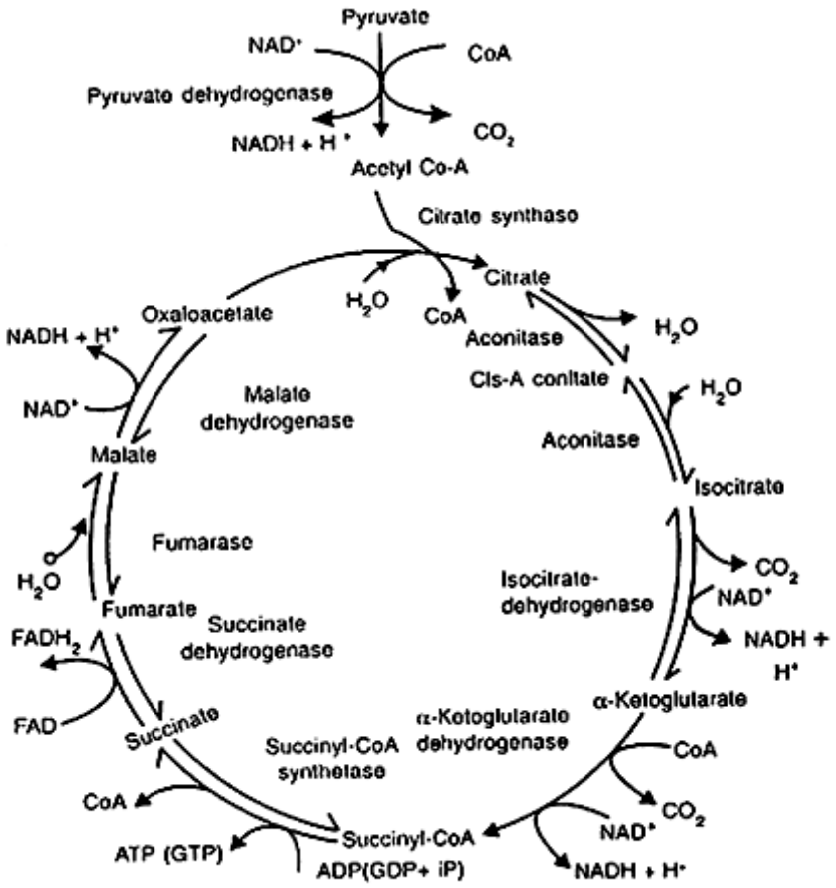


Fig. 4.3. Krebs cycle or citric acid cycle.

Regulation of the Citric Acid Cycle

- Pathway controlled by:

  (1) Allosteric modulators

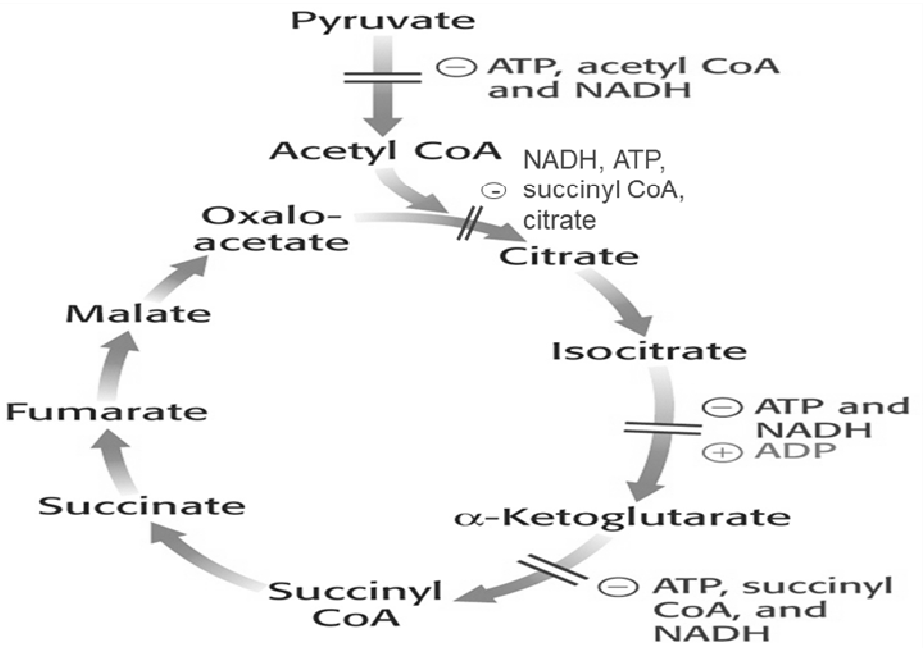
  (2) Covalent modification of cycle enzymes

  (3) Supply of acetyl CoA (pyruvate dehydrogenase complex)

Three enzymes have regulatory properties

- *citrate synthase* (is allosterically inhibited by NADH, ATP, succinyl CoA, citrate – feedback inhibition)
- *isocitrate dehydrogenase* (ICDH) (allosteric effectors: (+) ADP; (-) NADH, ATP. Bacterial ICDH can be covalently modified by kinase/phosphatase)
- *α-ketoglutarate dehydrogenase complex* (inhibition by ATP, succinyl CoA and NADH

Regulation of Kreb's cycle



Electron Transport System (ETS):

By the end of Krebs cycle, glucose molecule oxidises completely, but the energy does not release till NADH and $FADH_2$ oxidise through electron transport system (ETS). The metabolic pathway through which electron passes from one carrier to another, is called electron transport system (ETS). The electron transport system is also known as electron transport chain or mitochondrial respiratory chain.

The electron transport system consists of a series of coenzymes and cytochromes that take part in passage of electrons from a chemical to its ultimate acceptor. The passage of electrons from one-enzyme or cytochrome to the next takes place with a loss of energy at each step. Electron transport system is operative in the inner mitochondrial membrane.

The electron carriers include flavins, iron sulphur complexes, quinones and cytochromes. Most of them are prosthetic groups of proteins.

Electron transport system in mitochondria consists of four complexes which are found in bases of stalked particles in the inner mitochondrial membrane, and also ubiquinone (UQ) or coenzyme Q and cytochrome c which are not bound to stalked particles but act as mobile electron carriers between the complexes.

Complex-I:

Consists of NADH-dehydrogenase or NADH-Q reductase which contains a flavoprotein FMN (flavin mononucleotide) and is associated with iron-sulphur (Fe-S) proteins. This complex is responsible for passing electrons (also protons) from mitochondrial NADH to ubiquinone (UQ), located within inner mitochondrial membrane.

$$NADH + H^+ + FMN \longrightarrow FMNH_2 + NAD^+$$

$$FMNH_2 + 2Fe^{3+}\,S \longrightarrow FMN + 2Fe^{2+}\,S + 2H^+$$

$$2Fe^{2+}\,S + Q + 2H^+ \longrightarrow 2Fe^{3+}\,S + QH_2$$

Complex-II:

Consists of succinate dehydrogenase which contains a flavoprotein FAD (flavin adenine dinucleotide) in its prosthetic group and is associated with non heme iron-sulphur (Fe S) proteins.

This complex receives electrons (also protons) from succinic acid (which is oxidised in Krebs cycle to form fumaric acid) and passes them to ubiquinone (UQ). Ubiquinone also receives reducing equivalents via $FADH_2$ that is generated during oxidation of succinate, through the activity of energy succinate dehydrogenase, in Krebs cycle.

$$FADH_2 + 2Fe^{3+}\ S \longrightarrow 2Fe^{2} + S + 2H^{+} + FAD$$

$$2Fe^{2+}\ S + Q + 2H^{+} \longrightarrow 2Fe^{3+}\ S + QH_2$$

Complex-III:

Consists of ubiquinol, cytochrome c and cytochrome bc_1 .The reduced ubiquinone is called ubiquinol. Here ubiquinol is oxidised with the transfer of electrons to cytochrome c via cytochrome bc_1. Cytochrome c is a small protein attached to outer surface of the inner mitochondrial membrane and acts as a mobile carrier for transfer of electrons between complex III and complex IV.

This complex is called QH_2-cytochrome c reductase complex. This bears three components, i.e., cytochrome b, non-heme iron sulphur (Fe – S), and cytochrome c_1. Coenzyme Q is also involved between Fe-S and cytochrome c_1.

The equations are as follows:

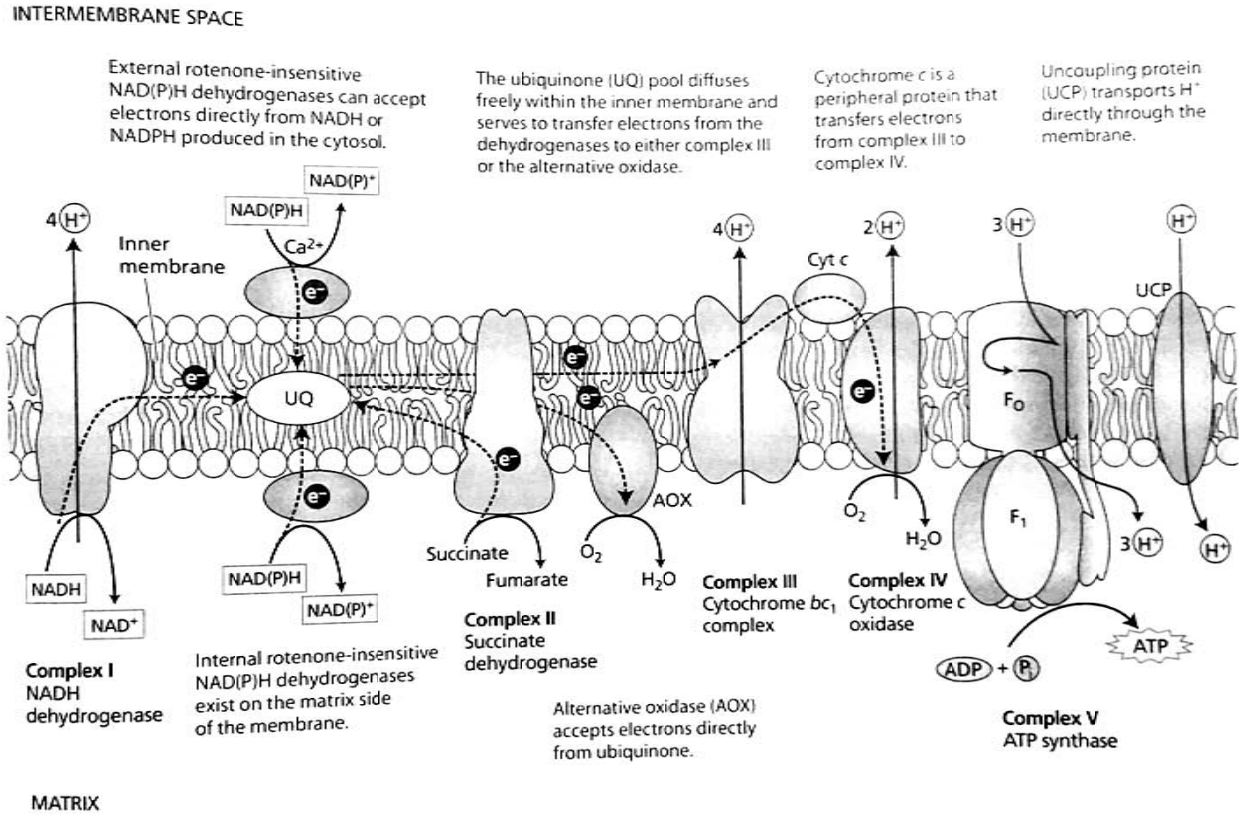
$$QH_2 + 2Fe^{3+}\ cy\ b \longrightarrow Q + 2H^{+} + 2Fe^{2+}\ cy\ b$$

$$2Fe^{2+}\ cy\ b + 2Fe^{3+}\ S \longrightarrow 2Fe^{3+}\ cy\ b + 2Fe^{2+}\ S$$

$$2Fe^{2+}\ S + Q + 2H^{+} \longrightarrow 2Fe^{3+}\ S + QH_2$$

$$QH_2 + 2Fe^{3+}\ cy\ c_1 \longrightarrow Q + 2H^{+} + 2Fe^{2+}\ cy\ c_1$$

Now, cytochrome c, transfers electrons to cy c. Like coenzyme Q, cy c is also mobile carrier of electrons.



Complex-IV:
Is known as cytochrome c oxidase complex. This contains cytochromes a and a_3, along with two copper centres. This complex receives electrons from cytochrome c and passes them to 1/2 O. Two protons are needed and Hp molecule is formed (terminal oxidation). Here, O_2 is ultimate acceptor of electrons. It combines with protons to form metabolic water or respiratory water.

$$\underset{(Fe^{++})}{2 \text{ Ferrocytochrome}} + 2H^+ + 1/2\ O_2 \rightleftharpoons \underset{(Fe^{+++})}{2 \text{ ferricytochrome } c} + H_2O$$

Complex-V:
When electrons are transferred from one carrier to next carrier via complexes 1 to IV in electron transport system (ETS), they are coupled to ATP synthase enzyme complex for production of ATP from ADP and inorganic phosphate (iP).
Here, number of ATP molecules synthesised during ETS, depends on nature of electron donor. Oxidation of one molecule of NADH gives rise to 3 molecules of ATP, and one molecule of $FADH_2$ gives rise to 2 molecules of ATP. ATP synthase complex is called complex V.
During transportation of electrons, hydrogen atoms split into protons and electrons. The electrons are carried by cytochromes. Before last stage, where hydrogen atom is accepted by oxygen to form water, the electrons again recombine with their protons. Oxygen acts as final hydrogen acceptor.
Oxidative Phosphorylation:
The whole process, where oxygen effectively allows the production of ATP by phosphorylation of ADP, is called oxidative phosphorylation. In other words, synthesis of ATP is called phosphorylation, and as it takes place in presence of oxygen, it is called oxidative phosphorylation.

$$2H \rightleftharpoons 2H^+ + 2e^-$$

$$NAD^+ + H^+ + H^+ + e^- + e^- \longrightarrow NADH + H^+$$

The enzyme required for synthesis of ATP, is called ATP synthase. This is located in F_1, or head piece of $F_0 - F_1$ or elementary particles. ATP synthase enzyme becomes active in ATP formation, where there is a proton gradient saving higher concentration of H_2.
ATP synthase, also known as complex V consists of two major components, i.e., F_1, and F_0. The F_1 headpiece is a peripheral membrane protein complex and contains the site for ATP from ADP and inorganic phosphate (iP).

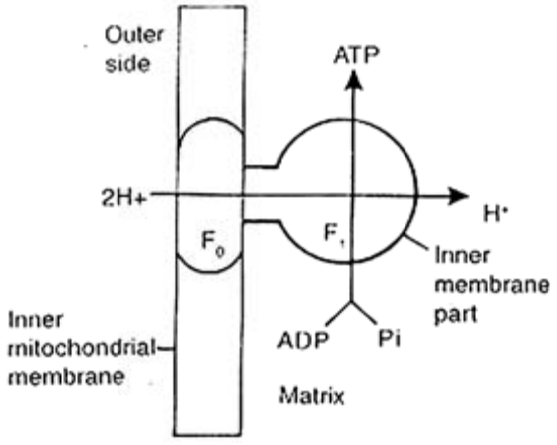


Fig. 4.5. Synthesis of ATP by inner membrane particles of mitochondrion.

Whereas, F_0 is an integral membrane mitochondrial-protein complex which forms the channel through which protons cross the inner membrane. The passage of protons through the channel is coupled to the catalytic site of the F_1 component for the production of ATP.
Oxidation of one molecule of $NADH_2$ produces 3 ATP molecules whereas a similar oxidation of $FADH_2$ produces 2 ATP molecules.

Net gain of ATP:

Complete oxidation of glucose to CO_2 and water shows that there is a net gain of 38 ATP. Each NADH + H^+ produces 3 ATP molecules, while $FADH_2$ forms only 2 ATP molecules at the end of reaction.

$$NADH + H^+ + 1/2\ O_2 + 3\ ADP + 3\ iP \longrightarrow NAD + 3\ ATP + H_2O$$

$$FADH_2 + 1/2\ O_2 + 2\ ADP + 2\ iP \longrightarrow FAD + 2\ ATP + H_2O$$

Thus, total gain of ATP in aerobic respiration is as follows:

However, in most eukaryotic cells, 2 molecules of ATP are required for transport of NADH produced in glycolysis into mitochondrion for further oxidation, and therefore, net gain of ATP is 36 molecules.

Significance of Krebs Cycle:

a. During Krebs cycle, carbon skeletons are obtained for use in growth and maintenance of the cell.
b. Many intermediate compounds are formed which are used in synthesis of other biomolecules, such as amino acids, nucleotides, chlorophyll, cytochromes and fats.
c. During this pathway amino acids are synthesised from α-ketoglutaric acid, pyruvic acid and oxaloacetic acid.
d. Here succinyl Co-A acts as starting molecule for synthesis of chlorophyll.
e. Krebs cycle is major pathway for generation of ATP molecules, which make energy currency of the cell.
f. Energy is released from glucose, and is used in various biochemical reactions.
g. Phenol, anthocyanin, etc., are produced from acetyl Co-A, whereas fatty acids are formed from glycerol.
h. Glutamic acid is formed from α-ketoglutaric acid; aspartic acid from oxaloacetic acid, and alanine from aspartic acid.
i. Amino acids are used in synthesis of proteins, nucleic acids, purines and pyrimidines.
j. Succinyl Co-A carries synthesis of pyrrole compounds of chlorophyll, cytochrome and phytochrome.
k. Krebs cycle is directly related to nitrogen metabolism, α-ketoglutaric acid, an intermediate of Krebs cycle is first acceptor molecule of NH_3 forming an amino acid, the glutamic acid. From glutamic acid various transamination reactions begin to form different amino acids which ultimately condense to form proteins.
l. Krebs cycle is also intimately related with fat metabolism. Dihydroxyacetone phosphate produced in glycolysis may be converted into glycerol via glycerol-3-phosphate and vice versa. After β-oxidation, fatty acids give rise to active 2-C units, the acetyl Co-A which enters the Krebs cycle.

Lipid metabolism

Digestion and Absorption:

Salivary juice produces a lipid hydrolyzing enzyme, especially in infants and children, known as lingual lipase. Lipids are not digested in the mouth, though lingual lipase is present because the high pH of the mouth does not favour the action of lingual lipase and also because the food remains here for a short period of time. As soon as the lipids enter the stomach, they get liquefied due to the heat of the stomach.

Further, the lipids get emulsified due to the peristaltic movements of the stomach. The gastric juice contains gastric lipase, which is inactive at the low pH of the stomach, whereas lingual lipase is active at this pH and it hydrolyses triacylglycerol's of short chain fatty acids (which are generally found in milk, so digestion in the stomach is seen only in children). The released fatty acids are absorbed via the stomach wall and enter the portal vein. The TAG with longer chain fatty acids dissolve and form fat droplets and finally enter the intestine.

In the intestine, the bile salts (sodium glycocholate and sodium taurocholate) and bile acids (cholic acid, chenodeoxycholic acid and cholesterol) help in emulsifying fats thereby making them susceptible to the digestive enzymes. The pancreatic secretions in the intestine contain pancreatic lipase which along with the help of a protein called co-lipase and lecithin acts on TAG at the water-oil interface.

Phospholipids are hydrolyzed by phospholipase A, B, C and cholesterol is hydrolyzed by cholesterol esterase.

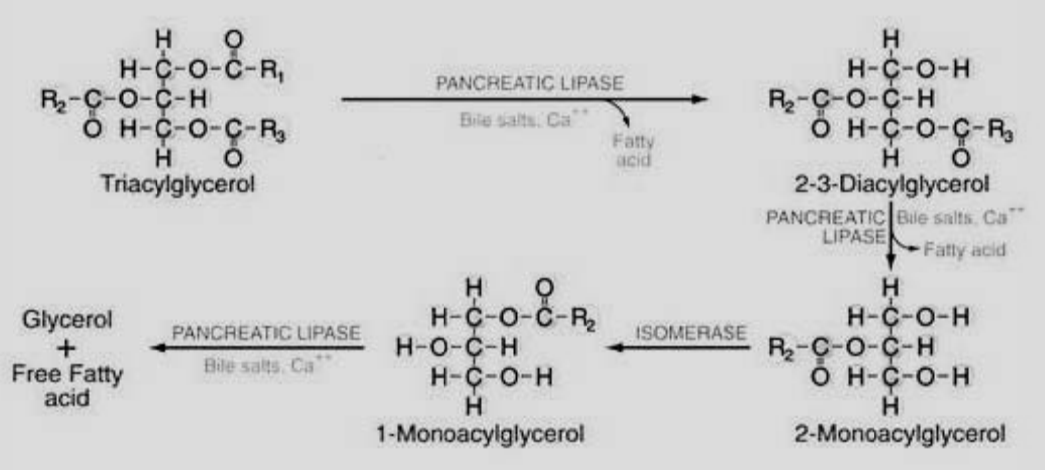
The end products of fat digestion are:
(1) Monoacylglycerols (MAG)

(2) Diacylglycerols (DAG)

(3) Triacylglycerol's (TAG)

(4) Free fatty acids and

(5) Glycerol.



Digestion of lipids takes place for a longer duration of time. Until and unless digestion of fats has not taken place, other food materials (carbohydrates and proteins) cannot be digested because the fats will cover the food and prevent enzymes reaching the food (hence take a fatty meal while going on a long journey).

The digested lipids enter the intestinal epithelium by diffusion or by a process called 'pinocytosis'. In the intestinal wall the free fatty acids and glycerol re-aggregate to form TAG i.e. here re-synthesis of digested TAG, phospholipids and cholesterol takes place (in order to maintain the concentration gradient of lipids).

The important pathways/cycles of lipid metabolism are briefly described:

1. Fatty Acid Oxidation:

The fatty acids in the body are mostly oxidized by α-oxidation. β-Oxidation may be defined as the oxidation of fatty acids on the p-carbon atom. This results in the sequential removal of a two carbon fragment, acetyl CoA (Fig. 67.10).

- **Knoop's β-oxidation of Fatty Acids:**

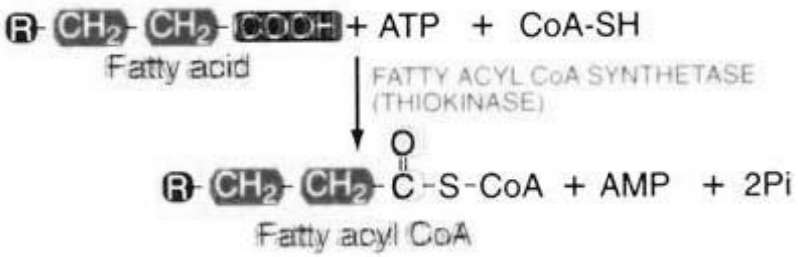
Oxidation of the fatty acids at the β-carbon atom to a carboxylic group is known as beta oxidation. This was proposed by the scientist Knoop, hence the name.

The steps involved in the β-oxidation of fatty acids are:

1. Activation of fatty acids
2. Formation of α-β unsaturated acyl-CoA (Enoyl-CoA)
3. Formation of β-hydroxy acyl-CoA
4. Formation of β-keto acyl-CoA
5. Thiolytic cleavage of keto acyl-CoA

1. Activation of fatty acids:

Oxidation of fatty acids can take place only if they are activated. The fatty acids are activated in the cytosol of the cell wherein the enzyme fatty acyl CoA synthetase (thiokinase) condenses the fatty acids with coenzyme-A by esterification which requires two high energy bonds.

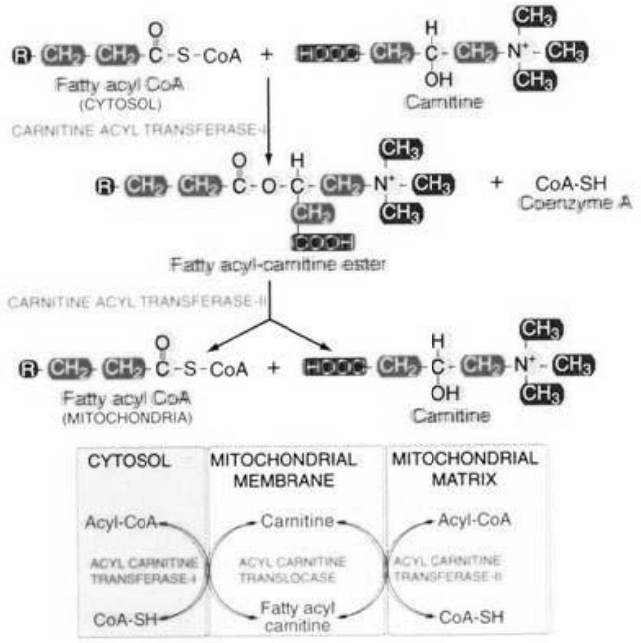


Further oxidation of fatty acids takes place in the mitochondrial matrix. The mitochondrial membrane is impermeable to the acylated fatty acids of the cytosol. Hence these are transported by carnitine mechanism after activation.

Carnitine mechanism:

Carnitine (beta-hydroxyl-gamma-trimethyl-ammonium butyrate) [$(CH_3)_3$-N^+– CH_2-CH(OH)-CH_2-COOH], is present in the inner mitochondrial membrane and it helps in the transport of fatty acids both form the cytosol to mitochondria and from mitochondria to the cytosol.

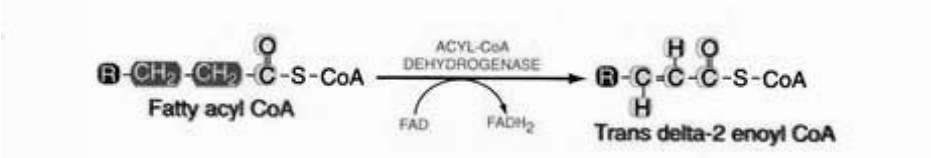
The mechanism of carnitine transfer is as follows:



Carnitine reacts with activated fatty acids in presence of an enzyme carnitine acyl transferase-I, forming fatty acyl-carnitine complex and CoA-SH. Now this complex is easily transported through the inner mitochondrial membrane through a transport protein called carnitine acyl carnitine translocate. In the inner surface of the membrane another enzyme carnitine acyl transferase-II hydrolyzes the fatty acyl carnitine to give fatty acyl-CoA and carnitine. Thereby carnitine is free for reutilization and fatty acyl- CoA undergoes further oxidation in the mitochondrial matrix.

2. Formation of α-β unsaturated acyl-CoA (Enoyl-CoA):

The fatty acyl-CoA undergoes dehydrogenation at the α and β-carbon atoms forming trans alpha-beta unsaturated acyl-CoA. These hydrogen's are taken up by the coenzyme, Flavin Adenine Dinucleotide (FAD) which gets converted to $FADH_2$. Oxidation of $FADH_2$ through electron transport chain produces two ATPs. The enzyme is fatty acyl CoA dehydrogenase.



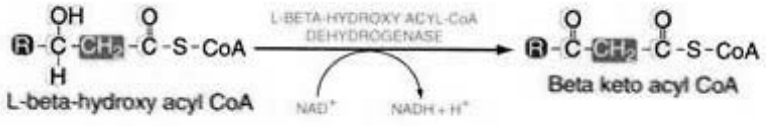
3. Formation of β-hydroxy acyl-CoA:

Enoyl-CoA is then hydrated by the enzyme crotonase (Enoyl-CoA hydratase) which adds water across the double bond. The product formed is P-hydroxy acyl-CoA.



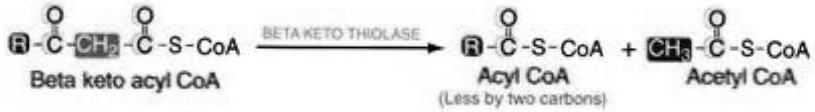
4. Formation of β-keto acyl-CoA:

β-Hydroxy acyl-CoA undergoes another dehydrogenation of the P-oxidation process forming β-keto acyl-CoA. The enzyme is β-hydroxy acyl-CoA dehydrogenase and its redox potential allows it to use NAD^+as the coenzyme releasing NADH + H^+ that produces 3 ATPs upon oxidation through ETC.



5. Thiolytic cleavage of β-keto acyl-CoA:

β-Keto acyl-CoA is then cleaved between the a and P-carbon atom releasing an acetyl-CoA and a fatty acyl-CoA shortened by two carbon atoms. The enzyme is β-keto thiolase that uses the coenzyme A for adding to the newly formed acyl-CoA.



The fatty acids undergo oxidative removal of two carbon units from the carboxylic side in the form of acetyl-CoA, which repeats till the fatty acid is completely converted to acetyl-CoA.

Calculation of Total ATP Produced When Palmitic Acid is Completely Oxidized to CO2 and H2O:

6 Carbon palmitic acid (i.e., the most abundant fatty acid in the human body) undergoes

7 such passes or cycles of P-oxidation, producing a total of 8 acetyl-CoA which are in turn oxidized through TCA cycle and the reducing equivalents produced are oxidized through ETC.

The number of ATPs produced by palmitic acid		
Name of the enzyme / process	**Number of reducing equivalents / ATP produced**	**Total number of ATP**
Fatty acyl CoA dehydrogenase	7 $FADH_2$ × 2	14
β-hydroxyacyl CoA dehydrogenase	7 NADH + H^+ × 3	21
	Total ATPs produced in β-oxidation	**35 ATP**
Acetyl CoA (8) oxidized through TCA cycle (each cycle=12 ATP)	8 × 12	96
	Total ATP produced both in β-oxidation and TCA cycle	**131**
ATPs utilized	Activation step ATP → AMP ATP → AMP + PPi ATP, ADP, ATP, ADP	2
	NET GAIN OF ATPs	**129**
Totally 129 ATP's are produced when palmitic acid is oxidized completely		

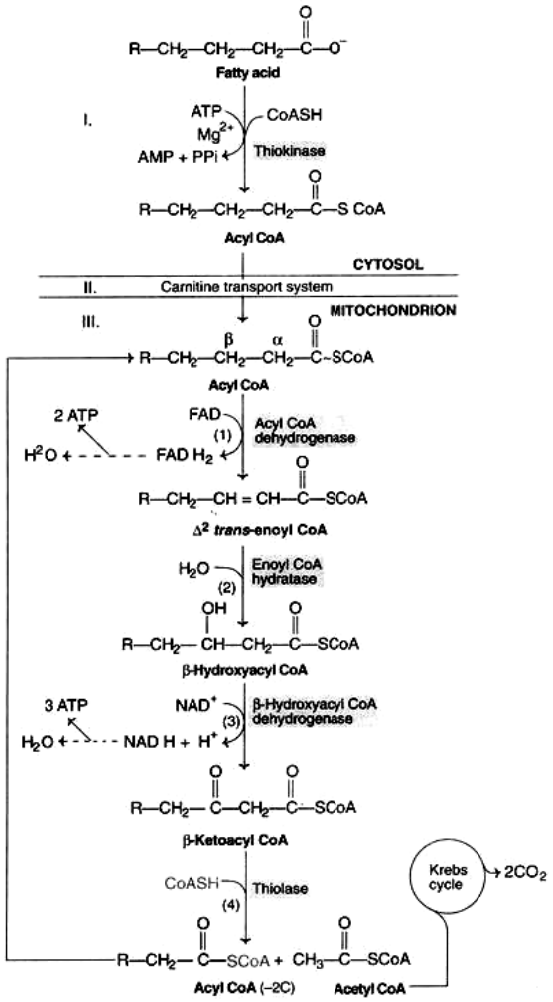


Fig. 67.10 : β-Oxidation of fatty acids : Palmitoyl CoA (16 carbon) undergoes seven cycles to yield 8 acetyl CoA [I-Activation; II-Transport; III-β Oxidation proper—(1) Oxidation, (2) Hydration, (3) Oxidation and (4) Cleavage]

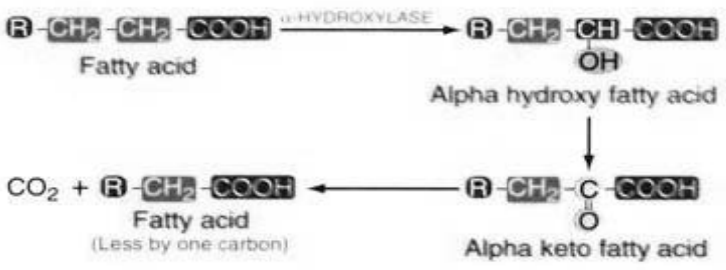
Fatty acids are oxidized by most of the tissues in the body. However, brain, erythrocytes and adrenal medulla cannot utilize fatty acids for energy requirement.

❖ **α-Oxidation of Fatty Acids:**

Oxidation of the fatty acids at the alpha carbon atom is known as alpha oxidation. During a-oxidation there is removal of one carbon atom at a time from the carboxyl end of the molecule. Alpha oxidation takes place for the alpha hydroxy fatty acids which are found in the brain.

During alpha oxidation, first a hydroxyl group is attached (if the fatty acid is devoid of alpha hydroxyl group) at the alpha carbon atom which is then oxidized to a keto group and then finally to a carboxylic group due to decarboxylation of the original carboxyl group giving rise to a fatty acid less by one carbon atom.

Characteristic Features of α-Oxidation:



1. It does not require CoA intermediates.
2. It does not generate high energy phosphate bonds.

Omega Oxidation:

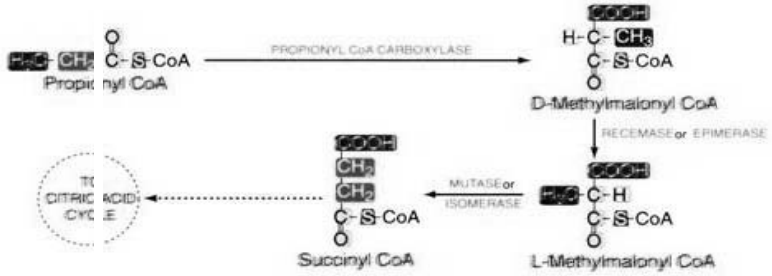
Oxidation of the omega carbon atom of the fatty acid is known as omega oxidation. During omega oxidation there is a successive removal of four

carbon atoms at a time. It predominantly occurs in the liver, whenever there is a high demand of energy as in the case of lactation, severe diabetics and starvation.

During omega oxidation first of all, with the help of hydroxylase enzyme a hydroxyl group is attached to the last carbon atom (-CH_3i.e., methyl group of the omega carbon atom), which is then further oxidized to a carboxylic group to form an alpha omega dicarboxylic acid. Now beta oxidation occurs from both the ends producing two acetyl-CoA at a time.

Oxidation of Fatty Acids with an Odd Number of Carbon Atoms:

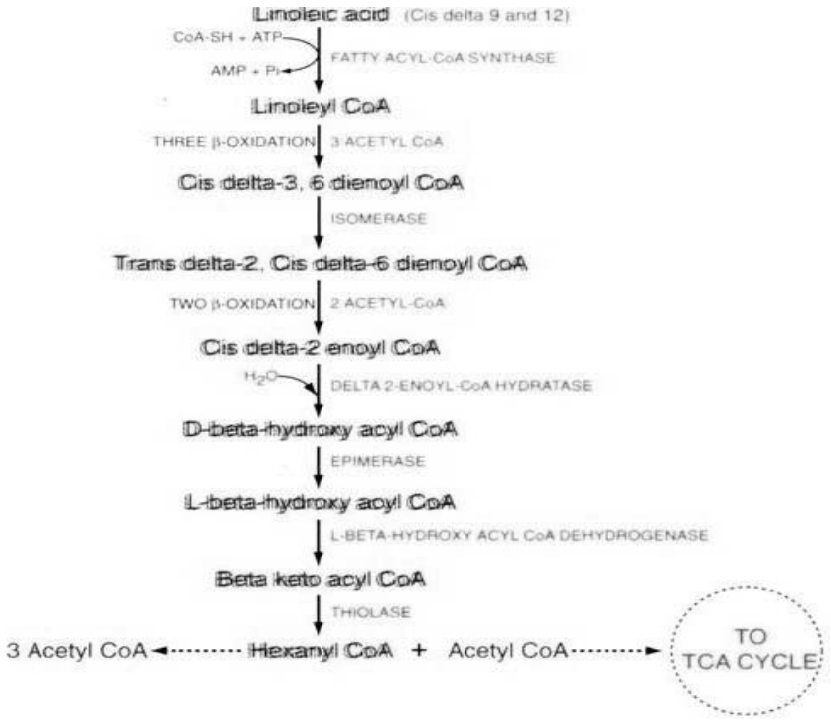
Fatty acids with an odd number of carbon atoms are normally oxidized by beta oxidation but the last cycle of the beta oxidation produces acetyl-CoA and propionyl-CoA (a 3 carbon moiety). This propionyl-CoA is further oxidized in the following manner:



Oxidation of Unsaturated Fatty Acids:

The 18 carbon linoleic acid is an unsaturated fatty acid, containing two cis double bonds (1) between carbon atoms 9 & 10 (cis Δ^9) and (2) between carbon atoms 12 & 13 (cis Δ^{12}). In its oxidation, three cycles of beta oxidation take place giving rise to 3 acetyl-CoA and cis- delta-3, 6-dienoyl-CoA, which is isomerized to trans-delta-2-cis-delta-6-dienoyl-CoA, which then undergoes two passes of beta oxidation forming two acetyl-CoA and nV-delta-2-enoyl-CoA.

This is converted to D-β-hydroxy acyl-CoA by the enzyme delta-2-enoyl-CoA hydratase. D-β-hydroxy acyl-CoA cannot be acted upon by the stereo-specific enzyme; L-P-hydroxy acyl-CoA dehydrogenase. Hence it is epimerized by an epimerase to give L-β-hydroxy acyl-CoA and then normal beta oxidation continues till the fatty acid is completely converted to acetyl-CoA.



2. Biosynthesis of Fatty Acids:

The dietary carbohydrates and amino acids, when consumed in excess, can be converted to fatty acids and stored as triacylglycerol's. De novo (new) synthesis of fatty acids occurs predominantly in liver, kidney, adipose tissue and lactating mammary glands.

The enzyme machinery for fatty acid production is located in the cytosomal fraction of the cell. Acetyl CoA is the source of carbon atoms while NADPH provides the reducing equivalents and ATP supplies energy for fatty acid formation.

The fatty acid synthesis may be learnt in 2 stages:

I. Conversion of acetyl CoA to malonyl CoA.

II. Reactions of fatty acid synthase complex.

I. Formation of malonyl CoA:

Acetyl CoA is carboxylated to malonyl CoA by the enzyme acetyl CoA carboxylase. This is an ATP-dependent reaction and requires biotin for CO_2 fixation. Acetyl CoA carboxylase is a regulatory enzyme in fatty acid synthesis.

II. Reactions of fatty acid synthase complex:

The remaining reactions of fatty acid synthesis are catalysed by a multifunctional enzyme known as fatty acid synthase (FAS) complex. In eukaryotic cells, including man, the fatty acid synthase exists as a dimer with two identical units. Each monomer possesses the activities of seven different enzymes and an acyl carrier protein (ACP) bound to 4′-phosphopantetheine.

Fatty acid synthase functions as a single unit catalysing all the seven reactions. Dissociation of the synthase complex results in loss of the enzyme activities. The sequence of reactions for the extra—mitochondrial synthesis of fatty acids (palmitate) is depicted in Fig. 67.11, and described below

1. The two carbon fragment of acetyl CoA is transferred to ACP of fatty acid synthase, catalysed by the enzyme, acetyl CoA-ACP transacylase. The acetyl unit is then transferred from ACP to cysteine residue of the enzyme. Thus ACP site falls vacant.

2. The enzyme malonyl CoA-ACP transacylase transfers malonate from malonyl CoA to bind to ACP.

3. The acetyl unit attached to cysteine is transferred to malonyl group (bound to ACP). The malonyl moiety loses CO_2 which was added by acetyl CoA carboxylase. Thus, CO_2 is never incorporated into fatty acid carbon chain.

4. β-Ketoacyl ACP reductase reduces ketoacyl group to hydroxyacyl group. The reducing equivalents are supplied by NADPH (from HMP shunt).

5. β-Hydroxyacyl ACP undergoes dehydration. A molecule of water is eliminated and a double bond is introduced between α and β carbons.

6. A second NADPH-dependent reduction, catalysed by enoyl-ACP reductase occurs to produce acyl-ACP. The four-carbon unit attached to ACP is butyryl group.

The carbon chain attached to ACP is transferred to cysteine residue and the reactions 2-6 are repeated 6 more times. Each time, the fatty acid chain is lengthened by a two-carbon unit (obtained from malonyl CoA). At the end of 7 cycles, the fatty acid synthesis is complete and a 16-carbon fully saturated fatty acid—namely palmitate—bound to ACP is produced.

7. The enzyme palmitoyl thioesterase separates palmitate from fatty acid synthase. This completes the synthesis of palmitate.

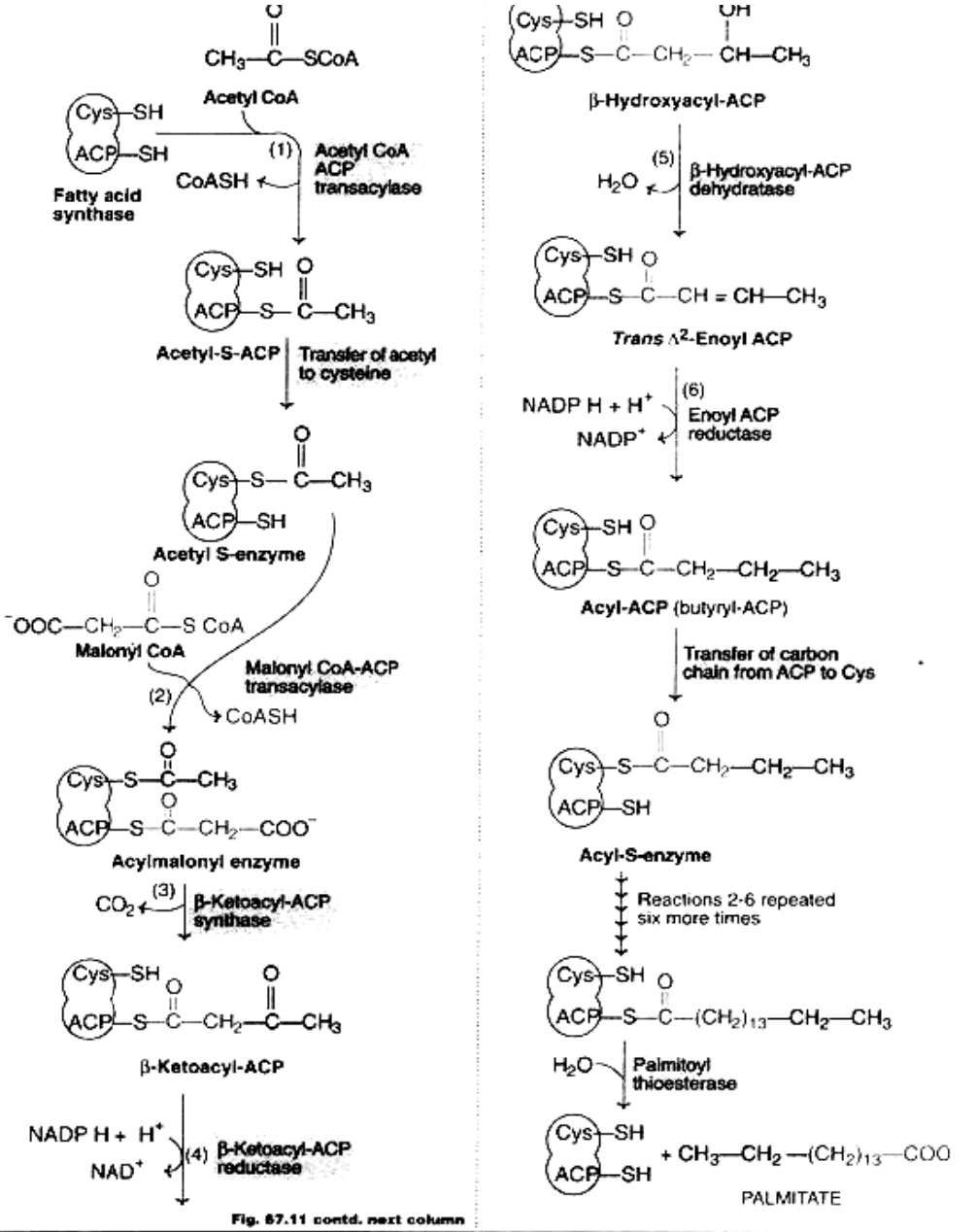


Fig. 67.11 : Biosynthesis of long chain fatty acid–palmitate. (Cys–Cysteine; ACP–Acyl carrier protein; The pathway repeats 7 times to produce palmitate; the first two carbons at the methyl end are directly from acetyl CoA, the rest of the carbons come from malonyl CoA).

18. Nitrogen metabolism: Biological and non biological nitrogen fixation, nitrate and ammonium assimilation.

Nitrogen metabolism

All the living organisms are basically composed of carbon, hydrogen, oxygen, nitrogen and many other forms of chemical elements. These elements contribute to finally organize various biomolecules present in a cell. Nitrogen is next to carbon in importance in living organisms. In a living cell, nitrogen is an important constituent of amino acids, proteins, enzymes, vitamins, alkaloids and some growth hormones. Therefore, study of nitrogen metabolism is absolutely essential because the entire life process is dependent on these nitrogen-containing molecules. In this lesson, you will learn about various aspects of nitrogen metabolism including nitrogen fixation and nitrogen assimilation in plants.

Molecular nitrogen

Nitrogen is primarily present in the atmosphere freely as dinitrogen or nitrogen gas. It is present in the combined form as Chile saltpetre or sodium nitrate and Chile in South America is the major source of this nitrate nitrogen. Molecular Nitrogen or diatomic nitrogen (N_2) is highly stable as it is triple bonded ($N^{o}N$). Because of this stability, molecular nitrogen as such is not very reactive in the atmosphere under normal conditions. In the atmosphere molecular nitrogen is 78.03% by volume and it has a very low boiling point (-195.8 oC) which is even lower than oxygen. Proteins present in living organisms contain about 16% nitrogen.

Nitrogen fixation (biological and abiological)

The conversion of molecular nitrogen into compounds of nitrogen especially ammonia is called nitrogen fixation. Nitrogen fixation, is a reductive process i.e., nitrogen fixation will stop if there is no reducing condition or if oxygen is present. This nitrogen fixation may take place by two different methods – abiological and biological.

Abiological nitrogen fixation

In abiological nitrogen fixation the nitrogen is reduced to ammonia without involving any living cell. Abiological fixation can be of two types : industrial and natural. For example, in the Haber's process, synthetic ammonia is produced by passing a mixture of nitrogen and hydrogen through a bed of catalyst (iron oxides) at a very high temperature and pressure.

In natural process nitrogen can be fixed especially during electrical discharges in the atmosphere. It may occur during lightning storms and nitrogen in the atmosphere can combine with oxygen to form oxides of nitrogen.

Biological nitrogen fixation

Chemically, this process is same as abiological. Biological nitrogen fixation is reduction of molecular nitrogen to ammonia by a living cell in the presence of an enzyme nitrogenase.

Nitrogen fixation of free living organisms and symbiotic nitrogen fixation

Nitrogen fixation is a distinctive property possessed by a select group of organisms, because of the presence of the enzyme nitrogenase in them. The process of nitrogen fixation is primarily confined to microbial cells like bacteria and cyanobacteria. These microorganisms may be independent and free living.

Mechanism of Biological Fixation of Nitrogen

Nitrogen fixation requires

(i) the molecular nitrogen

(ii) a strong reducing power to reduce nitrogen like FAD (Flavin adenine dinucleotide)

(iii) a source of energy (ATP) to transfer hydrogen atoms to dinitrogen and

(iv) enzyme nitrogenise

(v) compound for trapping the ammonia formed since it is toxic to cells.

The reducing agent and ATP are provided by photosynthesis and respiration. The overall biochemical process involves stepwise reduction of nitrogen to ammonia. The enzyme nitrogenase is a Mo-Fe containing protein and binds with molecule of nitrogen (N_2) at its binding site. This molecule of nitrogen is then acted upon by hydrogen (from the reduced coenzymes) and reduced in a stepwise manner. It first produces dimide (N_2H_2) then hydrazime (N_2H_4) and finally ammonia ($2NH_3$). NH_3 is not liberated by the nitrogen fixers. It is toxic to the cells and therefore these fixers combine NH_3 with organic acids in the cell and form amino acids.

Molecular nitrogen is a very stable molecule. Therefore, sufficeient amount of cell energy in the form of ATP is required for stepwise reduction of nitrogen to ammonia.In legumes, nitrogen fixation occurs in specialized bodies called nodules. The nodules develop due to interaction between the bacteria Rhizobium and the legume roots. The biochemical steps for nitrogen fixation are same. However, legume nodules possess special protein called LEGHEMOGLOBIN. The synthesis of leghemoglobin is the result of symbiosis because neither bacteria alone nor legume plant alone possess the protein. Recently it has been shown that a number of host genes are involved to achieve this. In addition to leghemoglobin, a group of proteins called nodulins are also synthesized which help in establishing symbiosis and maintaining nodule functioning. Leghemoglobin is produced as a result of interaction between the bacterium and legume roots. Apparently, Rhizobium gene codes for Heme part and legume root cell gene codes for Globin moiety. Both the coded products together consitute the final protein leghemoglobin.

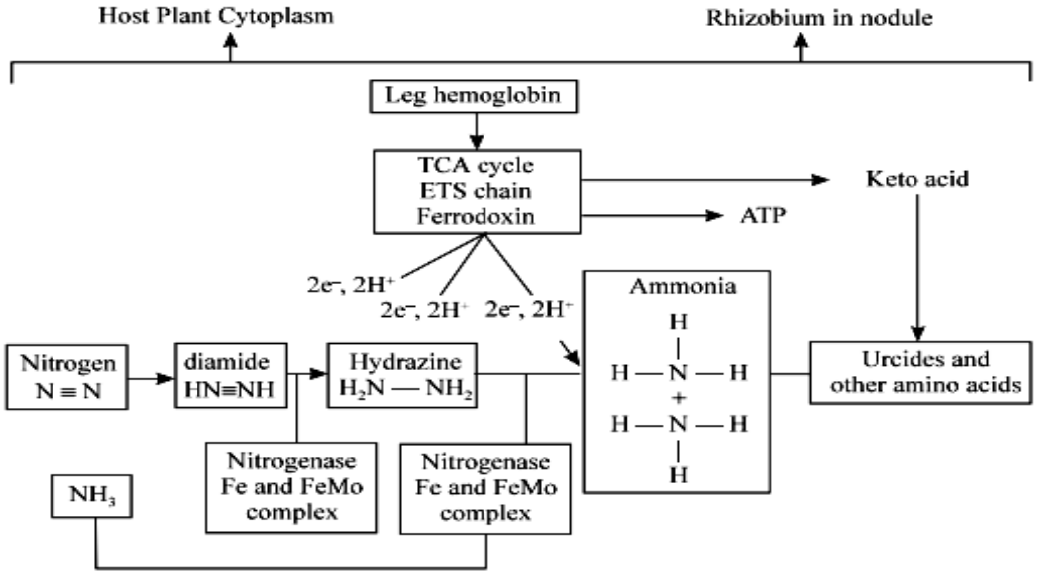


Fig. Simplified flow sheet of biochemical steps for nitrogen fixation

Leghemoglobin is considered to lower down the partial pressure of oxygen and helps in nitrogen fixation. However, this function is specific for legumes only because free living microbes do not possess nitrogen fixing leghemoglobin. Moreover, it has also not been found in cyanobacterial symbiosis with other plants.

Nitrate and ammonia assimilation by plants

As pointed in the previous section, nitrogen fixation is confined to selected microbes and plants. But all plants do require nitrogen because it has a role to play in the general metabolism. Therefore, plants which do not fix nitrogen, use other combined nitrogen sources such as nitrate and ammonia for carrying on metabolic activity. Nitrate is absorbed by most plants and reduced to ammonia with the help of two different enzymes. The first step conversion of nitrate to nitrite is catalyzed by an enzyme called nitrate reductase. This enzyme hasseveral other important constituents including FAD, cytochrome, NADPH or NADH and molybdenum.

The overall process of nitrate reduction take place in the cytosol and is an energy dependent reaction. The enzyme nitrate reductase has been studied in many plants and it is observed that the enzyme is continuously and synthesized and degraded. The enzyme nitrate reductase is inducible. This means that increase in nitrate concentration in the cytosol induces more of nitrate redutase to be synthesized. However, when excess NH_4^+ is produced then it has a negative effect on the synthesis of nitrate reductase. In plants, it has also been observed that light also increase nitrate reductase when nitrate is available. In the second step the nitrite so formed is further reduced to ammonia and this is catalyzed by the enzyme nitrite reductase. Nitrite present in the cytosol is transported into chloroplast or plastids where it is reduced to ammonia.

The enzyme nitrite reductase is able to accept electrons from sources such as NADH, NADPH or $FADH_2$. Besides, reduced ferredoxin has also been shown to provide electrons to nitrite reductase for reducing nitrite to ammonia. Ammonia so formed has to be utilized quickly by plants because accumulation of ammonia has a toxic effect. Some plants including algae leach out excess ammonia which can further be oxidized to nitrite and nitrate by microorganisms in the soil or water.

Amino acids synthesis by plants

As you have noticed that ammonia formation is achieved by plants either by (i.) nitrogen fixation or (ii) by reduction of nitrate to nitrite. Ammonium (NH_4^+) is the most reduced form of inorganic combined nitrogen. This ammonium now becomes the major source for the production of amino acids, which are the building blocks of enzymes and proteins. Amino acids have two important chemical groups. (i) amino group (NH) and (ii) carboxy1 group (-COOH).

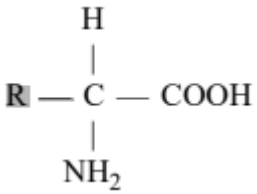


Fig. A typical amino acid with functional groups. R represents alkyl group

Ammonium so produced is the major source of amino group. However, the carboxyl group has to be provided by other organic molecule synthesized by the plants. There are two major reactions for amino acid biosynthesis in plants:

Reductive amination reaction

In this reaction, ammonia combines with a keto acid. The most important keto acid is the alpha ketoglutaric acid produced during the operation of Krebs cycle. The keto acid then undergoes enzymatic reductive amination to produce an amino acid.

$$\text{α-ketoglutaric acid} + NH_3 \xrightarrow{\text{glutamate dehydrogenase}} \text{Glutamic acid}$$

(keto acid) (amino acid)

Similarly another amino acid called aspartic acid is produced by reductive amination of oxaloacetic acid. It has been noted that reductive amination respresents the major 'port of entry' for ammonia into the metabolic stream in plants. This initiates synthesis of glutamic acid followed by other amino acids.

Transamination reaction

This is another very important reaction for amino acid biosynthesis. The reaction involves transfer of amino group, from already synthesized amino acid, to the keto acid.

$$\text{α-Ketoglutaric acid} + \text{Aspartic acid} \xrightarrow{\text{Transaminase}} \text{Glutamic acid} + \text{Oxaloacetic acid}$$

(Keto acid) (Amino acid) (Amino acid) (Keto acid)

In the above reaction, aspartic acid has transferred its amino group (NH_2) to the a-ketoglutaric acid to synthesize glutamic acid and release keto acid. The reaction is catalyzed by enzymes called transaminases. A large number of amino acids are synthesized by this transamination reaction. Amino acids are organic molecule containing nitorgen. The incorporation of amino group, from ammonium, into keto acids represents the major step for synthesis of nitrogenous organic biomolecules

19. Let's sum up

- Water potential is the chemical potential of water divided by the partial molal volume of water. The major factors influencing the water potential in plants are concentration, pressure, and gravity.
- In higher plants water is absorbed through root hairs which are in contact with soil water and form a root hair zone a little behind the root tips. During absorption of water by roots, the flow of water from epidermis to endodermis may take place through three different pathways: (i) Apoplastic pathway, (ii) Trans-membrane pathway and (iii) Symplast pathway.
- Three different theories of the mechanism by which ascent of water are brought about in plants. These hypotheses are (i) Root pressure (ii) Capillary action (iii) Cohesion-tension.
- A cell wall can have up to three parts— middle lamella, primary wall and secondary wall. The middle lamella is laid down first, formed from the cell plate during cytokinesis, and the primary cell wall is then deposited inside the middle lamella.

- Signal transduction is the process by which a chemical or physical signal is transmitted through a cell as a series of molecular events, most commonly protein phosphorylation catalyzed by protein kinases, which ultimately results in a cellular response. Proteins responsible for detecting stimuli are generally termed receptors, although in some cases the term sensor is used.
- In plants, red and blue light are especially effective in inducing a photo-morphogenetic response. Phytochrome is a blue protein pigment responsible for the perception of light in photo-physiological processes. It is possibly the only photoreceptor in photoperiodism and the flowering process.
- Plants produce signaling molecules, called hormones that have profound effects on development at vanishingly low concentrations. Until quite recently, plant development was thought to be regulated by only five types of hormones: auxins, gibberellins, cytokinins, ethylene, and abscisic acid.
- Embryogenesis transforms a single-celled zygote into a multicellular, microscopic, embryonic plant. Differentiation is the process by which a cell acquires metabolic, structural, and functional properties that are distinct from those of its progenitor cell. Germination is a process by which the embryo in the seed becomes activated and begins to grow into a new seedling.
- Senescence is the process of aging in plants. Plants have both stress-induced and age-related developmental aging. Chlorophyll degradation during leaf senescence reveals the carotenoids, and is the cause of autumn leaf color in deciduous trees. Leaf senescence has the important function of recycling nutrients, mostly nitrogen, to growing and storage organs of the plant.
- Flower initiation takes place by the transformation of vegetative apex into a reproductive structure. It signifies a transition from vegetative to the floral state. The shoot meristem is reduced and is also induced to develop sepals, petals, stamens and carpels in place of leaves. The ABC model of flower development in angiosperm demonstrates the presence of three classes of genes that regulate the development of floral organs. The genes are referred to as class A genes, class B genes and class C gene.
- Stress is usually defined as an external factor that exerts a disadvantageous influence on the plant. In most cases, stress is measured in relation to plant survival, crop yield, growth (biomass accumulation), or the primary assimilation processes which are related to overall growth.
- A buffer solution is an aqueous solutionconsisting of a mixture of a weak acid and its conjugate base, or vice versa. pH is defined as the decimal logarithm of the reciprocal of the hydrogen ion activity, aH^+, in a solution.
- Enzymes are proteins that act as catalysts within living cells. Catalysts increase the rate at which chemical reactions occur without being consumed or permanently altered themselves.

A chemical reaction is a process that converts one or more substances to another type of substance.

- Carbohydrates are a group of organic compounds consisting of C, H, O usually in the ratio of 1: 2: 1. Lipids are heterogeneous group of water insoluble compounds which are oily or greasy in consistency but soluble in non-polar solvents. Proteins are organic nitrogenous compounds in which a large number of amino acids are joined together by peptide linkages to form long polypeptide chains.
- Photosynthesis is the process that gives life to all living beings. The plants convert light energy into life energy. It is the only biological process that makes use of sun's light energy for driving the life machinery.
- In respiration carbohydrate is broken down, as a result the potential energy is transformed into kinetic form. Though it is a destructive or katabolic process, yet respiration is extremely beneficial, because it releases the necessary energy for performing the life functions.

21. Suggested Reading

1. Taiz, L., & Zeiger, E. Plant Physiology (4th ed.), 2006, Sinauer Associates, Inc. Publishers.
2. Lincoln Taiz, Eduardo Zeiger, Ian M. Møller, and Angus Murphy.Plant Physiology and Development. (6th ed.) Sinauer Associates.
3. Hopkins, W.G. & Hiiner, N.P. Introduction to Plant Physiology (3rd ed.) 2004, John Wiley & Sons.
4. Jain, V.K. Fundamental of Plant Physiology (7th ed.) 2004. S. Chand and Company.
5. Mathews, C.K., Van Holder, K.E. & Ahren, K.G. Bio-Chemistry (3rd ed.), 2000, Pearson Education.
6. Lehninger Principles of Biochemistry. Sixth Edition. 2013. David L. Nelson , Michael M. Cox. Freeman, Macmillan.Davies P.J. (ed.) Plant Physiology: Physiology, Bio-Chemistry & Molecular Biology, Academic Press.
7. Conn, E.E. and Stumpf, R.R. Outlines of Bio-Chemistry, Latest Ed., Wiley Eastern.
8. Hames, B.D. Bio-Chemistry (2nd ed.) Viva Books.
9. Sackheim, G. Chemistry for Biology Students (5th ed.) 1996, Benjamin/Cummings
10. Dainty, J. (1976) Water relations of plant cells. In *Transport in Plants*,Vol. 2, Part A: *Cells* (Encyclopedia of Plant Physiology, New
11. Series, Vol. 2.), U. Lüttge and M. G. Pitman, eds., Springer, Berlin, pp. 12–35.

12. Finkelstein, A. (1987) *Water Movement through Lipid Bilayers, Pores, and Plasma Membranes: Theory and Reality*. Wiley, New York.

13. Friedman, M. H. (1986) *Principles and Models of Biological Transport*. Springer Verlag, Berlin.

14. Hsiao, T. C. (1979) Plant responses to water deficits, efficiency, and drought resistance. *Agricult. Meteorol.* 14: 59–84.Loo, D. D. F., Zeuthen, T., Chandy, G., and Wright, E. M. (1996) Cotransport of water by the Na+/glucose cotransporter. *Proc.Natl. Acad. Sci. USA* 93: 13367–13370.

20. Assignmen

1. What will be the value of DPD in a fully turgid cell?

2. Where does photorespiration take place?

3. Discuss physiology of flowering with special reference to photoperiodism

4. Describe the mechanisms of enzyme actions.

5. Describe how plants use light and hormones to influence the germination of the seed.

6. How does phytochrome mediate the photomorphogenetic response?

7. Differentiate between cyclic and non-cyclic photophosphorylation.

8. Define buffer. Give an example.

9. Give an account of the secondary structure of protein.

10. What is Leghaemoglobin?

11. Write a short note on mode of action of auxin.

12. What do you mean by turgor pressure?

13. Explain the mechanism of water Absorption in plant.

14. What do you mean by Ascent of Sap?

15. What is the meaning of CAM? State the significance of it.

16. How does gibberellin induce α-amylase synthesis in aleurone layer of cereals?

17. Differentiate between apoplastic and symplastic pathway.

18. Describe the structure and functions of polysaccharides.

19. Explain why sucrose is called as invert sugar.

20. Classify aminoacids in various ways with suitable examples.

21. Write a short note on the isoelectric pH of protein and its importance.

22. Explain the different theories proposed for mechanism of enzyme substrate complex formation.

23. What is Electron Transport Chain? Explain the mechanism of ETC at mitochondrial Matrix?

24. Write about β-oxidation of Fatty acids? Calculate the net ATP in this process by taking Palmitic acid is an example.

25. Write the steps involved in Glycolytic pathway (or) EMP pathway?

All the materials are self writing and collected from e-book, journals and web sites

POST GRADUATE DEGREE PROGRAMME (CBCS)

IN

BOTANY

SEMESTER - II

Course: BOHCT 2.3

(Embryology, Plant Breeding and Biometry)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

KALYANI-741 235

WEST BENGAL

COURSE PREPARATION TEAM

Dr. Bapi Ghosh
Assistant professor
Department of Botany, DODL
Kalyani University

Dr. Pallab Kumar Ghosh
Assistant professor
Department of Botany, DODL
Kalyani University

Dr. Sudipta Roy
HOD & Associate professor
Department of Botany
Kalyani University

Dr. Zahed Hossain
Associate professor
Department of Botany
Kalyani University

Dr. Sudha Gupta
Assistant professor
Department of Botany
Kalyani University

Dr. Malay Kr. Adak
Assistant professor
Department of Botany
Kalyani University

Dr. Neera Sen Sarkar
Assistant professor
Department of Botany
Kalyani University

June, 2019

Directorate of Open and Distance Learning, University of Kalyani

Published by the Directorate of Open and Distance Learning,
University of Kalyani, Kalyani-741235, West Bengal and Printed by
Printtech, 15A, Ambika Mukherjee Road, Kolkata-700056

All right reserved. No part of this work should be reproduced in any form without the permission in writing from the Directorate of Open and Distance Learning, University of Kalyani.

Authors are responsible for the academic contents of the course as far as copyright laws are concerned.

Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2017 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Sankar Kumar Ghosh, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every member of PGBOS (DODL), University of Kalyani. Heartfelt thanks is also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and co-ordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

Self Learning Materials (SLMs) have been published by the Directorate of Open and Distance Learning, University of Kalyani, Kalyani-741235, West Bengal and all the copyright reserved for University of Kalyani. No part of this work should be reproduced in any from without permission in writing from the appropriate authority of the University of Kalyani.

All the Self Learning Materials are-self writing and collected from e-book, journals and websites.

Director
Directorate of Open and Distance Learning
University of Kalyani

SYLLABUS

COURSE–BOHCT 2.3

Embryology, Plant Breeding and Biometry

(Full Marks–75)

Course	Group	Details Contents Structure		Study hour
BOHCT 2.3	Embryology	**Unit 1. Plant development and Transition from vegetative to reproductive phase**	1. Plant development: Concept, definition and unique features of development in plants. 2. Transition from vegetative to reproductive phase: morpho-histochemical changes in shoot apex floral meristem and floral organ development in *Arabidopsis*.	1
		Unit 2. Molecular genetics in Plant Development and male gametophyte development	3. Molecular genetics in Plant Development: Techniques of studying plant developmental pattern: Mutants and transgenics in plant development. 4. Gametogenesis fertilization and early development: i. Development of stamen, anther, sporogenous tissue, microspores, pollen and male germ unit. Molecular basis of male gametophyte development.	1
		Unit 3. female gametophyte development, Self incompatibility, Fertilization, embryo and endosperm development	5. Gametogenesis fertilization and early development: ii. Development of carpel, ovule, megaspore, female gametophyte and female germ unit. Molecular basis of female gametophyte development. iii. Genetic and molecular basis of self incompatibility. Fertilization, double fertilization, triple fusion, imprinting and endosperm development in gymnosperms and angiosperms, embryogenesis in gymnosperms and angiosperms, establishment of symmetry in plants, seed formation.	1
		Unit 4. apomixis, polyembryony	6. Developmental routes of apomixis, polyembryony and its molecular aspects.	1

Course	Group	Details Contents Structure		Study hour
BOHCT 2.3	Plant Breeding & Biometry	**Unit 1. Breeding methods**	1. Breeding methods: Introduction and conservation of germplasm, mass selection, pure line selection, clonal selection, hybridization, selection after hybridization (bulk, pedigree, recurrent), heterosis & inbreeding depression.	1
		Unit 2. Mutation and polyploid breeding	2. An outline of mutation and polyploid breeding.	1
		Unit 3. Cytogenetics & breeding practices of crop plants	3. Cytogenetics & breeding practices of crop plants: rice, maize, potato.	1
		Unit 4. Breeding methods for disease resistance and MAS	4. An outline of breeding methods for development of disease resistance. 5. Molecular marker assisted breeding – an outline.	1
		Unit 5. Population samples, Central tendency, SD and SE	6. Population samples, sampling methods. 7. Frequency distribution, histogram, normal curve, mean, median, mode, variance, standard deviation, standard error.	1
		Unit 6. Probability, t-test and ANOVA	8. Probability & test of significance (Student & paired t) χ^2 test (detection of segregation ratio & linkage, test of independence & heterogeneity), analysis of variance (F test) CD, genetic advance, heritability estimation.	1
		Unit 7. Correlation & regression	9. Correlation & regression.	1
		Unit 8. Design of experiments	10. Design of experiments: general principles of field trails, randomized blocks, latin square, split plot designs, layout of breeding experiment.	1

Content

COURSE – BOHCT 2.3 Embryology, Plant Breeding and Biometry	Page No.
Unit 1. Plant development and Transition from vegetative to reproductive phase	2-9
Unit 2. Molecular genetics in Plant Development and male gametophyte development	9-27
Unit 3. Female gametophyte development, Self incompatibility, Fertilization, embryo and endosperm development	28-50
Unit 4. Apomixis, polyembryony	50-59
Unit 1. Breeding methods	61-87
Unit 2. Mutation and polyploid breeding	87-111
Unit 3. Cytogenetics & breeding practices of crop plants	111-120
Unit 4. Breeding methods for disease resistance and MAS	120-135
Unit 5. Population samples, Central tendency, SD and SE	135-155
Unit 6. Probability, t-test and ANOVA	156-178
Unit 7. Correlation & regression	178-184
Unit 8. Design of experiments	185-194

COURSE – BOHCT 2.3

(Embryology, Plant Breeding & Biometry)

Hard Core Theory Paper **Credit: (Groups A+B) = 3**

Group – Group A (Embryology)

Content Structure

---

1. Introduction
2. Course Objectives
3. Plant development: Concept, definition and unique features of development in plants.
4. Transition from vegetative to reproductive phase: morpho-histochemical changes in shoot apex floral meristem and floral organ development in *Arabidopsis*.
5. Molecular genetics in Plant Development: Techniques of studying plant developmental pattern: Mutants and transgenics in plant development.
6. Gametogenesis fertilization and early development:

   i. Development of stamen, anther, sporogenous tissue, microspores, pollen and male germ unit. Molecular basis of male gametophyte development.

   ii. Development of carpel, ovule, megaspore, female gametophyte and female germ unit. Molecular basis of female gametophyte development.

   iii. Genetic and molecular basis of self incompatibility. Fertilization, double fertilization, triple fusion, imprinting and endosperm development in gymnosperms and angiosperms, embryogenesis in gymnosperms and angiosperms, establishment of symmetry in plants, seed formation.
7. Developmental routes of apomixis, polyembryony and its molecular aspects
8. Let’s sum up
9. Suggested Reading
10. Assignment

1. Introduction

Plant embryology is one of the most important divisions of botany; its methods and goals are separate from those of plant morphology. General plant embryology explains the basic laws of the origin and development of generative and embryological structures (sporogenesis, gametogenesis, zygogenesis, endospermogenesis, embryogeny, apomixis). Comparative plant embryology studies embryological processes in various species in order to obtain data for solving problems of systematics and phytogeny. Specialized plant embryology is devoted to the study of embryological processes in the plants of individual systematic groups. Experimental plant embryology re-creates the course of development of plant organisms in order to reveal the functional, biochemical, and genetic nature of embryonic processes.

From this course, you will get the detail knowledge about various origin and development of embryological structure.

2. Course Objectives

You should gather knowledge after studying the course:

1. To know unique features of development in plants
2. Details explanation about floral meristem and floral organ development in *Arabidopsis*
3. Understand about plant demelopmental pattern in relation transgenic
4. Gathering knowledge about gametogenesis fertilization and early development.
5. Decribe developmental routes of polyembryony and its molecular aspects

3. Plant development: Concept, definition and unique features of development in plants.

Plant development is an umbrella term for a broad spectrum of processes that include: the formation of a complete embryo from a zygote; seed germination; the elaboration of a mature vegetative plant from the embryo; the formation of flowers, fruits, and seeds; and many of the plant's responses to its environment. Plant development encompasses the growth and differentiation of cells, tissues, organs, and organ systems. Plant development shares many similarities with developmental processes in animals, but the fact that plants are nonmotile, photosynthetic organisms requires certain novel developmental processes in addition to the common ones.

Development is the progression from earlier to later stages in maturation, e.g. a fertilized egg develops into a mature tree. It is the process whereby tissues, organs, and whole plants are produced. It involves: growth, morphogenesis (the acquisition of form and structure), and differentiation. The interactions of the environment and the genetic instructions inherited by the cells determine how the plant develops.

Growth is the irreversible change in size of cells and plant organs due to both cell division and enlargement. Enlargement necessitates a change in the elasticity of the cell walls together with an increase in the size and water content of the vacuole. Growth can be determinate—when an organ or part or whole organism reaches a certain size and then stops growing—or indeterminate—when cells continue to divide indefinitely. Plants in general have indeterminate growth.

Differentiation is the process in which generalized cells specialize into the morphologically and physiologically different cells. Since all of the cells produced by division in the meristems have the same genetic make-up, differentiation is a function of which particular genes are either expressed orrepressed. The kind of cell that ultimately develops also is a result of its location: Root cells don't form in developing flowers, for example, nor do petals form on roots.

Mature plant cells can be stimulated under certain conditions to divide and differentiate again, i.e. to dedifferentiate. This happens when tissues are wounded, as when branches break or leaves are damaged by insects. The plant repairs itself bydedifferentiating parenchyma cells in the vicinity of the wound, making cells like those injured or else physiologically similar cells.

Plants differ from animals in their manner of growth. As young animals mature, all parts of their bodies grow until they reach a genetically determined size for each species. Plant growth, on the other hand, continues throughout the life span of the plant and is restricted to certain meristematic tissue regions only. This continuous growth results in:

i. Two general groups of tissues, primary and secondary.
ii. Two body types, primary and secondary.
iii. Apical and lateral meristems.

Apical meristems, or zones of cell division, occur in the tips of both roots and stems of all plants and are responsible for increases in the length of the primary plant body as the primary tissues differentiate from the meristems. As the vacuoles of the primary tissue cells enlarge, the stems and roots increase in girth until a maximum size (determined by the elasticity of their cell walls) is reached. The plant may continue to grow in length, but no longer does it grow in girth. Herbaceous plants with only primary tissues are thus limited to a relatively small size.

Woody plants, on the other hand, can grow to enormous size because of the strengthening and protective secondary tissues produced by lateral meristems, which develop around the periphery of their roots and stems. These tissues constitute the secondary plant body.

Uniqueness of Plant Development

Although plants share many features of development with animals such as apical-basal polarity, regulation of the balance between cell growth and cell division, formation of distinctive patterns of organs, cells and tissues, and differentiation, some aspects of development are unique to plants. Among these are:

- The formation and maintenance of the perpetually embryonic regions, the apical meristems. The meristems have an indeterminate growth pattern that result in the occurrence of growth, organogenesis, and histogenesis throughout the life of the plant.

- Plant cells have rigid cell walls that prevent cell movement. Thus organogenesis and histogenesis must occur through differential growth and regulation of the planes of cell division. Cell-cell communication is important in plant development, but cell recognition is likely less important than it is in animals since plant cells keep the same neighbors throughout their life.
- Plant cells are totipotent; that is, able to differentiate as a different cell type if given the appropriate stimulus. Totipotency is likely a reflection of the plant's sedentary lifestyle. Plants can't escape predators and other kinds of damage, but they can readily repair wounds and reconnect vascular strands by differentiating the appropriate cell types.

4. Transition from vegetative to reproductive phase: morpho-histochemical changes in shoot apex floral meristem and floral organ development in *Arabidopsis*.

Introduction

The apical meristems function as the main sources of newcells to sustain plant growth. The regular recruitment ofmeristem cells to form new organs and tissues are balanced by cell proliferation within the meristem to maintain itssize relatively stable. This steady-state can persistthroughout the life of the plant, but in many cases themeristem is genetically programmed to stop producingnew cells at a specific developmental stage. In these cases,the meristem is said to be determinate. A determinatemeristem produces a part of the plant body with a predictablesize and form, such as the flower; whereas indeterminatemeristems produce parts of the plant whosesize and shape depend on the local environment, suchas branches and roots that grow to variable lengths. Thepositioning of determinate and indeterminate meristemsvaries between species and is a major determinant of plantarchitecture.

Maintenance of indeterminate meristems

The indeterminate growth of the vegetative and inflorescencemeristems is sustained by small groups of selfrenewingcells that are functionally similar to stem cells inanimals (Sablowski, 2004). These cells are located in thecentral zone (CZ) of the meristem, while some of theirdescendants are displaced to the peripheral zone (PZ),where they are recruited to form new organ primordial (Fig. 1). Below the CZ, the rib meristem (RM) sustainsstem growth. The CZ and PZ functions have been welltudied in Arabidopsis, although the RM has receivedmuch less attention. Superimposed on the CZ/PZ organization,the typical tunica/corpus structure found inangiosperms can also be distinguished, with two externallayers (L1 and L2) in which most cell divisions areoriented tangentially to the meristem surface, while the inner layer does not have clearly oriented divisions .Although the functional significance of the L1–L3 layering is not well understood, the existence of these clonally distinct layers has been essential to reveal the role of intercellular communication in meristem function.

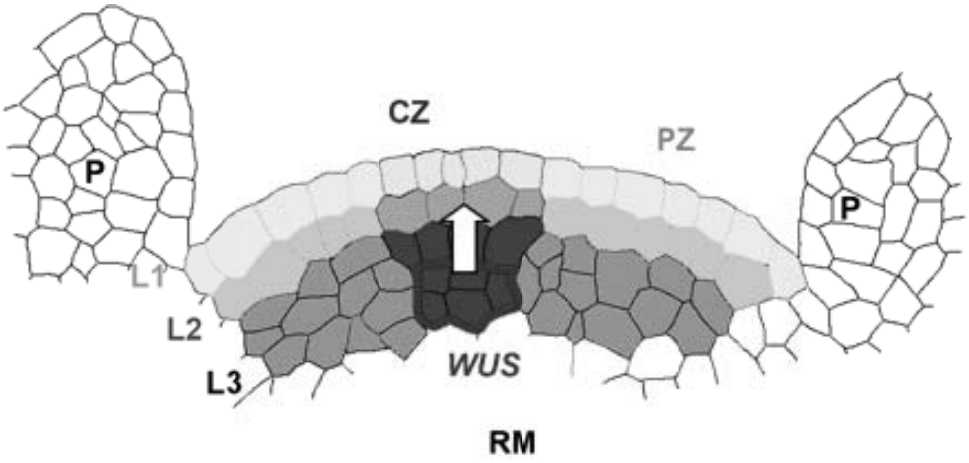


Fig-Structure of the shoot apical meristem. The concentric L1, L2, and L3 cell layers are indicated by different shades of colour. The central zone (CZ) is marked in blue, the peripheral zone (PZ) in green, and the position of the rib meristem (RM) is indicated below the meristem dome. The region that expresses WUS is circled in red; the white arrow represents the intercellular signal produced by the WUS-expressing cells to maintain cell identity and proliferation in overlying CZ. P indicates organ primordia produced on the flanks of the meristem.

SHOOT MERISTEMLESS (STM) and WUSCHEL (WUS) are two regulatory genes with central roles in shoot meristem development. STM and WUS genes function synergistically during meristem development and are required not only for the establishment of the shoot meristem during embryogenesis, but also for subsequent maintenance of the vegetative, inflorescence and floral meristems.

STM encodes a homeodomain protein expressed throughout the meristem and has been proposed to delay differentiation to allow enough cells to bulk up before recruitment into organogenesis

WUS also encodes a homeodomain-containing protein and is essential to specify the stem cells present in the CZ: in wus mutants, the defective CZ cannot keep up with organ recruitment in the PZ, and the meristem is quickly consumed Shoot meristem activity is eventually reinitiated in the axils of the leaves, only to terminate again; this intermittent meristem activity can carry on to the reproductive phase, when incomplete flowers are produced because of premature termination of the floral meristem. Not only is WUS necessary to maintain the CZ cells, but ectopic expression of WUS is also sufficient to convert cells in organ primordia and even root meristems into cells with characteristics of the shoot meristem CZ. Although WUS is required to maintain stem cells in all layers of the CZ, it is expressed only in a few L3 cells in the centre of the meristem (Mayer et al., 1998) (Fig. 1). Because its effects are seen in cells that do not express WUS, an intercellular signal is believed to mediate these effects. The size and location of the WUS-expressing region of the meristem are maintained in spite of the continuous cell proliferation within the meristem, implying that WUS expression must be adjusted constantly according to the position of cells in the meristem.

From vegetative to floral meristems

The dynamic balance between shoot meristem activity and organ initiation is maintained during most of the plant's growth, but it is eventually tipped in favour of organogenesis during floral development. The suppression of indeterminate growth in the floral meristem depends on floral-specific regulatory genes, whose expression is embedded within a programme of gene expression that is initiated at the transition to reproductive development. During this transition, the vegetative meristem is initially converted into the inflorescence meristem, which then produces floral meristems on its flanks. The transition from vegetative to reproductive development is controlled by multiple environmental and endogenous signals that ultimately converge on key regulators of floral identity: APETALA1 (AP1)/CAULIFLOWER (CAL) and LEAFY (LFY).

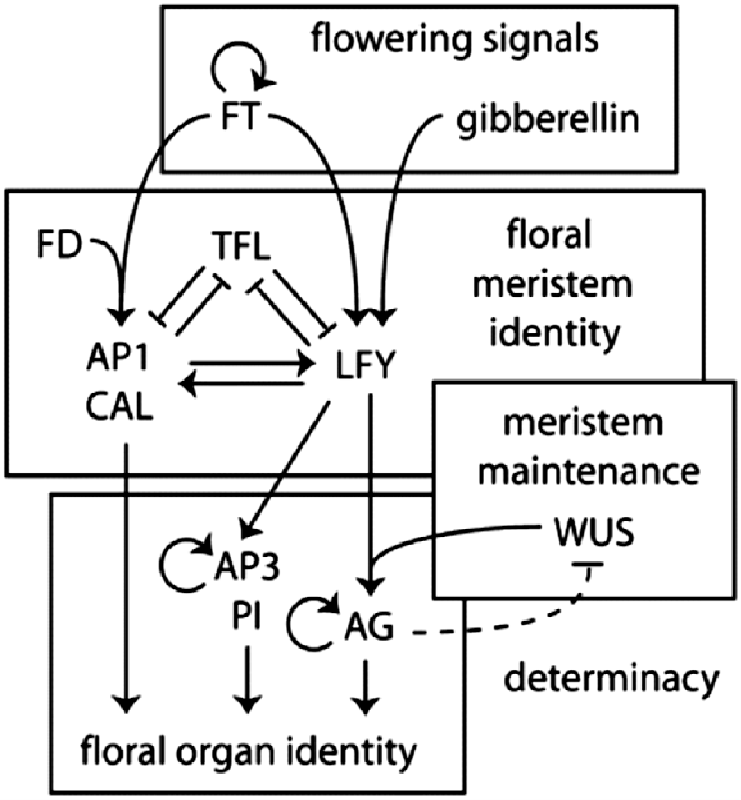


Fig. 2. Diagram of the interactions between regulators of floral meristem identity, floral organ development, and determinacy. Activation is indicated by an arrow and repression by a blunted line. The repression of WUS by AG is indicated by a dashed line to emphasize that it is likely to be indirect.

AP1 and CAL encode closely related MADS-domain transcription factors that are necessary and sufficient for the transition from inflorescence to floral meristem. In the double mutant ap1-1 cal-1, the primordia produced on the flanks of the inflorescence meristem fail to develop as flowers, and instead function as new inflorescence meristems, which go on to produce their own primordia, repeating the process until a large mass of meristems accumulates at the inflorescence apex, which resembles a cauliflower curdConversely, overexpression of AP1 is sufficient to convert the

inflorescence meristem into a terminal flower (Mandel and Yanofsky, 1995). Consistent with their role in specifying floral meristems, AP1 and CAL are expressed as soon as the floral primordium emerges from the inflorescence meristem.

LFY also encodes a transcriptional regulator that specifies floral identity and consequently promotes determinacyThis has been shown both by loss of lfy function, which converts floral meristems to inflorescence shoots, and by the effect of ectopic LFY expression, which converts the inflorescence meristem into a terminal floral meristem. LFY promotes the transition from inflorescence to floral meristem largely by activating AP1, but subsequently has a central and AP1-independent role in controlling floral development.

In addition to being activated by LFY, AP1/CAL are redundantly activated by the FT gene. Recent evidence suggests that FT is expressed in leaves and that its RNA or protein is transported to the apex as part of a mobile flowering signal that is produced in response to long days. After reaching the apex, FT is believed to interact with the bZIP protein FD to activate AP1/. LFY itself is also activated by FT besides being activated by gibberellin, which also functions as a signal to promote the shift to reproductive development.

As mentioned above, to maintain the indeterminate inflorescence meristem, AP1/CAL and LFY must be activated only in the floral primordia. The expression of LFY and AP1/CAL in the inflorescence meristem is prevented by TERMINAL FLOWER (TFL), which encodes a homologue of FT but has the opposite function, i.e. it antagonizes floral development.

In the flower of angiosperms there are usually four concentric whorls of organs, i.e. sepal, petal, stamen and carpel that are formed in whorl 1, whorl 2, whorl 3 and whorl 4 respectively, the whorl 1 being on the peripheral side.

In the whorl 1 class A genes when expressed induce the development of sepals. The interaction between class A and class B genes induce the development of petals in the whorl 2. Stamens are formed in the whorl 3 as a result of interaction between class B and class C genes.

In the whorl 4 class C gene induces the formation of carpel. So the summary of ABC model is: class A genes together and class C gene alone are responsible for the development of sepals and carpel respectively. The class B genes and class A genes function cooperatively to determine the development of petals. The class B genes and class C gene act together to induce the development of stamens

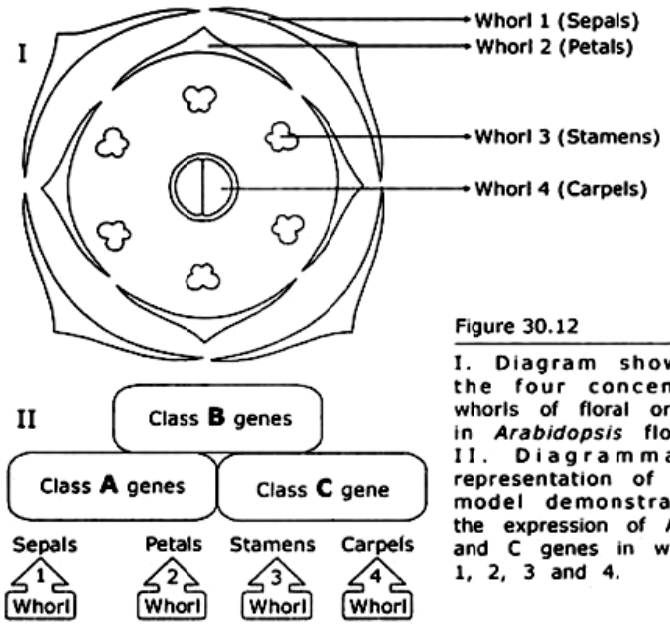


Figure 30.12

I. Diagram showing the four concentric whorls of floral organs in *Arabidopsis* flower. II. Diagrammatic representation of ABC model demonstrating the expression of A, B and C genes in whorls 1, 2, 3 and 4.

The following two have led to formulate ABC model:

(1) The discovery of homeotic mutants (homeotic genes identify specific floral organs and help the organ to develop in respective whorl. The homeotic mutant has

inappropriate expression—that is, it induces right organ to develop in wrong whorl. As for example — petals emerge in the whorl where normally stamens develop).

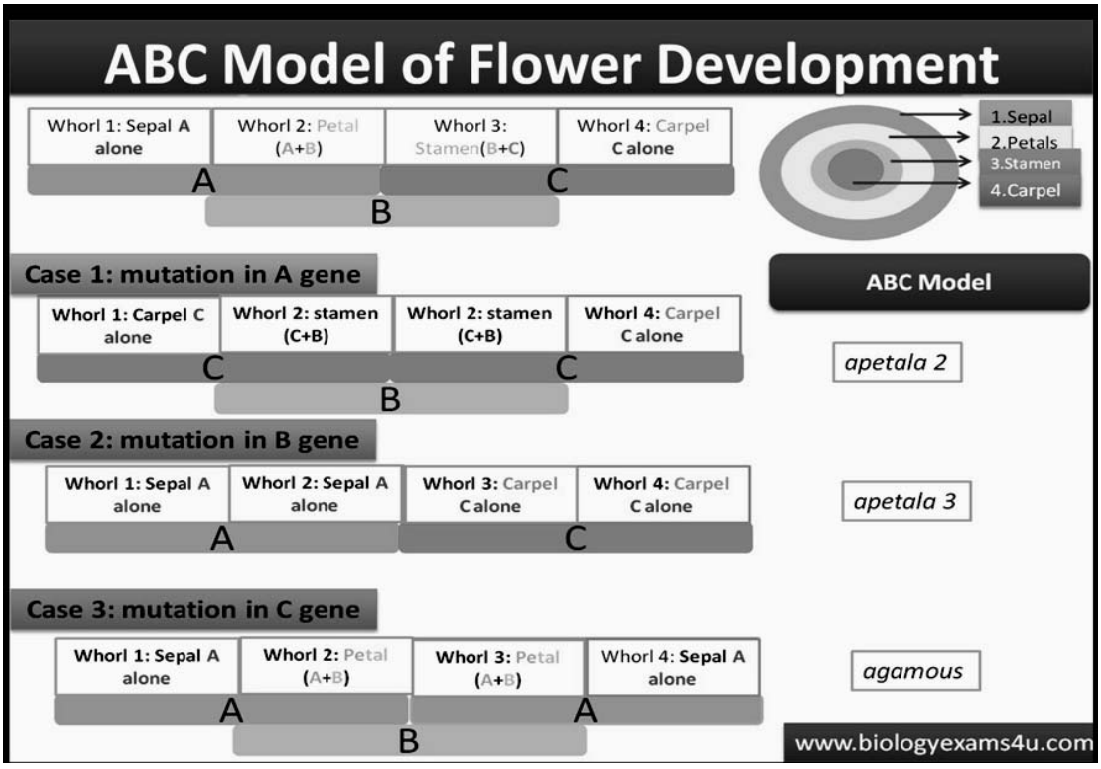
(2) The observation that each of the genes that induce the formation of an organ in a flower has an effect on two groups of floral organs, i.e. sepal and petals or petals and stamens.

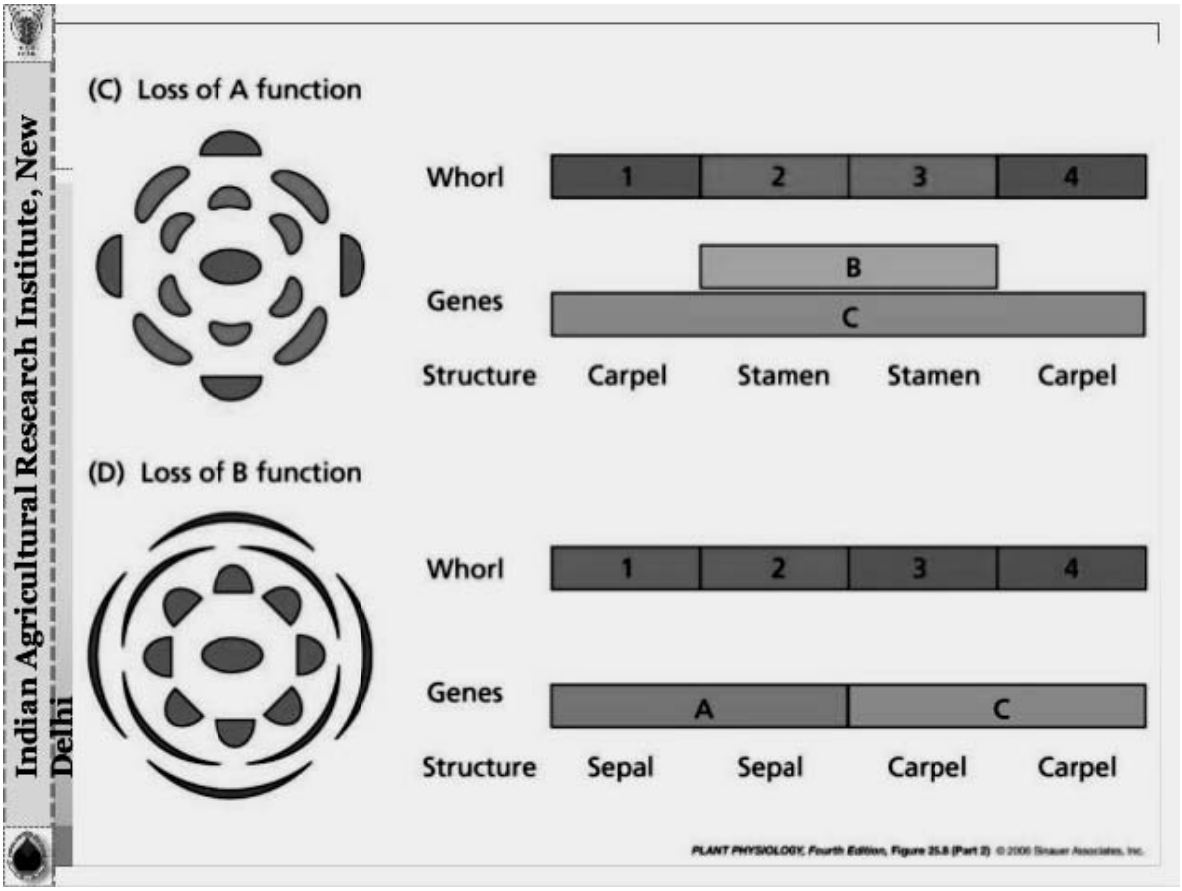
Class A, B and C genes are homeotic genes. They determine the identity of different floral organs and induce the organs to develop in their respective whorls.

The homeotic mutants have defects in floral organ development and induce the right organs to develop in wrong whorls/place, i.e. one floral organ develops in the whorl, which is the normal position of another floral organs. Petals, for example, develop in the whorl where stamens are normally to be formed.

In each whorl of a flower there is one or more homeotic genes and their cooperative functions determine the organ to be formed in that whorl. For example, the activity of class A genes is restricted to whorls 1 and 2. The class B genes have function in whorls 2 and 3. The class C gene functions in whorls 3 and 4.

Another way of describing the function of class A, B and C genes is that—in whorl 1, the class A gene-function alone determines the formation of sepals; in whorl 2, class A and B gene-functions both determine the formation of petals; in whorl 3, class B and C gene-functions both determine the emergence of stamens and in whorl 4, class C gene-function alone determines the carpel formation.
In *Arabidopsis* there are two genes in class A, two genes in class B and one gene in class C. The most characteristic feature of these homeotic genes is in the identification of floral organs and in the determinacy of position / whorl of their emergence in a floral meristem. The two genes of class A and the two genes of class B act cooperatively.





5. Molecular genetics in Plant Development: Techniques of studying plant developmental pattern: Mutants and transgenics in plant development.

Introduction

Transgenic plants are developed by transferring or modifying genes from another organism by a diverse technique like physical, chemical and biological methods. Transgenic plants are obtained by introducing a gene into its genome with the help of vectors in order to develop a plant with new characteristics. This process of recombinant DNA technology is used for developing genetically modified plants to embolden the variety against pests, diseases and stress or to improve the quality of the product. These can also be achieved by conventional breeding if the genes for the desired characters are present in close or wild relatives else transgenesis is the only viable alternative. Apart from this, genetic engineering can overcome the other limitations of conventional breeding such as large space requirement, time consuming, and uncertainty of the result.

Steps Involved In Production of Transgenic Plants

There are several steps involved in production of transgenic plants which is given

Steps Involved in production of transgenic plants

1. Isolate the gene of interest

2. Add DNA segment or promoter to initiate or enhance gene expression

3. Add selectable markers

4. Introduce gene construct into plant cell

(Transformation)

5. Select transformed cells or tissues

6. Regenerate whole plant

Isolation and Cloning the Gene of Interest

Identification and cloning of the gene of interest is a first limiting step in the transgenic development process. Locating, identifying, characterizing, and cloning genes of agricultural importance requires, a huge effort both in terms of human and financial capital. One of the earliest development is the introduction of insect resistance by transgenic technology. The discovery of Bt Genes has revolutionized plant transgenics. Spores of the soil bacterium Bacillus thuringiensis (Bt) contain a crystal (cry) protein (δ-endotoxin). Inside insect gut, the crystals break apart and release a toxin that binds to and creates pores in the intestinal lining. Instead of whole gene truncated cry gene is used in Bt crops.

Control of Gene Expression

The level of gene expression is determined by regulatory sequences such as promoters as well as 5' UTR elements (Described in detail in module 5- lecture 5).

Transgene Promoters: Most commonly used is the CaMV 35S promoter of cauliflower mosaic virus. It is a constitutive promoter (turned on all the time in all tissues), that gives high levels of expression in plants.

Most commonly used terminator sequence is the nopaline synthase (nos) gene from Agrobacterium tumefaciens.

Selectable Markers

Various selectable markers are used for the selection of transgenic plants.

Plant Tissues Used For Transformation

The tissue must be capable of generating callus (undifferentiated tissue), from which the complete plant can be produced. The choice of tissue depends on the species. Some commonly used tissues are immature embryos, leaf disks, and apical meristems.

Introduction of Gene Construct into Plant Cells (Transformation)

The basic methods and techniques used for plant cell transformation are as follows:

i) Agrobacterium mediated transformation

ii) Agro-infection

iii) Chloroplast transformation

iv) Indirect gene transfer

v) Electroporation

vi) Biolistic (Gene gun) Method

vii) Chemical method of gene transfer

viii) Microinjection

ix) Pollen Transformation

x) Direct DNA uptake by mature zygotic embryos

Selection of Transformants

Cells/tissues in which new genes are incorporated into plant's DNA are identified and then grown in media containing antibiotics or herbicides.

So during transformation, selectable marker gene (antibiotic resistant etc.) is incorporated such that, cells which are devoid of this plasmids get killed or their growth is arrested.

Kanamycin, an antibiotic capable of killing plant cells.

Since transformed plants contain kanamycin resistance gene, they only can survive.

In the *Agrobacterium* infiltration method, the seed of the infiltrated plants are plated on agar containing kanamycin – the plant seed containing the introduced DNA will germinate (less in number) and will grow on agar plates containing Kanamycin.

Regeneration of Transformant

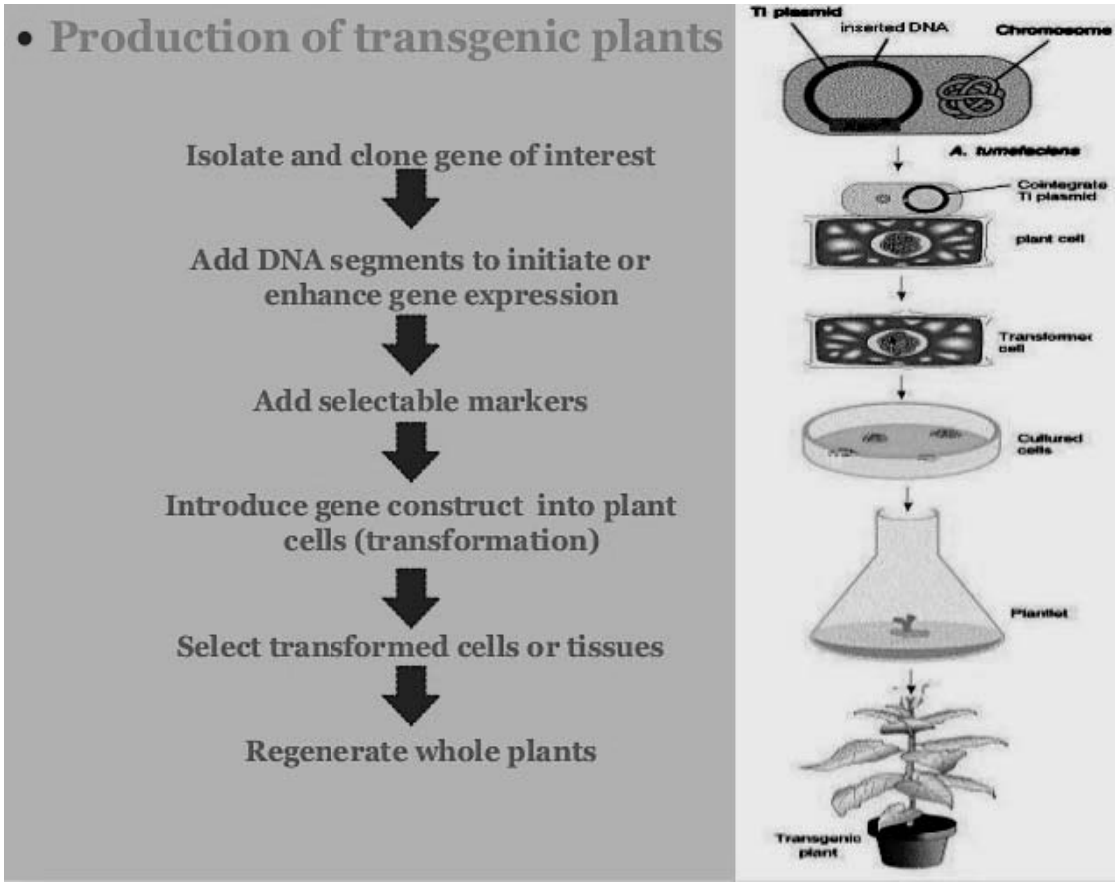
During regeneration, whole plants with inserted genes are developed through tissue culture.

Cultured transformed plant cells are regenerated under appropriate conditions.

Auxin/cytokinin ratio is important for plant regeneration.
In the culture media high concentration of auxin (plant growth regulator), causes rooting of the cultured transformed cells. Further, shooting is initiated by increasing the concentration of cytokinin (plant growth regulator-phytohormones) and new plantlet develops from the culture.

After that plantlets are transferred to soil for proper anatomical and physiological developments, and for better acclimatization to environment.

Confirmation of transformed plants

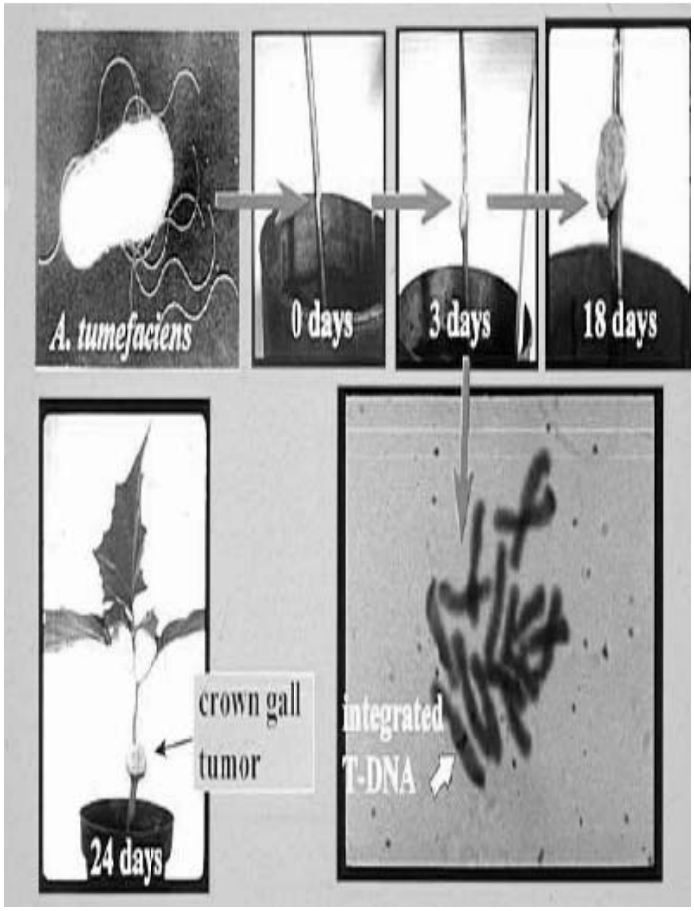


The presence and activity of introduced gene is confirmed by observation of phenotype and by advanced methods as listed below.

1) Southern blot: In Southern blotting separated DNA fragments obtained after electrophoresis, are transferred to a filter membrane and subsequent fragment detection is accompanied by probe hybridization.

2) Northern blot: It is used to study gene expression by detection of mRNA (or isolated mRNA) in a plant sample.

3) Western blot: It is used to study the gene expression by detection of the protein produced by the transformed regenerated plants.



Gene transfer methods

Biological methods

Agrobacterium mediated gene transfer

Plant virus vectors

Physical methods

Electroporation

Gene gun

Microinjection

Liposome Fusion

Chemical methods

Polyethylene glycol mediated

Diethylaminoethyl dextran mediated

***Agrobacterium tumefaciens*-mediated transformation** is another common technique. *Agrobacteria* are natural plant parasites. Their natural ability to transfer genes provides another engineering method. To create a suitable environment for themselves, these *Agrobacteria* insert their genes into plant hosts, resulting in a proliferation of modified plant cells near the soil level (crown gall). The genetic information for tumor growth is encoded on a mobile, circular DNA fragment (plasmid). When *Agrobacterium* infects a plant, it transfers this T-DNA to a random site in the plant genome. When used in genetic engineering the bacterial T-DNA is removed from the bacterial plasmid and replaced with the desired foreign gene. The bacterium is a vector, enabling transportation of foreign genes into plants. This method works especially well for dicotyledonous plants like potatoes, tomatoes, and tobacco. *Agrobacteria* infection is less successful in crops like wheat and maize.

Ti plamid:

The size of Ti plasmid is approx. 200 kb.

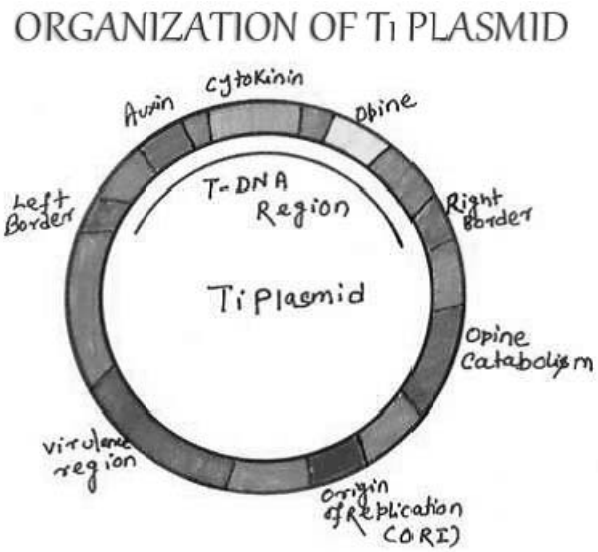
The Ti plasmid has three important regions:

(i) T-DNA region

(ii) Virulence region

(iii) Opine catabolism region

There is ori region that is responsible for the origin of DNA replication.



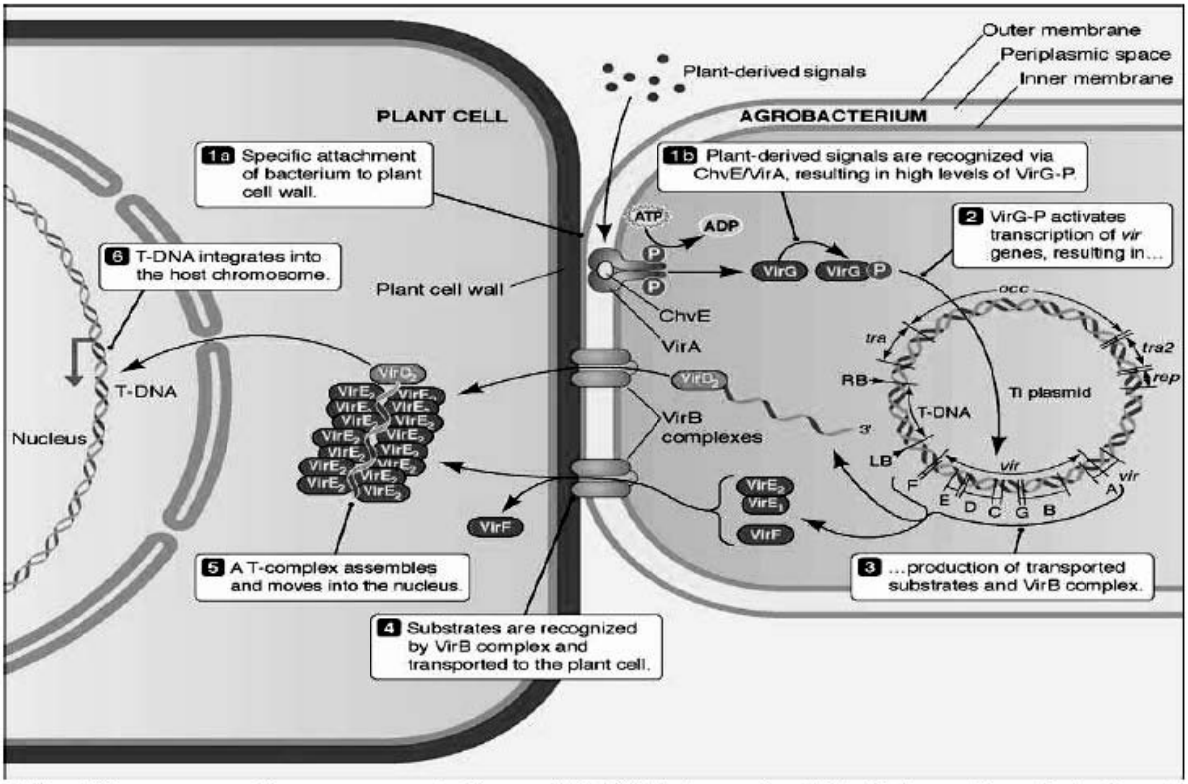


Fig: Diagrammatic representation of T-DNA transfer &its integration into host plant cell genome

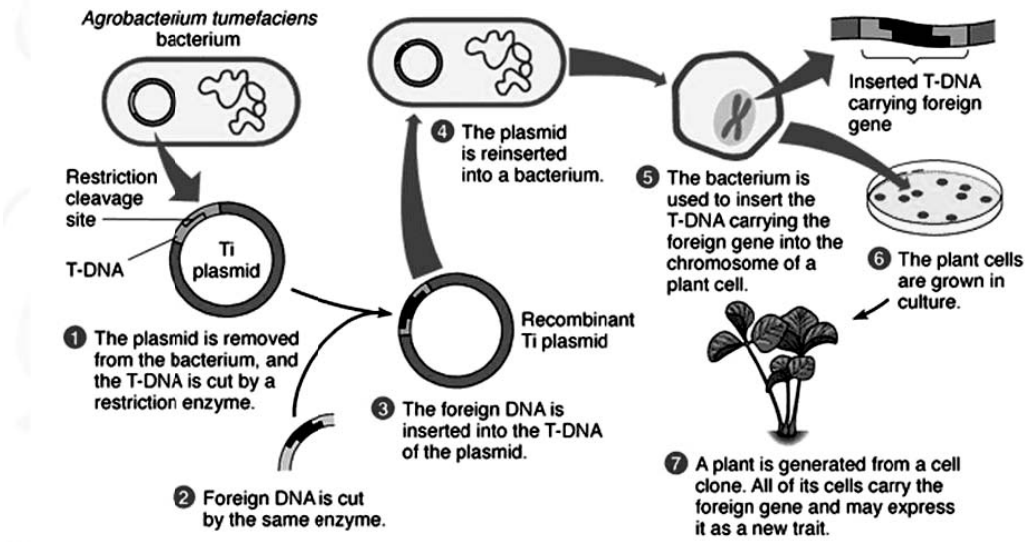


Fig.1: *Agrobacterium*-mediated genetic transformation.

Plant virus vectors

Plant viruses are considered as efficient gene transfer agents as they can infect the intact plants and amplify the transferred genes through viral\ genome replication.

It has some limitations like that vast majority of plant viruses have genome not of DNA but of RNA. Two classes of DNA viruses are known to infect higher plants, caulimovirus and geminivirus & neither is ideally suited for gene cloning.

I) Caulimovirus:

They contain circular dsDNA and are spherical in shape. The caulimovirus group has around 15 viruses & among these Cauliflower Mosaic Virus(CaMV) is the most important for gene transfer. CaMV infects many plants & can be easily transmitted. The infection is systemic. It's genome does not contain any non-coding regions.

It has certain limitations

i) CaMV vectors has a limited capacity for insertion of foreign genes.

ii) Infective capacity of CaMV is lost if more than a few hundred nucleotides are introduced.

iii) Helper viruses cannot be used since the foreign DNA gets expelled and wild-type viruses are produced.

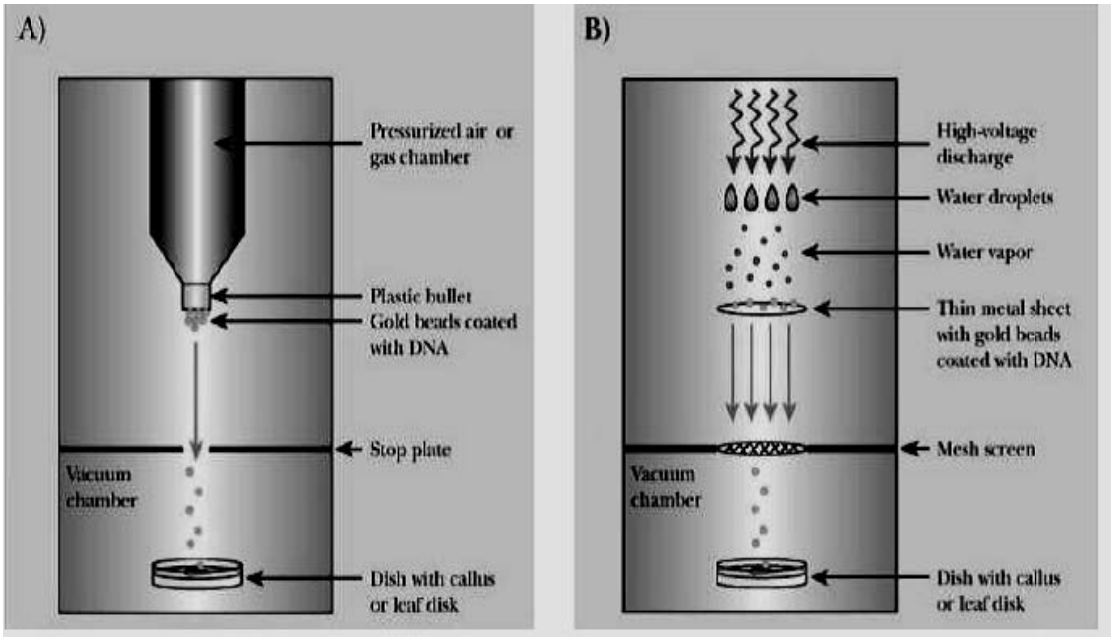
II) geminivirus:

They contain one or two circular ssDNA. They are particularly interesting because their natural host include plant size such as maize & wheat. They could therefore be potential vector for these and other monocots.

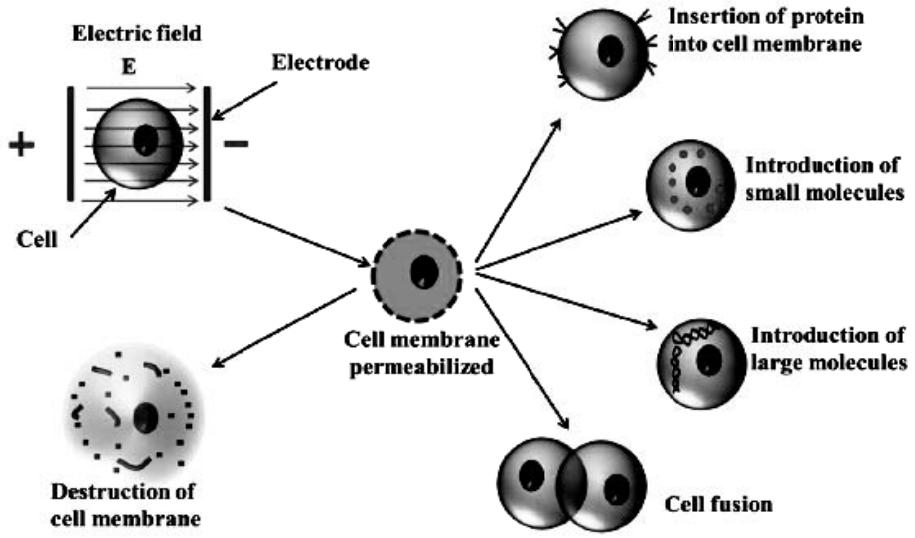
They have their own set of difficulties- It is very difficult to introduce purified viral DNA into the plants.

Physical method:

Gene guns (also known as biolistics) target genes into plant cells. It is the most common method. DNA is bound to tiny particles of gold or tungsten which is subsequently shot into plant tissue or single plant cells under high pressure. The accelerated particles penetrate both the cell wall and membranes. The DNA separates from the metal and is integrated into plant DNA inside the nucleus. This method has been applied successfully for many cultivated crops, especially monocots like wheat or maize, for which transformation using *Agrobacterium tumefaciens* has been less successful. The major disadvantage of this procedure is that serious damage can be done to the cellular tissue.

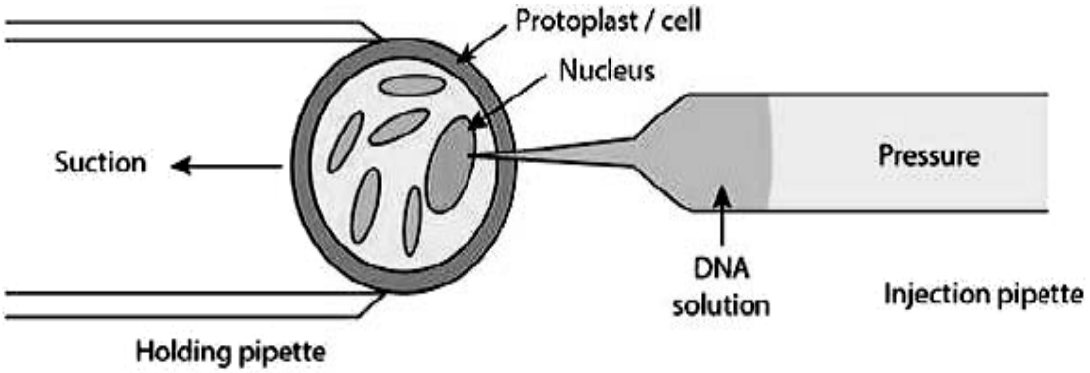


Electroporation is used when the plant tissue does not contain cell walls. Electroporation involves the creation of pores in the cell membrane using electric pulse of high field strength. If DNA is present in the buffer solution at sufficient concentration, it will be taken up through these pores.



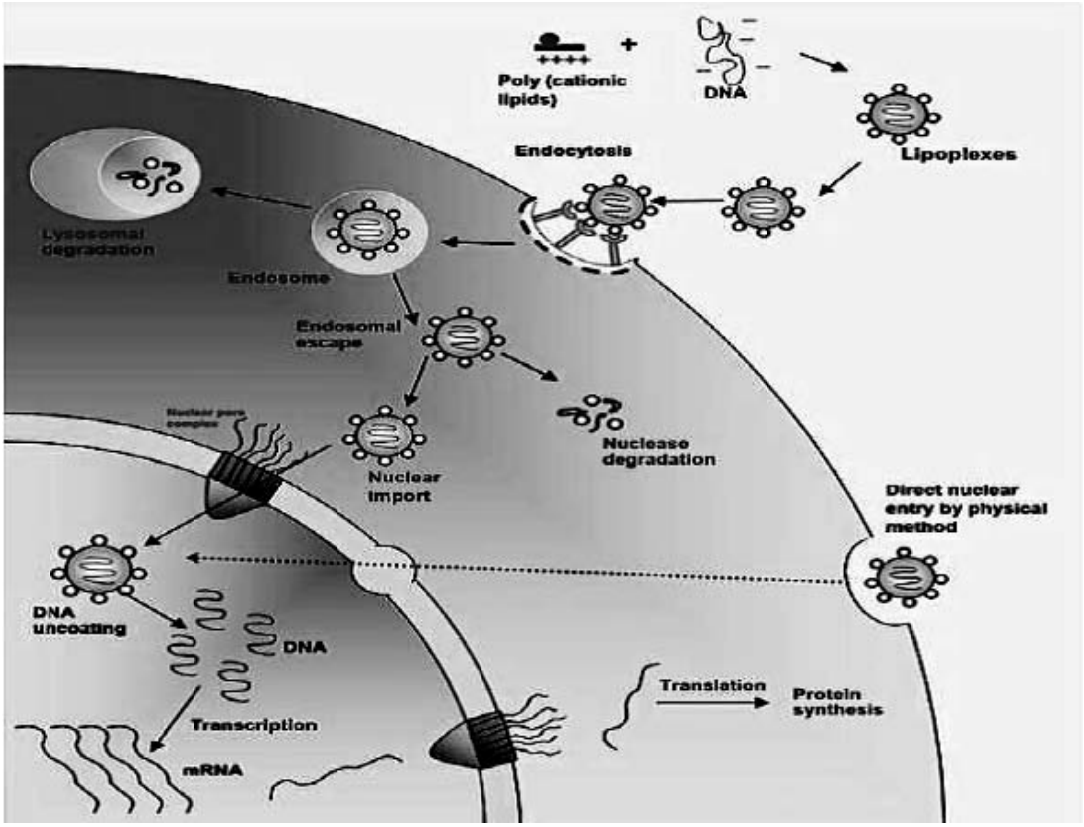
Microinjection is a direct physical method involving the mechanical insertion of the desirable DNA into a target cell.

- The technique of microinjection involves the transfer of the gene through a micropipette into the cytoplasm or nucleus of a plant cell or protoplast.
- The most significant use of this is the introduction of DNA into the oocyte and the eggs of animals, either the transient expression analysis or to generate transgenic animals.
- The major limitations of microinjection are that it is slow, expensive, and has to be performed by trained and skilled personnel.



4) Liposome mediated transformation

Liposome mediated transformation involves adhesion of liposomes to the protoplast surface, its fusion at the site of attachment and release of plasmids inside the cell.



Chemical gene mediated transfer

1) Polyethylene glycol- mediated transformation

- Polyethylene glycol (PEG), in the presence of divalent cations, destabilizes the plasma membrane of protoplasts and renders it permeable to naked DNA.
- A large number of protoplasts can be simultaneously transformed.
- This technique can be successfully used for a wide variety of plant species.
- It has certain limitations:
    a. The DNA is susceptible for degradation and rearrangement.
    b. Random integration of foreign DNA into genome may result in undesirable traits.
    c. Regeneration of plants from transformed protoplasts is a difficult task.

2) Deae-dextran mediated transfer

The desirable DNA can be complexed with a high molecular weight polymer diethyl amino ethyl(DEAE)dextran and transferred.

The major limitation of this approach is that it does not yield stable transformants.

Detection of Mutations in Plants:

In plants, two methods are used for detection of mutations depending upon the visibility of mutations.

These methods are briefly described below:

i. Detection of Visible Mutations:

Visible mutations generally occur in qualitative or oligogenic characters. Such mutations are detected on the basis of altered phenotype.

This technique consists of following steps:

a. The seeds are treated with a mutagen. For this purpose an improved variety or strain is used.

b. The treated seeds are grown in the experimental field. These plants are known as M1 plants or M1 generation. These M1 plants are selfed to avoid outcrossing. The seeds obtained from M1 plants represent M2 generation of seed.

c. The seeds obtained from M1 plants are grown to obtain M2 plants. A sufficiently large population should be raised in M2 generation to obtain mutant phenotypes which generally occur at a low frequency.

d. A search is made to identify or to detect plants which differ from the parent variety. Such plants are isolated and their frequency is estimated. Such mutations are called macro- mutations.

In maize, a different procedure is used for detection of visible mutations. In maize, some stocks are homozygous for several recessive genes and other stocks are homozygous for several dominant genes. The seeds of homozygous dominant lines are treated with a mutagen and M1 plants are raised. These M1 plants are crossed with homozygous recessive stock.

The mutagen treated plants are used as females due to presence of some degree of male sterility in these plants as a consequence of mutagenic effect. The F1 progeny of such cross is grown and a search is made to detect plants with recessive phenotype for a specific gene. Presence of plants with recessive phenotype for a gene confirms induction of mutation.

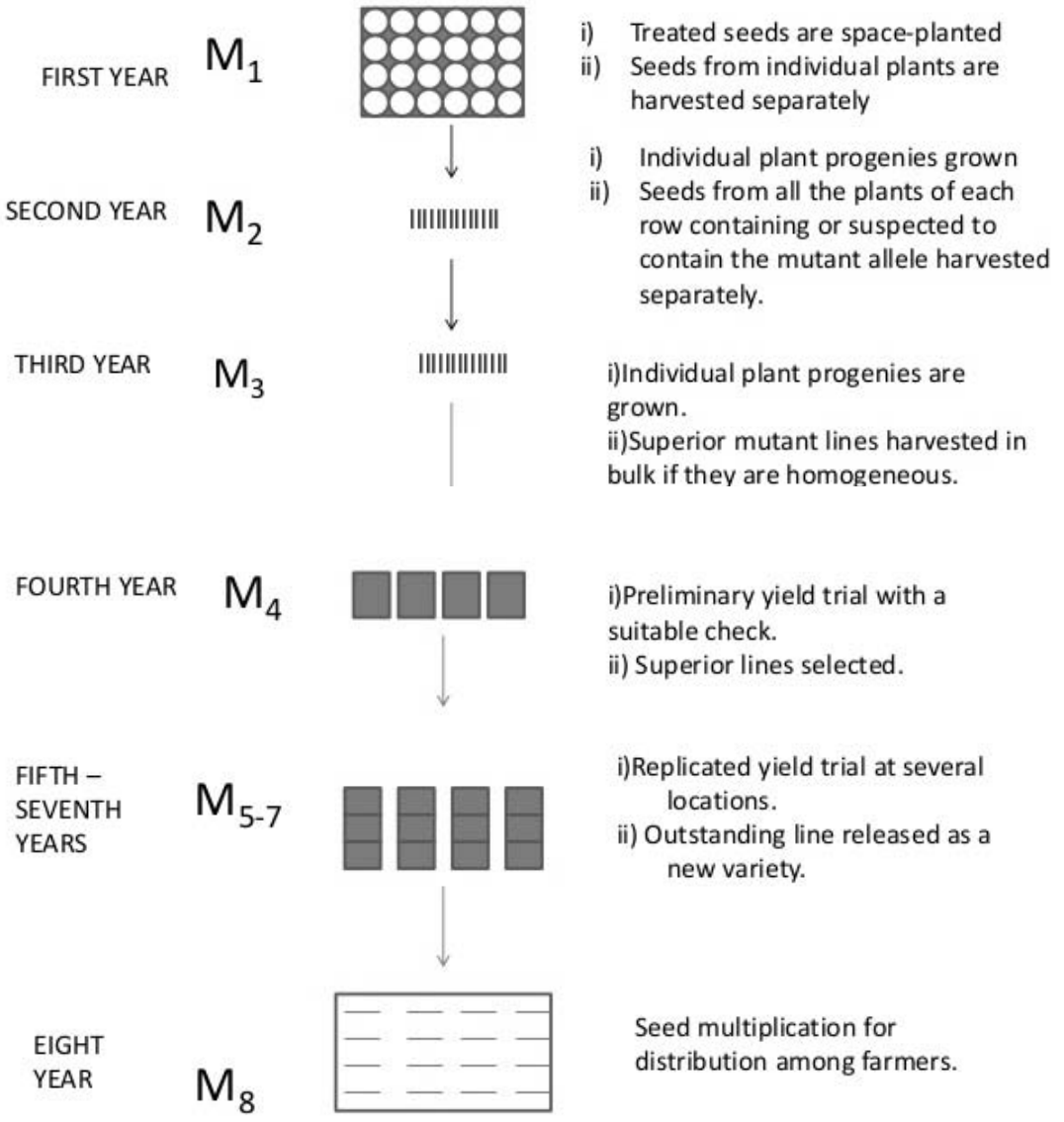


Fig: A generalised scheme for mutation breeding for oligogenic trait.

ii. Detection of Invisible Mutation:

Invisible mutations usually occur in quantitative or polygenic characters like yield and protein content. Detection of such mutations requires quantitative measurement of such characters. For yields, the mutagen treated and untreated variety is grown in replicated trials.

If the yield of treated and untreated treatments differs significantly, the presence of mutation is indicated. Similarly, if the protein content of treated material differs significantly from the parent variety, it indicates that mutation has taken place. Such mutations are called as micro-mutations.

Nutritional Deficiency Method of Mutations:

This method of detection of induced mutations is used in micro-organisms like Neurospora. The normal strain is treated with a mutagen and then cultured on minimal medium. A minimal medium

contains sugar, salt, inorganic acids, nitrogen and vitamin biotin. The normal strain of Neurospora grows well on the minimal medium, but a biochemical mutant fails to grow on such medium.

This confirms induction of mutation. Then minimal medium is supplemented with certain vitamins or amino acids, one by one and the growth is observed. The medium which results in normal growth of mutagen treated mould indicates that the mutant lacks synthesis of that particular vitamin or amino acid, addition of which to the minimal culture medium has resulted in normal growth of treated strain.

Applications of Mutations in Crop Improvement:

Induced mutations are useful in crop improvement in five principal ways, viz:

i. Development of Improved Varieties:

More than 2000 improved varieties (some directly and some by use of mutants in hybridization) have been developed through induced mutations in various field crops all over the world.

In India, induced mutations have been instrumental in developing improved varieties in wheat (NP 836, Sarbati Sonor'a, Pusa Lerma), barley (RDB 1), rice (Jagannath, IIT 48, NT 60), tomato, castor bean (Aruna, Sobhagya), cotton (MCU 7, MCU 10, Indore 2), groundnut (TGI), sugarcane (Co 8152, 8153) and several other crops.

Besides high yield, varieties have been developed with better quality, earliness, dwarfness, disease resistance and low toxin contents in various crops.

Improvement in quality has been achieved for protein content in wheat and rice, oil content in mustard and sugar content in sugarcane. Earliness has been achieved in castor (from 270 days to 140 days), rice and soybean. Dwarf varieties have been developed through the use of mutant parents in wheat, rice, Sorghum and pearl millet.

Disease resistance has been induced in oats to Victoria blight and crown rust; in wheat for strip rust; in barley for mildew; in groundnut for leaf spot and stem rust; in sugarcane for red rot; in apple for mildew, etc. Low toxin content varieties have been developed in rapeseed and mustard for erusic acid and in Lathyrus sativa for neurotoxin content.

ii. Induction of Male Sterility:

Induced mutations have been useful in induction of male sterility in some crop plants. Genetic male sterility has been induced in durum wheat using radiations. CMS mutants have been induced in barley, sugarbeet, pearl millet and cotton. Use of GMS and CMS lines helps in reducing the cost of hybrid seed production.

iii. Production of Haploids:

Use of X-ray irradiated pollens has helped in production of haploids in many crops. Chromosome doubling of these haploids results in the development of inbred lines which can be utilized in the development of commercial hybrids.

iv. Creation of Genetic Variability:

Induced mutations are very effective in creating genetic variability for various economic characters in crop plants. Induced mutations have been used for increasing the range of genetic variability in barley,

oats, wheat and many other crops. In asexually propagated crops like sugarcane and potato, somatic mutations may be useful, because the mutant plant can be multiplied as a clone.

v. Overcoming Self-Incompatibility:

Mutation of S gene by irradiation offers a solution to the production of self-fertile plants in self-incompatible species. This has been successful in case of Prunusovium. Besides this practical application in crop improvement, induced mutations are of fundamental interest in genetical studies.
Induced mutations have some limitations also. Most of the mutations are deleterious and undesirable. Identification of micro-mutations, which are more useful to a plant breeder is usually very difficult. Since mutations are produced at a very low frequency, a very large plant population has to be screened to identify and isolate desirable mutants.

6. Gametogenesis fertilization and early development:

i. Development of stamen, anther, sporogenous tissue, microspores, pollen and male germ unit. Molecular basis of male gametophyte development.

Stamens are the male reproductive organs of flowering plants. They consist of an anther, the site of pollen development, and in most species a stalk-like filament, which transmits water and nutrients to the anther and positions it to aid pollen dispersal. Within the anther, male sporogenous cells differentiate and undergo meiosis to produce microspores, which give rise to pollen grains, whereas other cell types contribute to pollen maturation, protection, or release. Pollen development involves an array of extraordinary events, including cell division and differentiation independent of a conventional meristem, the transition from sporophytic to gametophytic generation, and modifications of cell division to produce structures that are unusual in plant development, including coenocytic tissues (the tapetum and the microsporocyte mass), and subsequently free cells (microspores) that give rise to self-contained units for genome dispersal (pollen grains).

Anther development

Development of anther (microsporangium):

(a) Development of microsporangia is eusporangiate type (i.e, from a group of initial cells)

(b) Few cells in the hypodermal region become differentiated as archesporial cells. In *Boerhavia* and *Dionaea*, there is only one archesporial cell.

(c) The archesporial cell divides periclinally (along the periphery) to form outer – primary parietal layer and inner – sporogenous layer.

(d) The primary parietal layer lies just beneath the epidermis and divides again periclinally to form 3-5 concentric layers. These layers give raise the wall of the sporangium, along with epidermis.

(e) The innermost layer of the wall is called tapetum, which serves to provide nourishment to the developing pollen grains.

(f) The layer just below the epidermis is called endothecium.

(g) The sporogenous layer may function directly as pollen mother cell or it may divide to form many pollen mother cells.

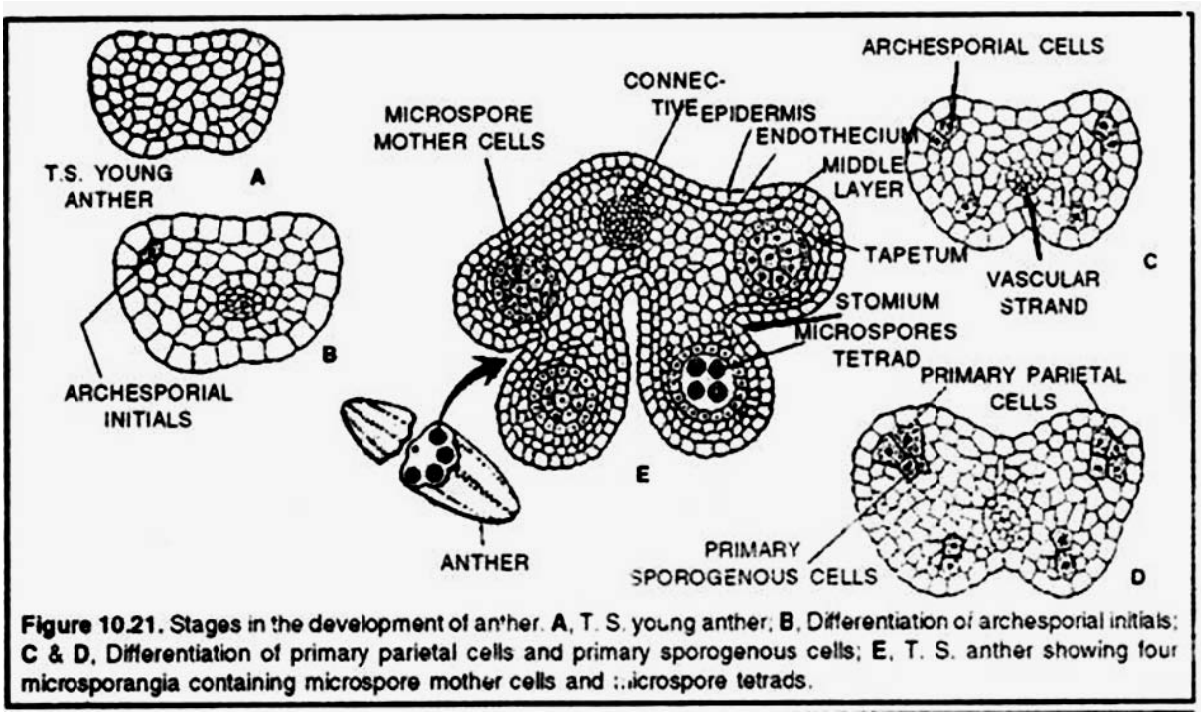


Figure 10.21. Stages in the development of anther. **A**, T. S. young anther; **B**, Differentiation of archesporial initials; **C & D**, Differentiation of primary parietal cells and primary sporogenous cells; **E**, T. S. anther showing four microsporangia containing microspore mother cells and microspore tetrads.

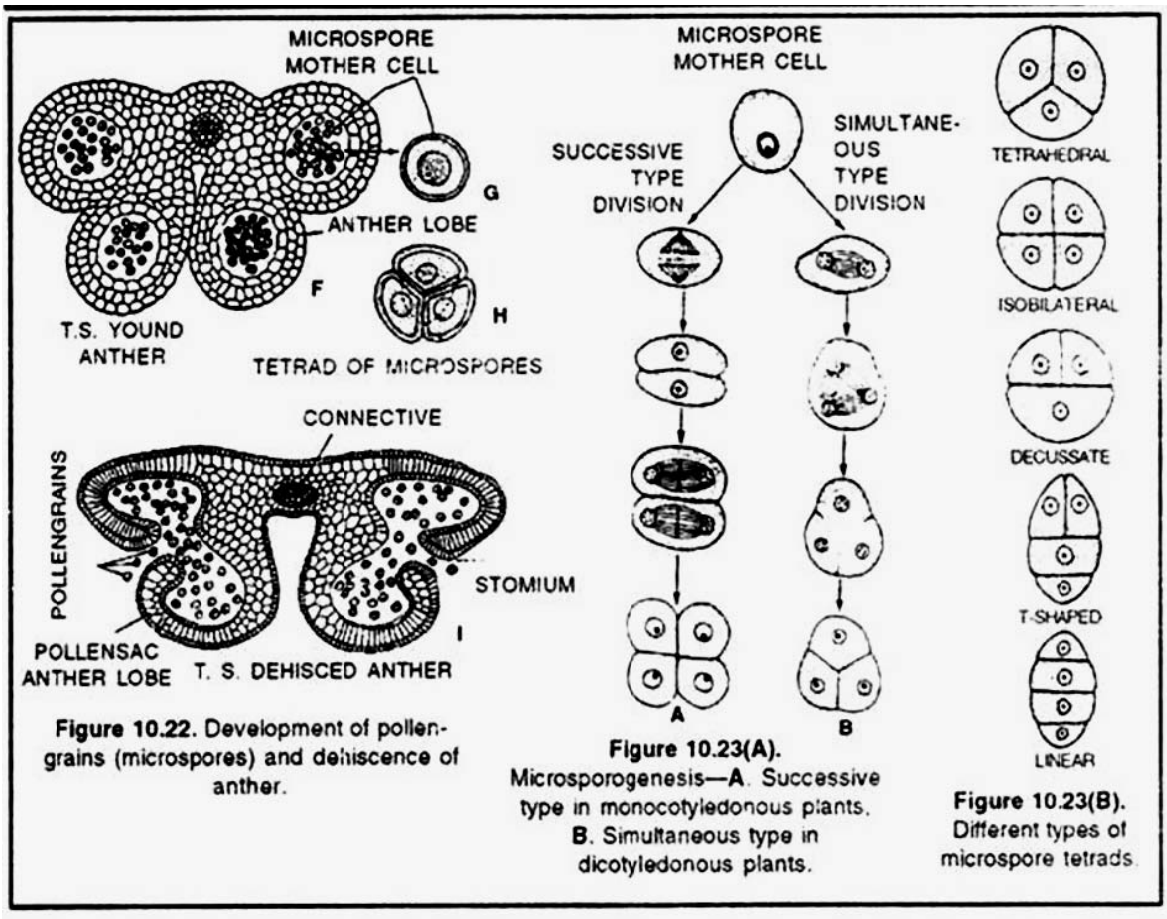


Figure 10.22. Development of pollen-grains (microspores) and dehiscence of anther.

Figure 10.23(A). Microsporogenesis—**A**. Successive type in monocotyledonous plants. **B**. Simultaneous type in dicotyledonous plants.

Figure 10.23(B). Different types of microspore tetrads.

Anther wall:

(a) The mature anther wall comprises of an epidermis, followed by an endothecium, 2-3 middle layers and innermost tapteum.

(b) Endothecium consists of radially elongated cells, which possess fibrous bands and these are hygroscopic (moisture absorbing) in nature. These help in dehiscence of anther (splitting of anther to release spores).

Tapetum:

It is the innermost nourishing layer of the anther wall present below the middle layer. It is usually single layered and is rich in reserve food material.

It is polyploidy & serves to provide food to the developing sporogenous tissue (microspores) based on its behaviour, tap turn is of two type:

A. Glandular (or secretory) tapetum:

It is also called parietal tapetum. In this type the tapetum cells remains as such in their original position, throughout the microspore development. These cells secrete nutrient materials which are given to the developing spores. It is more common in angiosperms. In the initial stages of .the development, the cells of the glandular tapetum, contains, small bodies, called pro-ubisch bodies, which are involved in the external thickening of the exine of the spore wall.

B. Amoeboid tapetum:

In this type, the breakdown of the inner and radial walls take place and the cytoplasm, containing food material moves into the inner anther cavity and forms the peri-plasmodium mass, which provide nourishment of the sporogenous cells. It is found usually in hydrophytes.

2. Formation of microspores or pollen grains (micro-sporogenesis):

(a) The sporogenous tissues, formed by archesporial cells divide many times to form pollen mother cells (or microspore mother cells) these are diploid cells.

(b) Each such cell divides meiotically (by meiosis) and forms four haploid microspores or pollen grains.

(c) The division is of two types in various angiosperms – simultaneous type and successive type.

(d) In simultaneous division, the M1 of meiosis is not followed by cytokinesis, wall formation takes place only after the completion of both Mi (Meiosis-I) and M2 (Meiosis-ll).

(e) On the other hand, in successive type of division, cytokinesis occurs after both divisions M1 as well as M2

Microspore mother cells →M1 2 haploid cell →M24 Microspore (pollen grain)

The microspore so formed, remain associated with each other for some time. This group of 4n (pollen grains) is called microspore tetrad. Different plants represent 5 types of tetrads –

1. Tetrahedral tetrad (most common),

2. Isobilateral tetrad,

3. Decussate tetrad,

4. Inverted T shaped tetrad and

5. Linear tetrad.

In *Aristolochiaelegans*, all the 5 types of microspore tetrads are found. The enzyme callase disintegrates the callose (polysaccharide) present in pollen tetrad.

Structure of (microspore) pollen grain (microspore):

(a) Microspores (or pollen grains) are the unicellular, uni-cleated, haploid and spherical structures, which develop to give rise to male gametophyte. Smallest pollen are present in myosotis and largest pollens are present in *Mirabilis*.

(b) Microspores represent the first cell of male gametophyte.

(c) Microspores are surrounded by a two-layered wall.

(d) The outer layer is called exine, which is thick and cuti-cularised layer.

(e) The inner layer is called intine, which is smooth, thin and cellulosic.

(f) The exine shows different types of outgrowths. It contains a resistant fatty substance, called sporopollenin. Exine consists of extexine (outer) and endexine (inner) Extectine further coisists of a foot layer, baculate layer(middle) and an outermost tectum. Tectum characteristic texture to exine. The morphology and texture of exine is important from taxonomic point of view. The study of pollen and its exine structure is called palynology. Father of palynology is Erdtman & Indian palynology is P.K. Nair.

(g) Chemically the pollen grains are composed with carbohydrates (25-48%), protein (7-26%), and water (7-16%), Fats (1-15%).

(h) The germ pores are important because these mark the origin of pollen tube,

(f) Normally there are three germ pores in dicots, while only one in monocots.

Pollinium (Translator Apparatus) *Corpusulum*:

In some plants of family Asclepidiaceae, (as in Calotropis procera) orchidaceae (orchids) the spores remain together in a single mass, called pollinium. Pollinium occurs in pair forming balloon like structures. These structures are called pollinia. Each pollinium consists of a stalk (called corpusculum), caudicle (disc like) and two pollinia carries mass of pollen grains.

Germ pores:

(a) These are the weak portions of the exine of the pollen grain. These are the points where from germ tube (pollen tube) emerges out, during germination.

(b) Some pollen has one germ pore, while others have two or three germ pores. If only one germ pore is present the pollen is called monocolpate.

(c) If two pores are present the pollen **is** called bicolpate and three germ pores are called tricolpate.

Development in Microspore and Formation of Male Gametophyte:

Development of male gametophyte starts when the pollens are within the anther lobes. Thus initial development of male gametophyte takes place inside the anther lobes (micros-porangia). But when half of the germination (i.e, up to 2-3 cell stage) gets completed, the anther dehisces and the germinating pollens (male gametophyte) are shed. Now pollination takes place (i.e, these pollens are transferred to the stigma of the carpel of a flower). Further development of male gametophyte take place on the stigma of carpel.

The development of the male gametophyte involves following steps –

(a) Development before pollination.

(b) Dehiscence of anther and liberation of developing pollens.

(c) Pollination and

(d) Development after pollination

(a) Development of male gametophyte before pollination (Micro-gametogenesis)

1. Development of male gametophyte before pollination occurs inside the anther (microsporan gia)

2. During germination, the nucleus of the microspore is displaced from centre to one side. This displacement is always in a particular direction and marks the position of the germi-native cell.

3. The cytoplasm becomes highly vacuolated.

4. Microspore undergoes mitotic division and forms two unequal cells:

a. The larger cell, called vegetative cell and

b. The smaller cell, called generative cell or germinative cell.

5. The vegetative cell on germination gives rise to pollen tube (after pollination, on stigma)

6. The germinative cell produces two sperms (male gametes) by one more mitosis, later.

7. The germinative cell is initially attached to the wall of pollen grain, but later comes to lie freely in to the cytoplasm of vegetative cell.

8. Generally the pollens are shed (fall) at two-cell (vegetative and germinative cell stage), and further development of the male gametophyte takes place on the stigma, after pollination. In some cases, the pollen is shed at three celled stage (vegetative cell and two sperms stage). Thus, in these plants, the sperm formation takes place before pollination.

(b) Dehiscence of anther and liberation of developing pollens. When pollens get matured (usually at 2 celled stage), they exert some pressure on anther wall. Consequently the wall of the anther gets burst and sets free the germinating pollen grains. This is called dehiscence of anther.

Thus, the gametcphyte is reduced to three nuclei, only one of which is not a gamete.

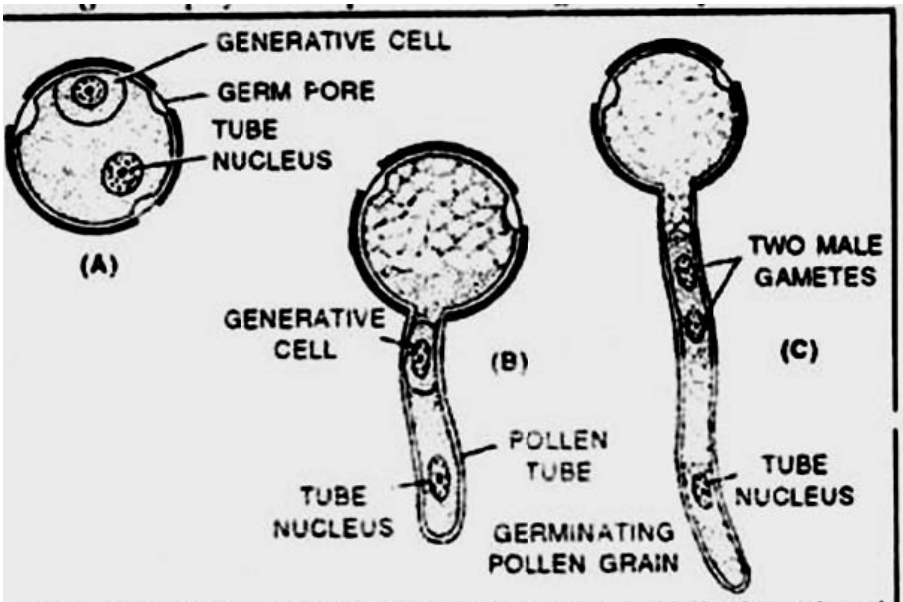


Figure 10.24 Development of male gametophyte with the formation of two male gametes (This figure too should be studied and given in continuation with the earlier figure 1.25)

Development of male gametophyte after pollination:

It involves following steps:

(i) Formation of male gametes (sperms):

(a) Only a right kind of spore germinates on the right kind of stigma, (i.e.; for germination, both pollen and the stigma must be of the plants, that belong to the same species or genus.)

(b) After pollination, the male gametophyte (germinating pollen) is usually at two cell stage. It contains a vegetative cell and a germinative cell or generative cell.

(c) The germinative cell contains a large amount of protoplasm. It acquires a characteristic vermi form appearance. It now divides mitotically, to form two elongated, haploid male gametes or sperms.

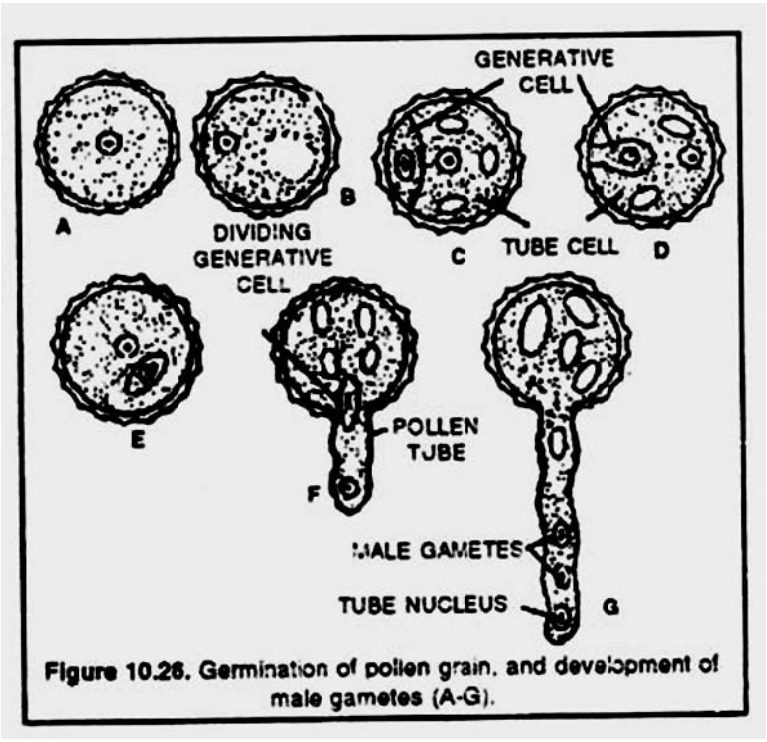


Figure 10.26. Germination of pollen grain, and development of male gametes (A-G).

Anther development in Arabidopsis has been divided into 15 stages, which commence from division of a single archesporial cell; defined cell types and adaxial–abaxial polarity are established, resulting in the formation of the mature microsporangia. The Arabidopsis floral meristem, as for theshoot apical meristem, comprises three cell layers; the stamen primordia are usually initiated by periclinal divisions in the L2 layer .Ultimately these divisions result in an anther comprising four maternal cell layers and an internal layer of sporogenous cells. The initial divisions arise from single L2 archesporial cells, which divide periclinally to form primary parietal cells (PP) subjacent to the L1 and inwardly facing primary sporogenous cell (PS). The PS cells undergo a number of divisions to generate the meiocytes, whereas the PP divides periclinally to form an endothecial cell subjacent to the L1 and a secondary parietal cell . Further periclinal divisions of the SPC generate the middle cell layer, endothecium and tapetum. In maize, the peripheral L2-derived (L2-d) cells undergo asymmetric cell division to generate the endothecium and secondary parietal cell SPC; SPCs then undergo symmetrical divisions to generatethe middle and tapetum layers, whereas the centralized L2- derived cells form sporogenous cells. The linear array of different cell types then arises from periclinal

divisions of the single archesporial cell. It has been proposed that the sporogenous cells play a key role in organization of the radially symmetrical microsporangium and that a radial field of signals is formed by the PS, inducing periclinal division and development in adjacent cells. The PP subsequently divides resulting in the endothecium cell and the meristematic SP, which undergo a further division to form the tapetum and middle cell layer.

Pollen development

Pollen development comprises three major stages: (i) microsporogenesis (differentiation of the sporogenous cells and meiosis); (ii) post-meiotic development of microspores; and (iii) microspore mitosis. Sporogenous cells (pollen mother cells or meiocytes) are encased in a tapetum-derived callose wall and undergo meiosis to form tetrads of haploid microspores. This callose layer appears essential for development and formation of the scaffold for the pollen wall, it then breaks down and the microspores are released into the anther locule to begin male gametophyte development (post-meiotic development). The free micropsores then go through one/ two rounds of mitotic divisions and pollen wall formation continues. The final mature pollen may be tricellular, or as seen in some species it can be bicellular with the final mitotic division occurring in the pollen tube. The anther filaments elongate rapidly and the flowers and anthers then open, enabling effective release of the pollen. The sporophytic anther tissues, in particular the tapetum cell layer, play an essential part in this developmental process both in the regulation and coordination of development, but also in providing materials for pollen wall formation. During pollen mitosis I, the microspore divides asymmetrically to produce a vegetative and a generative cell. Following the first mitotic division, a second mitosis produces two sperm cells enabling double fertilization to produce the embryo and endosperm.

Male gametophyte development at molecular level

During the past decade, major advances in genetic and genomic technologies have fuelled significant progress in understanding male gametophyte development at the molecular level.

Numerous regulatory genes have been identified in *Arabidopsis* as responsible for anther cell differentiation and pollen formation.The SPOROCYTELESS/NOZZLE (SPL/NZZ) transcription factor is one of the early acting regulators that is required for the initiation of cell division and differentiation in stamen and carpels. It is induced by the MADS box transcription factor AGAMOUS (AG). AG shows prolonged expression, with early roles in floral initiation via stamen and carpel specification and during later development via induction of DEFECTIVE IN ANTHER DEHISCENCE1 (DAD1) which catalyses jasmonic acid (JA) biosynthesis and results in stamen filament extension and flower opening. AG function is conserved in rice and associated with OsMADS53 and OsMADS58.NZZ/SPL acts in the anther L2 cell layer during archesporial division. In the mutant, formation of pollen mother cells (PMCs) and the surrounding cell layers fails, suggesting that SPL/NZZ determines anther and ovule development via interaction with the surrounding cell layers. In maize, the MULTIPLE ARCHESPORIAL CELLS1(MAC1) gene acts on archesporial cells and thus appears to function earlier than SPL/NZZ. The mac1 mutant produces

Mutational analysis is a powerful approach to identify structural and regulatory components that participate in a particular developmental pathway. Mutants affecting pollen development also provide excellent material to analyze processes of cell fate specification and cellular function. Currently we know about 45 such gametophytic mutants for which 13 corresponding genes have been identified.

Seven of these were identified through forward genetics, by positional cloning (GEM1, DUO1, TIO), or by isolating flanking sequence tags (UNG3, AGM, HAP5, HAP12). The remainder were functionally identified using reverse genetics approaches (AGP1, AtPTEN, ADL1C, AGM, AHA3, GPT1, VHA-A). Proteins with a variety of cellular roles have been defined including those with roles in cell division (GEM1, DUO1, TIO), nutrition and metabolism (AHA3, GPT1), pH regulation and ion transport (VHA-A, HAP5), and other groups with roles in transcriptional regulation (DUO1, HAP12) or posttranslational (AtPTEN, TIO) intracellular signaling events.

Gemini pollen1 (gem1) affects male and female transmission and displays a range of microspore division phenotypes including equal, unequal and partial divisions (Park et al.

1998). GEM1 was the first male gametophytic gene to be positionally cloned, revealing its identity to the MOR1 (Whittington et al. 2001). MOR1/GEM1 is homologous to the MAP215 family of microtubule-associated proteins that stimulate plus-end microtubule growth.

In the two-in-one (tio) mutant microspores complete nuclear division, but fail to complete cytokinesis resulting in binucleate pollen grains. In contrast to gem1, tio shows normal nuclear polarity before pollen mitosis I, but displays cytokinesis-specific defects including the formation of incomplete callosic cell plates that fail to expand. TIO, localises to the phragmoplast midline and was recently identified as the plant homologue of the Ser/Thr protein kinase FUSED (Oh et al. 2005), which is a key component of the hedgehog signalling pathway in fruitflies and humans (Lum and Beachy 2004). The duo pollen (duo) mutants complete normal asymmetric division at PMI, but fail to enter or complete generative cell division (Durbarry et al. 2005). Heterozygous duo mutants produce ~50% bicellular pollen containing a vegetative cell nucleus and a single ‘generative-like’ cell with condensed nuclear chromatin. DUO1 was recently identified as a novel R2R3 MYB protein specifically expressed in generative and sperm cells (Rotman et al. 2005). DUO1 represents the first germ line specific regulator to be identified and DUO1 homologues in maize, rice and tobacco all possess a supernumerary lysine signature in the R3 MYB domain that defines the DUO1 family (Rotman et al. 2005). Mutant generative cells in duo1 pollen fail to enter PMII and enter a partial endocycle, suggesting that DUO1 may be a specific regulator of genes required for G2 to M-phase transition (Durbarry et al. 2005).

Analysis of T-DNA insertion mutants in the single A-type cyclin-dependent kinase (CDKA;1) in Arabidopsis revealed an essential role in germ cell division (Fig. 1) (Iwakawa et al., 2006; Nowack et al., 2006). In cdka;1 mutants, germ cell division fails and DNA synthesis (S) phase of the cell cycle is delayed. A very similar phenotype to the cdka;1 mutant is observed when the F-box-Like 17 (FBL17) gene is disrupted (Fig. 3) (Kim et al., 2008). F-box proteins associate with Skp1 and CUL1 to form SKP1-CUL1-F-box protein (SCF) E3 ubiquitin protein ligase complexes.

Germline specification begins shortly after PMI, when the germ cell possesses factors that promote the germline differentiation pathway. Such factors may include suppressors of GRSF expression, which, in turn, would alleviate the repression of germline-specific genes like LGC1. Positive factors are proposed to activate DUO1, which, in turn, activates genes required for sperm cell specification, therefore integrating differentiation with cell cycle progression. Ultimately, the co-ordinated association of these parallel pathways results in a pair of fully differentiated sperm cells equipped with a complement of essential germline factors such as GCS1.

ii. Development of carpel, ovule, megaspore, female gametophyte and female germ unit. Molecular basis of female gametophyte development.

Development and fertilization of the female gametophyte

1. Development of Ovule (Megasporangium):

(a) Ovule arises as a small mound of homogenous tissue on the inner wall of the ovary (placenta).

(b) This mound develops to form the inner central part of the ovule, called nucellus.

(c) Integumentary' processes arise close to the base of this mound, which forms outer covering of the ovule.

Structure of ovule (mega sporangium):

(a) Each ovule is attached to the inner wall of the ovary (placenta), by a slender stalk, called funicle.

(b) The point of attachment of ovule to its funicle is called hilum.

(c) Main body of the ovule is formed by inner central mass i.e., nucellus. Nucellus consists of living parenchymatous cells.

(d) Each mature ovules the nucellus serves to cover and provide nutrition to the embryo sac (female gametophyte).

(e) Each ovule has two distinct ends-a micropyle end (it also called opening of ovule during fertilisation) and b. Chalaza end (the posterior end, opposite to micropylarend).

(f) Externally the nucellus is covered by one or two protective covers, called integuments. These integuments arise from the chalazal end.

(g) When only one integument is present, the ovule is called unitegmic, and if the ovule consists of two integuments, it is called bitegmic very rarely tri-tegmic (with three integuments) is present in plants like Asphodelus. In some plants such as Santalum, etc, ategmic (no integument) condition may be present.

(h) In mature ovules, the female gametophyte or embryo sac is present in the centre. The embryosac consists of egg cell (female gamete), synergid cells, antipodal cells and polar nuclei, (this is described a little later).

Female gametophyte development occurs over two phases referred to as megasporogenesis and megagametogenesis.More than 15 different patterns of female gametophyte developmenthave been described.The developmental pattern exhibited by most species is referred to as the Polygonum type because it was first described in *Polygonum divaricatum*.

Megasporogenesis

The ovule or the megasporangium develops as a small protuberance of the placental tissue. In the very young ovule a single hypodermal cell is differentiated as the archesporium.

This archesporium cell may or may not cut off some parietal cells and then becomes the megaspore mother cell. The megaspore mother cell now undergoes meiosis or reduction division, and, usually, a linear row of four haploid megaspore cells ('linear tetrad') is formed.

Meanwhile, two integuments develop from the base of the ovule. Of the linear tetrad of megaspores, usually the lower- most one enlarges and becomes the functional megaspore while the three on top disinte-grate.

The functional megaspore now develops the female gametophyte or the embryo sac. In Angiosperms, the development of the female gametophyte is completely endosporous, i.e., within the megaspore. In a typical case, the nucleus of the embryo sac, which is the same as the functional megaspore, divides into two, then four and finally, eight daughter nuclei (four of which are located at each pole.

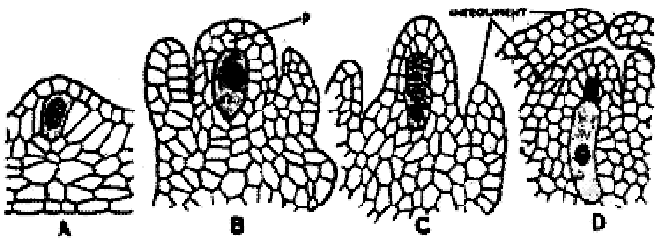


FIG. 411. Stages of ovule development and megasporogenesis. A. Protuberance of placental tissue and differentiation of archesporium (shaded). B. Parietal cells (*p*) and megaspore mother cell (shaded). One integument is developing. C. Linear tetrad of megaspores (shaded). D. Disintegration of the three upper megaspores and enlargement of the functional megaspore. Both integuments have developed.

Then, one nucleus from each pole moves to the centre of the embryo sac and fuses there forming the fusion or secondary nucleus. Finally, the embryo sac or the female gametophyte becomes organized.
Three nuclei at the base form the antipodal cells. The secondary nucleus remains at the centre. On the top, three cells from the egg apparatus which consists of two flask-shaped synergids and a round egg cell (or ovum or oosphere) hanging between and below them.
The synergids usually are somewhat notched by an indentation and they show striations at the tip (‘filiform apparatus’). There is prominent vacuolation below the synergid nuclei and above the egg nucleus, showing accumulation of cytoplasm at different regions.
While the above method of gametophyte formation is usually described as the typical or normal type it has been found that many Angiosperms do not conform to this rule but show variations.

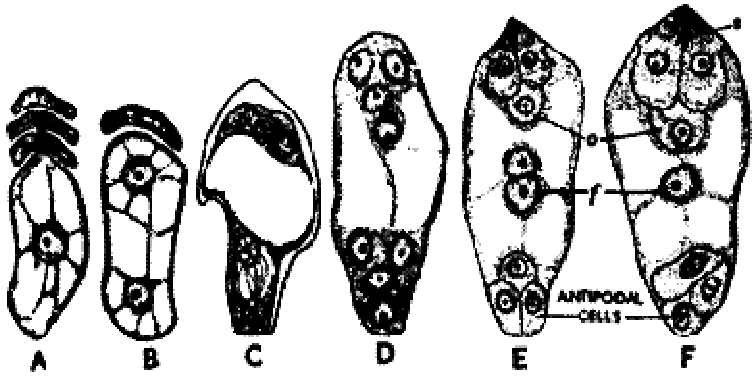
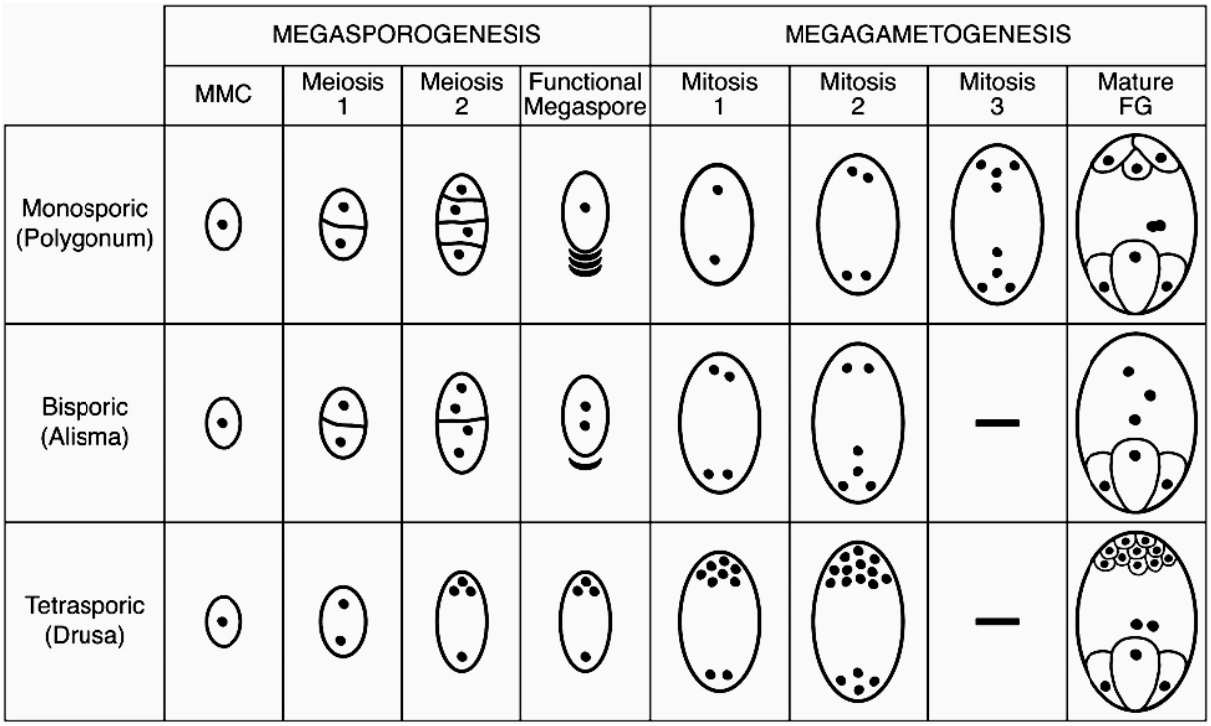


FIG. 412. Development of the female gametophyte or the embryo sac. A. Functional megaspore. Disorganising megaspores on top. B. 2-nucleate stage. C. 4-nucleate stage. D. 8-nucleate stage. E. Coming together of two nuclei at centre and organisation of embryo sac. F. Mature embryo sac showing egg apparatus with two synergids (*s*) and an egg cell (*e*) at micropylar end, fusion nucleus (*f*) at centre and antipodal cells at chalazal end.

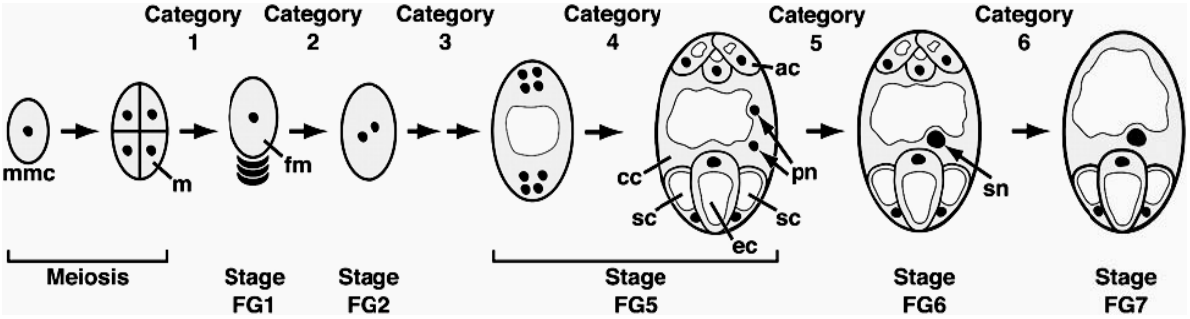
During megasporogenesis, the diploid megaspore mother cell undergoes meiosis and gives rise to four haploid nuclei. Angiosperms exhibit three main patterns of megasporogenesis, referred to as monosporic, bisporic, and tetrasporic. These three patterns are summarized in Figure 2. The three types differ mainly in whether cell plate formation occurs after these divisions, thus determining the number of meiotic products that contribute to the formation of the mature female gametophyte. In the monosporic pattern, both meiotic divisions are accompanied by cell plate formation, resulting in four one-nucleate megaspores. Subsequently, three megaspores, generally the micropylar-most megaspores, undergo cell death. In the bisporic pattern, cell plates form after meiosis I but not meiosis II.The result is two two-nucleate megaspores, one of which degenerates. In the tetrasporic pattern, cell plates fail to form after both meiotic divisions, resulting in one four-nucleate megaspore.Thus, these three patterns give rise to a single functional megaspore that contains one (monosporic), two (bisporic), or four (tetrasporic) meiotic nuclei. The monosporic pattern is the most common form and is represented within the *Polygonum* pattern.



Megagametogenesis

During megagametogenesis, the functional megaspore gives rise to the mature female gametophyte. Initially, the megaspore undergoes one or more rounds of mitosis without cytokinesis, resulting in a multinucleate coenocyte. Subsequently, cell walls form around these nuclei, resulting in a cellularized female gametophyte. For example, in the Polygonum-type pattern, a single nucleus undergoes two roundsof mitosis, producing a four-nucleate cell with two nuclei at eachpole. During a third mitosis, phragmoplasts and cell plates formbetween sister and nonsister nuclei, and soon thereafter, thefemale gametophyte cells become completely surrounded bycell walls. During cellularization, two nuclei, one from each pole(the polar nuclei), migrate toward the center of the developingfemale gametophyte and fuse together either before or uponfertilization of the central cell. These events result in a sevencelledstructure consisting of three antipodal cells, one centralcell, two synergid cells, and one

egg cell .The monosporic,*Polygonum* type of female gametophyte is typically aseven-celled structure at maturity. However, this structure maybe modified by cell death or cell proliferation events in variousspecies. For example, in *Arabidopsis*, the antipodal cellsundergo cell death immediately before fertilization (Figure 3),whereas in grasses (e.g., maize), the antipodal cells proliferate.



Molecular basis of female gametophyte development:

The small size of the female gametophyte and its inaccessibility, which is owing to it being embedded within the sporophytic tissues of the ovule, have made it a particularly challenging and intractable system for developmental studies. Large-scale forward genetic screens have been employed, using insertional mutagens such as T-DNA or transposons, to identify genes with important functions in the embryo (Bonhomme et al., 1998; Christensen et al., 1998; Feldmann et al., 1997; Howden et al., 1998; Pagnussat et al., 2005). These screens have revealed genes that affect embryo sac development, its capacity for fertilization and the post-fertilization development of the seed. In the majority of cases, the mutations are in genes that control essential functions, such as cellular metabolism or the cell cycle, although mutations in putative regulatory or signaling genes can also be found (Pagnussat et al., 2005). However, such screens are generally limited to the discovery of single genes that result in phenotypes when mutated, and are less effective in the identification of multiple redundant genes that might be required for a developmental process.

From both forward and reverse genetics approaches, functions for several transcription factors involved in cell-type differentiation have been uncovered. For example, the ATMYB98 transcription factor was identified by its synergid-specific expression and appears to be required for the formation of the filiform apparatus (see Glossary, Box 1) by the synergid cells (Punwani et al., 2007; Punwani et al., 2008). AGL80 and AGL61, two Arabidopsis genes that are expressed specifically in the central cell and that belong to the type I MADS-box family of transcription factors, appear to be important for the normal differentiation and function of the central cell. Plants that carry mutations in these genes fail to express central cell markers, to achieve fertilization of the central cell or to initiate endosperm development (Bemer et al., 2008; Portereiko et al., 2006). Because these genes are expressed relatively late after cellularization, it is probable that they establish central cell identity and function after cell specification. Mutations in another type I MADS-box transcription factor gene, AGL23, arrest embryo sac development at the FM stage; however, the phenotype is incompletely penetrant, and the exact role of this gene in gametophyte development remains to be determined (Colombo et al., 2008).

Mutants that alter cell-fate specification are very infrequent, and only four such mutants have been described. In the eostre mutant, the mis-expression of the Knox-TALE homeodomain gene BEL-like homeodomain 1 (BLH1) in the embryo sac produces nuclear migration abnormalities from stage FG3 onwards that correlate with the production of an extra functional egg cell in place of a synergid

(Pagnussat et al., 2007). These observations suggested that the determination of egg cell fate might depend on a location-specific mechanism within the syncytial female gametophyte (see below). However, eostre is a gain-of-function mutation, and as the loss-of-function of BLH1 has no phenotype, and as BLH1 is not expressed in wild-type embryo sacs, it is probable that another BLH gene from the same family of ~13 genes in Arabidopsis functions in nuclear positioning in the wild-type embryo sac. The Knox-TALE family of transcription factors is found in both animals and plants (Burglin, 1997), and members of this family typically function as heterodimers. In plants, the BLH proteins interact with Knotted-like homeobox (Knox) proteins to activate transcription (Bellaoui et al., 2001). Interestingly, the gain-of-function eostre mutant is suppressed by loss-of-function of KNAT3, which encodes a type II Knox protein; these form a sub-class of Knox proteins conserved even in lower plants, but with as yet no defined functions. It is possible that nuclear positioning during embryo sac development is mediated by KNAT3 in association with an unidentified BLH gene product.

Three other genes, LACHESIS (LIS), GAMETOPHYTIC FACTOR1 [GFA1; CLOTHO (CLO)] and ATROPUS (ATO) have been found to be required to restrict gametic cell fate (Coury et al., 2007; Gross-Hardt et al., 2007; Moll et al., 2008). Mutants in these genes show changes in cell identities inside the embryo sac that only become apparent after cellularization, after an initially normal development, including normal nuclear positioning within the syncytium (Gross-Hardt et al., 2007; Moll et al., 2008). In the embryo sacs of lis, gfa1 (clo) and ato mutants, cells that are normally specified as synergid cells can express egg cell-specific markers, and cells that are normally specified as antipodal cells can express central cell-specific markers. In addition, in clo and ato mutants, cells that are normally specified as central cells can express egg cell-specific markers. Also, pollen tube attraction is reduced and no zygotes are produced, which suggests pleiotropic effects on other embryo sac functions, including fertilization. It has been proposed that a mechanism of lateral inhibition by the gametic cells, i.e. the egg cell and the central cell, operates to prevent the formation of excess gametic cells inside the female and to maintain the cell fate of the accessory synergids and antipodals (Gross-Hardt et al., 2007). All three genes, LIS, GFA1/CLO and ATO, encode pre-mRNA splicing factors that are conserved among all eukaryotes (Bartels et al., 2003; Horowitz et al., 1997) and are expressed throughout the Arabidopsis plant. In the mature embryo sac, CLO and ATO are expressed in all cells, whereas LIS is restricted to gametic cells. It is not clear why such altered cell-fate phenotypes arise from mutations in genes that encode components of the core splicing machinery. It is possible that the splicing defects might disrupt signaling mechanisms required for the maintenance of cell fates specified at cellularization.

Genetic and molecular basis of self incompatibility. Fertilization, double fertilization, triple fusion, imprinting and endosperm development in gymnosperms and angiosperms, embryogenesis in gymnosperms and angiosperms, establishment of symmetry in plants, seed formation.

Self-incompatibility or intraspecific incompatibility is a well-designed genetic mechanism by which certain plants recognize and reject their own pollen thus forcing outbreeding. It is defined as "**inability of the plant producing functional gametes to set seed upon self-pollination**",. Lundqvist (1964) defined it as "the inability of fertile hermaphrodite seed plant to produce zygotes after self-pollination".

Its genetic system is based on a single locus, the sterility (S) locus, with multiple alleles. Pollen germination or pollen tube growth is blocked when the pollen grain and the stigma upon which it

lands have the same allele at the same locus. Besides the genetic factors, intraspecific incompatibility is also associated with different lengths of stamens and style in flowers on same plant.

This self- incompatibility is acquired nearly one or two days before anthesis as well as in open flowers. Nearly two-thirds of the families of angiosperms exhibit self- incompatibility. The significance of SI in the evolutionary context cannot be overstated, since its possession leads to obligate outbreeding and the maintenance of heterozygosity within a species.

In the crop and ornamental plants, most of the perennial grasses, forage, legumes, and members of Brassicaceae, Asteraceae, Rosaceae, and Solanaceae have SI mechanism of varying kinds and degrees of effectiveness.

Self-incompatibility system can be divided into two basic groups: 1. Heteromorphic Systems 2. Homomorphic Systems.

1. Heteromorphic Incompatibility:

This incompatibility refers to those plants where the same species produces more than one morphological types of flower, where the relative length of the style (distyly and tristyly) and stamens differ. In distyly the plants produce two forms of flowers, called long styled or pin morph and short styled or thrum morph.

Distyly is controlled by a single gene complex, S, with two alleles S arid s. The allele for short-style (S) is dominant over the long-style (s) allele. The long-styled plants are homozygous recessive (ss), and short-styled are heterozygous (Ss).

Thus compatible pollination (Ss x ss) results in progeny of nearly equal number of long-styled and short-styled form. Lewis (1954) believes that the S gene (super gene) has six linked genes, viz., G S I' I" P A, where, G= length of style, S = surface of stigma, I' = pollen incompatibility, I" = stylar incompatibility, P = pollen size and /or shape, and A = height of stamen.

The tristyly plants produce three floral morphs, namely, long-styled, mid-styled and short-styled. Each of these morphotypes produces anthers at different levels that correspond to the level of stigma in the other two forms.

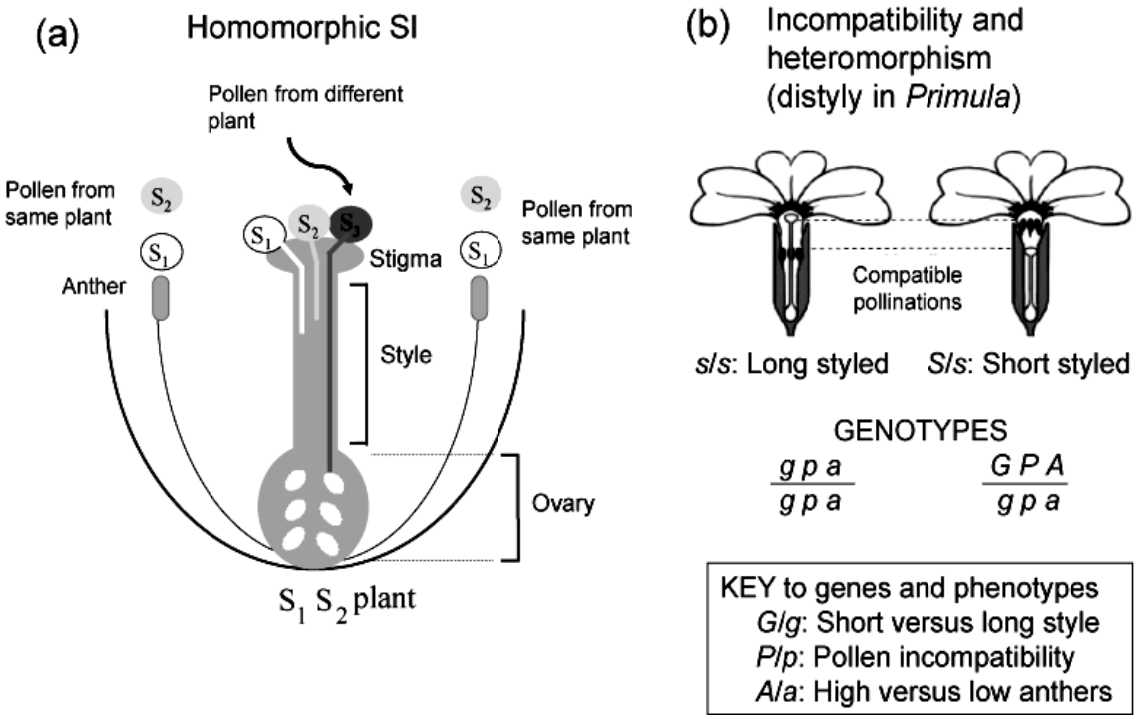
In a successful pollination pollen from long stamens of short-styled morph is compatible on stigma of long-styled morph and not on mid-styled morph, while those from mid- stamens are compatible on stigma of mid-styled morph and not on long-styled morph (Fig 6.9b).

Tristyly is controlled by two genes, M and S where S is epistatic to M, and both the two alleles. The long-styled morph is homozygous recessive for S (ss) and homozygous dominant or heterozygous for M (ss MM/ ss Mm). The short-styled morph is heterozygous for S, and M (Ss mm/Ss Mm/Ss MM).

The zone of inhibition is an important factor in incompatibility and is co-related with the pollen cytology or crosses among different floral morph. For instance the 3-celled pollen of *Linum* and *Limonium* show stigmatic inhibition, whereas the 2-celled pollen of *Primula* shows stylar inhibition. In Fagopyrum, inhibition in thrum x thrum pollination is in the stigma, while in pin x pin pollination it is in the style.

It has been seen that in *Primula vulgaris* incompatible pollen is inhibited at different sites, viz., on stigmatic surface due to failure of germination or pollen tube penetration through the stigmatic head or transmitting tract of the style. In *Linum thrum* or short-styled stigma, support adhesion and germination of both thrum and pin pollen, however, only thrum pollen is effectively inhibited in the stigma.

The involvement of a stylar protein dialysates in the intercellular secretions of the transmitting tissue in *Primula vulgaris* for incompatibility responses was recorded by Shivanna (1978a) in both the morph types. The protein inhibited tube growth of pollen of the same morph more strongly than that of the other.



Homomorphic Incompatibility:

In this incompatibility the flowers produced by different plants do not show morphological variations. The physiological barriers act in such a way that pollens do not germinate on a stigma of similar genetic constitution or their growth is so slow that by the time the pollen tube reaches the embryo sac, the latter withers out.

Almost all the molecular work on self-incompatibility has come from homomorphic systems which have been reported in over 250 genera.

East and Mangelsdorf (1925) have given a satisfactory genetic interpretations of homomorphic incompatibility in Nicotiana. They found that the rate of pollen tube growth on stigma and style of *Nicotiana* is governed by a gene S with multiple alleles (S1, S2, S3, S4,……. Sn) at a single locus. Each plant has only two of these alleles.

Cells of styles and stigmas are diploid and carry both the alleles of the plant. Pollen grains are haploid and, therefore, carry only one of the two alleles. A pollen grain carrying a self sterility allele does not grow well on a female style carrying the same allele, but grows and fertilizes a plant that carries the other allele. For instance, a plant with the genotype S1, S2 will produce two types of pollen grains, S1 and S2, none of which will grow on a style with these alleles.

Homomorphic incompatibility is divided into gametophytic and sporophytic control. In the gametophytic self-incompatibility phenotype is determined by the genotype of the pollen grain, whereas in the sporophytic type, pollen rejection is imposed by the genotype of the pollen parent plant and not by the pollen genotype.

Table 6.3 : Here both S_1 and S_2 pollen tubes are inhibited in the S_1S_2 style. In cases where there is no match of alleles, for instance, S_1S_2 pollen grains on an S_3 and S_4 pistil, pollen grains of both genotypes grow through the style to the ovule and embryosac. The S_2 pollen grains will also grow successfully through the S_1S_3 style

Gametophytic Incompatibility			
Expression of genotype of individual pollen grains	S_1 S_2	S_1 S_2	S_1 S_2
Pistil Genotype	S_1 S_2	S_1 S_3	S_3 S_4

Table 6.4 : Here a match of the alleles of the pollen parent with those of the pistil, for example S_1S_2 or S_1S_3, cause arrest of pollen germination. In the absence of a match, for example, S_3S_4 style with $S_1S_2S_1S_2$ pollen grains, there is germination and growth through the style and into the embryosac. In case of the central panel the allele S_1 is dominant to, or codominant with S_2 in the pollen and S_3 in the style. If S_3 is dominant to S_1 in the style or S_2 is dominant to S_1 in the pollen, the pollen from the S_1S_2 parent will be compatible

Sporophytic Incompatibility			
Expressed genotype is that of pollen parent	S_1S_2 S_1S_2	S_1S_2 S_1S_2	S_1S_2 S_1S_2
Individual pollen grains genotype (haploid)			
Pistil genotype (diploid)	S_1S_2	S_1S_3	S_3S_4

Molecular Basis of Self-Incompatibility:

The overwhelming support for the presence of specific proteins that arbitrate in self-incompatibility, the major arena of research then centered on the isolation of incompatibility genes, identification of the protein products (S- proteins), and characterization of S-gene expression in wild type and transgenic plants.

i. Molecular Basis of Gametophytic Self-Incompatibility:

The molecular biology of self-incompatibility has been worked most extensively in Nicotiana alata. Sequential expression of S-genes and their protein products in the female floral parts perfectly matches with the site and the time of incompatibility.

Northern blot analysis of RNA from flower buds of different ages probed with a cDNA clone encoding S-linked proteins showed that, gene expression is maximum in mature styles, comparatively weak in the ovary and totally absent in the immature styles.

Similar analysis at the tissue and cell level showed that the gene transcripts are localized in the stigmatic papillae and the stylar transmitting tissue of mature flowers and in the epidermis of the placenta.

In fact the gene transcripts are localized in those tissues and cells, through which the pollen tube makes its journey to reach the embryo sac. S- gene expression and self-incompatibility in *Nicotiana alata* by immunocytochemical localization of allele-specific antibodies has removed all doubt about this relation. It showed a remarkable coincidence between accu-mulation of gene products and gene transcripts in the same tissue and cells of the carpel.

The S-gene products were found to be homologous to the fungal RNases, thus the stylar product of the S-gene was designated as S-RNases. Several physiological factors and molecular approaches have succeeded in holding S-RNase responsible for self- incompatibility.

The high expression of S- RNases in a transgenic hybrid developed between two self-compatible line of Nicotiana, viz., Nicotiana alata and N. langsdorfii leaves no doubt that pollen rejection is due to RNase action. On the other hand, antisense suppression of S-RNase activity in a transgenic hybrid between Nicotiana plumbaginifolia and N. alata allowed the acceptance of self pollen.

Besides antisense suppression of S-RNase and prevention of self pollen rejection, there is another way to do so by introducing a S-gene construct, into Petunia inflata where one of the histidine residues, which is the heart of RNase molecule essential for its catalytic activity, is replaced by asparagine by site-directed mutagenesis.

The transgenic plant produced the modified protein lacking the S-RNase activity but failed to reject the self pollen bearing the same allele. Mc-Clure (1990) explained the mechanism of RNase function in a self-incompatible system.

They found that RNA extracted from styles of incompatibly pollinated Nicotiana alata flowers suffered a massive degradation and appeared as a streak of low molecular mass in an agarose gel run under denaturating condition.

Since S- RNase is easily taken up by the pollen tubes grown in vitro, cytotoxic effects of the enzymes on the pollen tube RNA may be responsible for RNA degradation. Thus ensuring the death of the pollen tube in an incompatible pollination.

Changes in the level of cytosolic Ca^{2+} have been found to be responsible for self- incompatibility reaction in Petunia rhoeas. It has been seen that by raising the endogenous level of Ca^{2+} by ultra violet activation, the growth of pollen tube was inhibited even in the absence of incompatible stigmatic extract. This elevated Ca^{2+} level induced by the S-locus proteins might moderate the transcriptional activation of genes necessary for the initiation of self- incompatibility reactions in the species.

ii. Molecular Basis of Sporophytic Self-Incompatibility:

Sporophytic self-incompatibility has been best characterized in *Brassica oleracea* and *Brassica campestris* and the stigmatic protein responsible for the incompatibility is a S-locus- specific protein called glycoproteins (SLGs). Separation of cDNA clones encoding SLGs from different homozygous lines of *Brassica oleracea* initiated the molecular analysis of sporophytic self-incompatibility.

The SLG genes transcribe closely related glycoproteins that are secreted into the walls of the stigmatic papillae. Experimental regulation of an SLG gene in a transgenic plant gives support to the existence of a correlation between cellular and developmental expression of the gene with the site and the timing of inhibition of pollen germination.

An SLG gene from Brassica oleracea incorporated into Nicotiana tabacum is expressed at the protein level in the stylar transmitting tissue. Southern blot analysis of genomic DNA of Brassica oleracea has exposed the existence of several sequences with different degrees of homology to the SLG gene.

A gene designated as S-locus related (SLR) is not linked to S-locus gene series, but like the SLG gene its expression is restricted to the papillar cells of the stigma concurrent with the achievement of self-incompatibility.

In spite of considerable similarity with the SLGs in supposed signal sequence, N-glycosylation sites and a cystein- rich domain has a subsidiary role in incompatibility. It appears that another gene designated as S-locus receptor kinase (SRK) maybe involved in the mechanism that activates self-pollen rejection in Brassica oleracea.

This gene is genetically linked to the SLG gene at the S-locus and has a complex structure consisting of an SLG-like domain, presumed serine/threonine protein kinase domain, and a stretch of transmembrane domain linking the SLG-like region with the kinase moiety.

Hiscock (2002) proposes a model for the mechanism of SSI in Brassica oleracea. Inhibition of incompatible pollen occurs at the stigma surface, usually before germination of the pollen, and appears to involve a deregulation of water flow to the pollen from the stigma during hydration of the pollen grain (Dickinson, 1995). Here, pollen from an S1S2 individual is inhibited on an S1S3 stigma as a consequence of a haplotype-specific interaction between male (pollen) and female (stigma) products of the S1, haplotype.

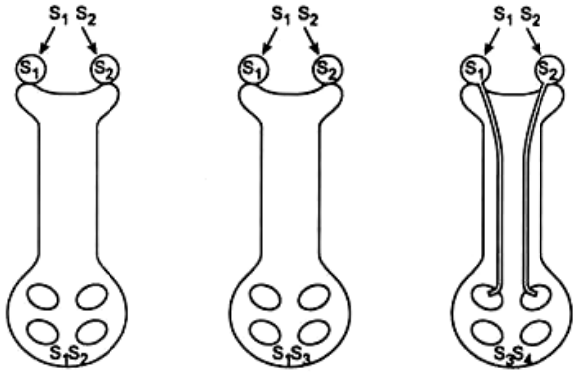


Fig. 6.15 : In SSI the SI phenotype of the pollen is determined by the diploid genome of its parent plant, so if S alleles are expressed co-dominantly 'in the pollen', 100% of the pollen from a S_1S_2 individual will be inhibited by an S_1S_3 pistil (in SSI, pollen is usually inhibited on the stigma). SSI is possible because pollen S alleles are expressed sporophytically in diploid cells of the anther tapetum that supplies the S proteins to the pollen coating.

This S proteins are colour coded: S1, green; S2, blue; and S3, pink. SCR1, a pollen S protein is recognized by, and binds to the extracellular receptor domain of SRK1, a stigma S protein, thereby inducing dimerization of SRK1, and autophosphorylation denoted by P, on serine and threonine residues in the kinase domain (shown as a zig-zag tail). 'Activation' of the SRK protein then initiates an intracellular signaling cascade within the stigma that leads to localized rejection of the pollen.

SLG acts as an accessory protein in the formation of the receptor complex PCP-A1, a small cysteine-rich pollen-coat protein similar to SCR, but not genetically linked to S, binds nonspecifically to SLGs from all haplotypes and may function as an additional accessory protein alongside SLG during formation of the receptor complex.

Signaling downstream of SRK has not yet been characterized, but essential for the SI response is ARC1, an arm-repeat protein, that binds to the kinase domain of SRK in a phosphorylation-dependent manner, but whose function is as yet unknown.

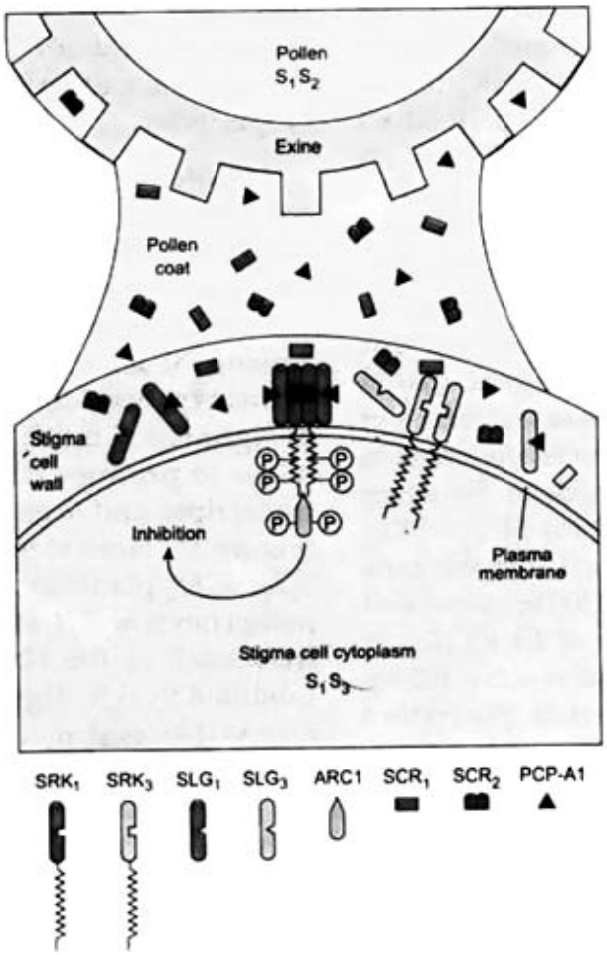


Fig. 6.16 : Model for the mechanism of SSI in *Brassica oleracea.*

Nasrallah (1994) provided evidence that the binding of a pollen-borne cell surface ligand to the SLG-SLK receptor leads to a series of resultant reactions mediated by a protein kinase, which regulate the sporophytic incompatibility system. The strongest support is that the amino acid sequence of the SRK gene product has characteristics of a plasma membrane based protein.

The specific targeting of the SRK protein to the plasma membrane of the stigmatic cells of the transgenic tobacco further support the view the molecular role of protein kinase as pivotal in the incompatibility response.

In an another experiment it was found that the spontaneous mod ("modifier") mutation in *Brassica campestris* produce self-compatible plants characterized by the absence of an aquaporin-like gene encoding a water protein channel.

Ikeda (1997) from this observation concluded that aquaporin regulates the self- incompatibility reaction in wild -type plants as a component of the SRK signaling system, by obstructing the flow of water from the stigmatic papillae into pollen grains.

Determinants of Self-Incompatibility:

The self-incompatibility phenomenon is controlled by a complex and polymorphic locus.

Among the genes at this S-locus is a pair of sequence related genes, the cell wall localized S- locus glycoprotein (SLG) gene and the plasma membrane-spanning receptor protein kinase (SRK gene), both of which are expressed specifically in the stigma epidermal cells. Different variants of the S-locus, designated as haplotypes, are characterized by highly polymorphic alleles for SLG and SRK.

Although one function of SLG is to stabilize SRK, it itself is viewed as a ligand- activated receptor kinase and the primary female determinant of self-incompatibility. Selective binding of pollen-borne ligand is thought to initiate a signal transduction cascade resulting in the SI pollen rejection reaction.

Recombination analysis of the Brassica campestris S8 haplotype has shown that the male and the female SI determinant are contained in a 65-kb chromosomal segment encompassing SLG8 and SRK8.

During sequence analysis of the 13-kb region between SLG8 and SRK8 it was found that the 400-base pair Hind III-Xba I restriction fragment indicated in Figure 1A (arrange sequence) contained a small segment with an unusual high frequency of cystein residue in one of the deducible reading frames. RNA gel blot analysis with the 400 bp Hind III- Xba I fragment as probe revealed that this segment is part of a transcribe gene that is expressed specifically in anthers.

Screening of an Ss anther cDNA library allowed the isolation of a 450-bp full length cDNA clone. Sequence comparison of this clone and the SLG8– SRK8 intergenic segment determined that the gene consists of two exons (110 and 300 bp) separated by an unusually large intron of 4.1 kb.

In DNA gel blot analysis, the 400-bp Hind III-Xba I probe detected restriction fragment with sizes predicted by the restriction map (figure B, below) Thus a single copy, 5 locus-encoded, anther expressed gene, which was named 5 locus cystein-rich protein (SCR) gene.

To test that whether SCR is indeed that male determinant of SI, a detail analysis of both its expression and function was done by Schopfer,1999. They analyzed the expression of wild type self-incompatibility plants by RNA gel blot analysis.

SCR shows an anther specific, developmentally regulated expression. SCR transcripts can be detected in anthers only after the generation of haploid microspores, with transcripts accumulating in the microspores. Therefore, the SCR gene is active postmeiotically and gametophytically.

SCR transcripts in anthers isolated from a self-incompatible mutant Brassica oleracea strain, designated ml600, generated by gamma- irradiation of an S13S13homozygote. Reciprocal pollination between ml600 resulted in the loss of S13 specificity in pollen but not in stigma.

Gel blot analysis of anther RNA demonstrated that in contrast to plants carrying the wild type S13 haplotype, ml600 anthers lacked detectable SCR transcripts (Figure above 3A, lanes 8 to 10). The correlation between loss of pollen S13 specificity and absence of detectable SCR,, transcripts in the ml 600 mutants strain provides strong evidence that SCR is necessary for pollen SI specificity.

In order to establish the function of SCR as the pollen determinants of SI Schopfer (1999) transformed a B. oleracea S2S2 homo-zygous strain with a chimeric gene consisting of the SCR6 promoter fused to the SCR6 cDNA. Among the 14 hygromycin resistant plants, 12 plants expressed

the SCR6 transgene and were designated S2S2/SCR+6 where two plants failed to produce detectable levels of SCR6 transcripts and were designated S2S2/SCR+6.

Pollen from the two S2S2/SCR+6 plants germinated and produced pollen tubes on S6S6 stigmas. In contrast pollen from each of the 12 S2S2/SCR+6 plants was inhibited by S6S6 stigmas, even though pollen was viable as demonstrated by its ability to produce pollen tubes on stigmas homozygous for an unrelated S-haplotype such as S22. Thus pollen of S2S2 /SCR+6 transformants had acquired S6 specificity not only proves the involvement of SCR in SI, but also demon-strates that this gene is sufficient for determining male SI specificity.

Significance of Self-Incompatibility:

Self-incompatibility is by far the most efficient outbreeding mechanism. It has been envisaged as one of the main causes for the rapid evolution of angiosperms. In many crop plants the major limitation to exploit hybrid vigour is the lack of male sterile line, and the absence of suitable chemicals to induce effective male sterility without affecting the female fertility.

Thus, here self-incompatibility can be used effectively in producing hybrid seeds, without undergoing emasculation, nuclear or cytoplasmic sterility or restoring to gametocides. Thus it is very essential to select strictly self- incompatible lines, which will not demonstrate pseudo-compatibility under reasonably varied environmental conditions.

Besides its importance in breeding programmes, it sometimes becomes essential to overcome self-incompatibility. These include, production of pure lines of the selected parents in the plant breeding programmes; maintaining hybrid characters in species which are difficult to propagate vegetatively or susceptible to viral infection; in fruit orchards self-incompatible is a rule, however, it becomes necessary to include varieties that with cross-compatible, because lack of proper weather conditions for the activity of pollinating insects may drastically reduce yield.

Fertilization:

Fertilization is angiosperms, is unique. It is called **double fertilisation**. This is because there are two sperms per pollen tube. One of the sperms fertilises the egg cell to form zygote, while remaining sperm fertilises two polar nuclei, resulting in the formation of a triploid endosperm cell. The phenomenon of fertilization was first reported by **Strasburger** (1884) in *Monotrapa*. The male gametes are brought to the egg present in female gametophyte by a pollen tube. This phenomenon is called **siphonogamy** which was discovered by G. B. Amici in *Protulaca* plant.

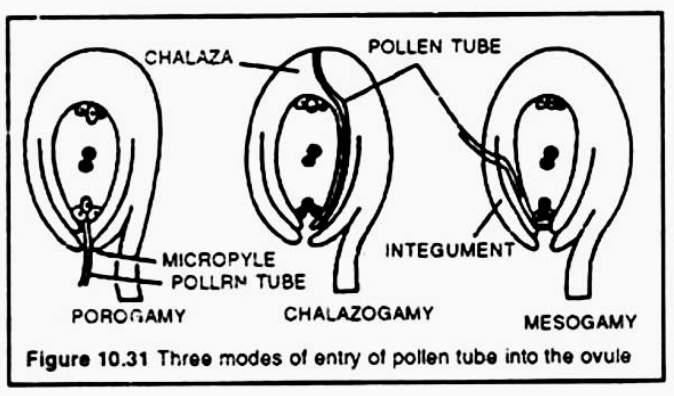


Figure 10.31 Three modes of entry of pollen tube into the ovule

Important events in fertilization are following:

1. Entry of pollen tube into ovule and embryo sac:

(a) After arriving in the ovary, the pollen tube finds its way through style and enters the ovule.

(b) Depending on the place, the retry of pollen tube into the ovule, can be of following three type:

(i) Porogamy:

It is the most common type. Here the pollen tube enters the ovule from the micropylar end. According to some workers, the porogamy is directed by filiform apparatus (of synergid).

(ii) Chalazogamy (Basigamy):

In this case the pollen tube enters the ovule from chalazal end; however the entry into the embryo sac occurs through micropyle. Ex- *Casuarina*.

Mesogamy:

In this case the pollen tube enters the ovule through the funiculus or integuments. However the final entry into the embryo sac occurs through micropyle. But the pollen tube with two mole gametes enters into the embryosac only through the micropyle.

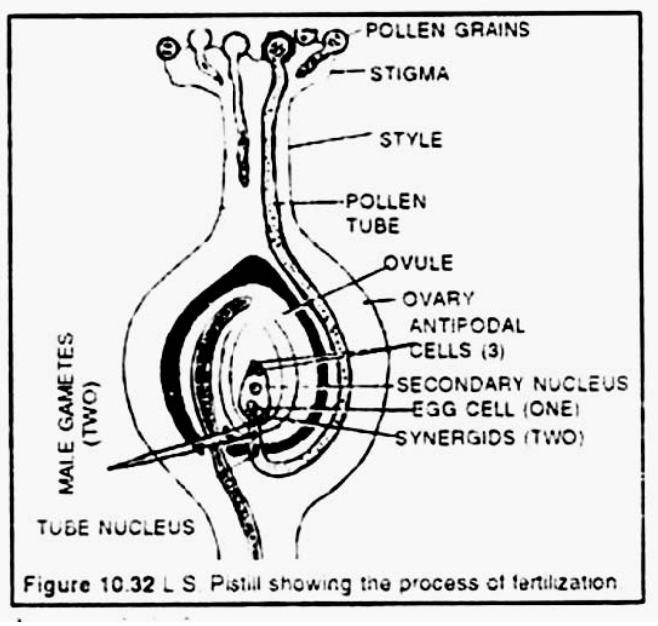


Figure 10.32 L S. Pistil showing the process of fertilization

2. Discharge of male gametes (sperms):

(a) The pollen tube contains two sperms (each is a haploid male gamete).

(b) When pollen tube enters the embryo-sac (inside the ovule), it bursts to release its contents i.e., two sperms along with certain amount of protoplasm .The first male gamete discharge in one of the synergid.

3. Double fertilization:

(a) Both the male garnet present in pollen tube utilises in fertilisation process of angiosperm is known as double fertilisation. It is the characteristic feature of angiosperms except Family Orchidaceae, Podostemaceae and Trapaceae.

It involves two types of fusion –

a. Syngamy (fusion of egg cell and one male gamete) and

b. **Triple fusion** (fusion of remaining male garnet and two proper nuclei).

c .It was first observed by Nawaschin (1898) in Fritilaria and Lilium. It was supported by Guignard (1899).

(1) Syngamy:

(a) One of the two sperms goes to fertilize the egg cell. This fusion is called syngamy.

(b) It results in the formation of zygote, which gives rise to proper embryo.

(2) Triple fusion:

(a) The remaining sperm now fuses with the two haploid polar nuclei (present in the centre of embryo sac).

(b) This fusion is called as triple fusion (as three nuclei i.e., one male garnet and 2 polar nuclei, are fused).

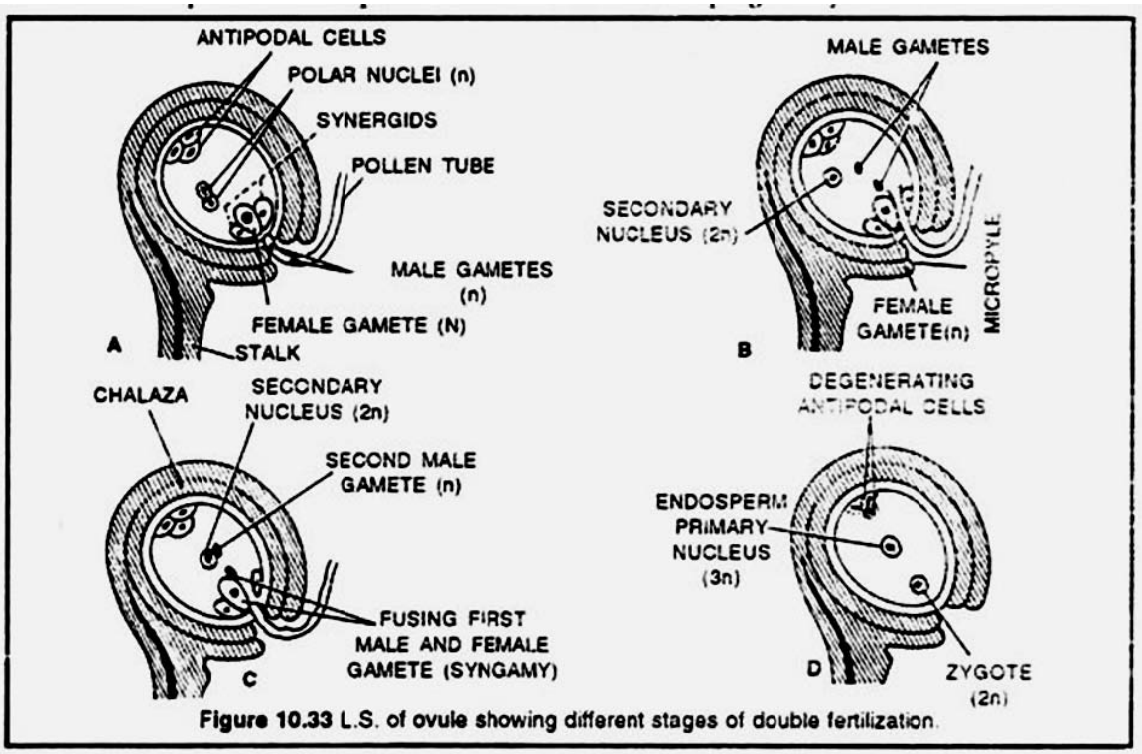


Figure 10.33 L.S. of ovule showing different stages of double fertilization.

(c) It results in the formation of triploid endosperm nucleus, which on development (Repeatd mitosis) form the endosperm.

(d) Endosperm is therefore triploid in angiosperms (It is a characteristic feature of angiosperms)

(e) Endosperm serves to provide nutrition to the developing embryo.

Development of Embryo (Embryogeny):

As written earlier also, the highly organized body of a seed plant represents the sporophytic phase of the life-cycle. It begins its existence usually with the fertilized egg, the zygote, which develops into the embryo by characteristic steps showing characteristics of future adult organization of the plant. Embryo, as a whole, assumes a specific form in which an axis and one or more leaf like appendages, the cotyledons, can be recognised.

Because of its location below the cotyledons, the stem like axis is called hypocotyl. At its lower end (i.e., the root pole), the hypoectyl bears the incipient root, at its upper end (i.e., the shoot pole), above the caryledons, the incepient shoot. The root may be represented by its meristem (apical meristem of the root) or by a primordial root, the radicle. Similarly, the apical meristem of the shoot located at the shoot pole may or may not initiate the development of a shoot above the cotyledons. If a primordial shoot is present, it is called epicotyl or plumule.

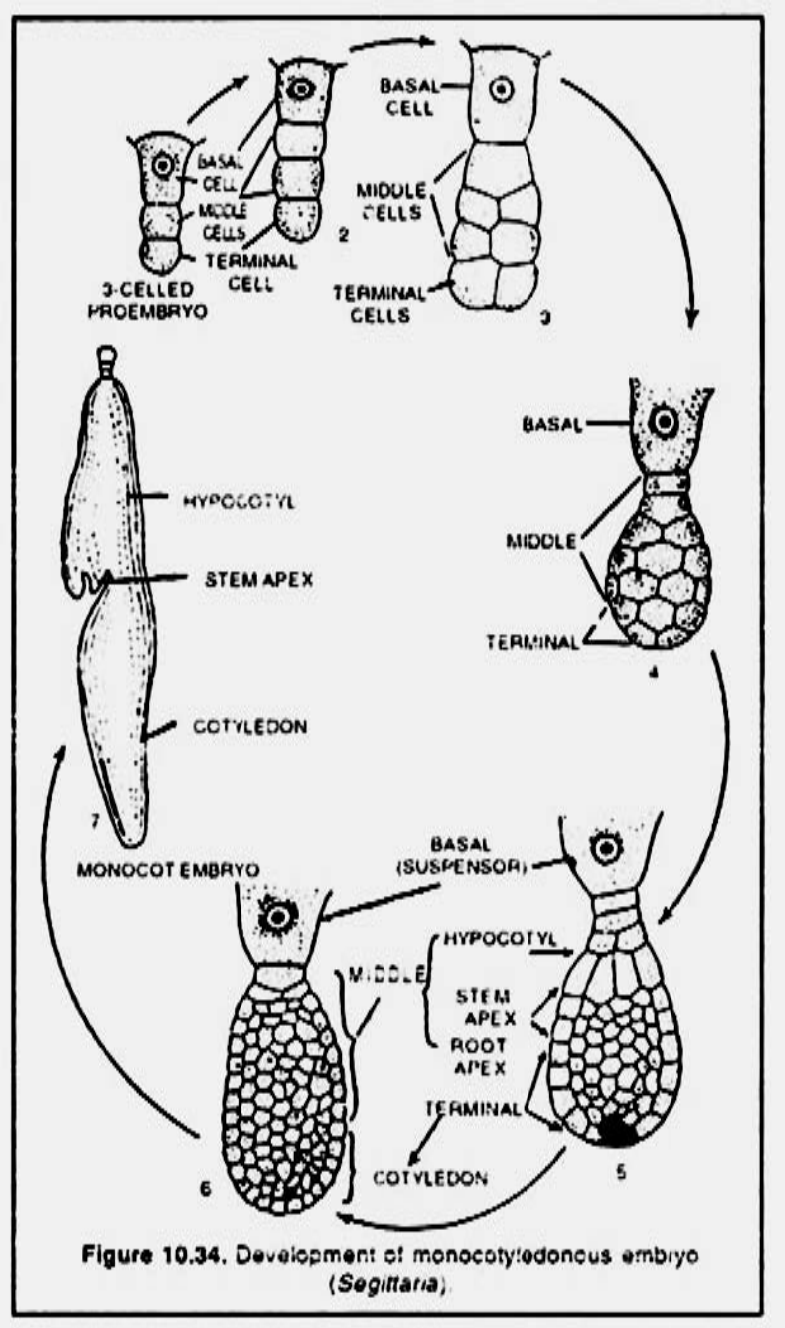


Figure 10.34. Development of monocotyledonous embryo (*Sagittaria*).

(i) Development of Embryo in Monocot Plants:

In monocotyledonous plants, a good deal of variation is found in the stages of development of embryo. It is rather difficult to find a single plant in which the development of embryo may be considered as typical of monocotyledons. Development of embryo in *Sagittaria* (family, Allismaceae) has close resemblance to that in other monocots and is thus described here.

Soon after fertilization, the fertilized egg or oospore greatly enlarges in size and divides transversely to form a 3-celled proembryo. These three cells are basal, middle and terminal. The basal cell, which is cut off towards the micropylar end, enlarges very much and forms major portion of the suspensor. The middle cell undergoes repeated transverse and vertical divisions thus differentiating few suspensor cells, radicle, plumule and hypocotyl. The terminal cell also undergoes a number of divisions in various planes and forms a single cotyledon.

Here the cotyledon is a terminal structure and the plumule is laterally situated in a depression. In some monocots, like Colocasia, there is no suspensor in the embryo. Suspensor usually pushes the embryo into the endosperm from where the former gets nourishment. The suspensor may also serve as

haustorium to absorb food from the nucelius. In some monocotyledonous plants like *Agapanthus* (family, Liliaceae) two cotyledons are formed instead of one.

(II) Development of Embryo in Dicot Plants:

Development of embryo in dicotyledonous plant basically follows a uniform pattern except for slight variations. The fertilized egg or oospore greatly enlarges in size, elongates and divides transversely into a suspensor cell (towards the micropylar end) and an embryonal cell (towards the middle of the embryo sac). As the development proceeds, the suspensor cell further divides and forms a 8 to 10-celled suspensor which pushes the developing embryo into the food storage tissue, endosperm. The lowermost cell of the suspensor, which is called hypophysis, undergoes more divisions to form the radicle

These changes in the suspensor cell are associated with the enlargement of the embrynonal cell. The embryonal cell also divides; the first division is longitudinal followed by one more longitudinal at right angle to the first and the other transverse forming an octant (8-celled proembryo). This is followed by a periclinal division thus forming a 16-celled structure which may be differentiated into posterior or hypobasal octant (towards the suspensor) and anterior or epibasal octant. The epibasail cells, as a result of further divisions and differentiation, form plumule and cotyledons, while the hypocotyl is formed from the hypobasal cells.

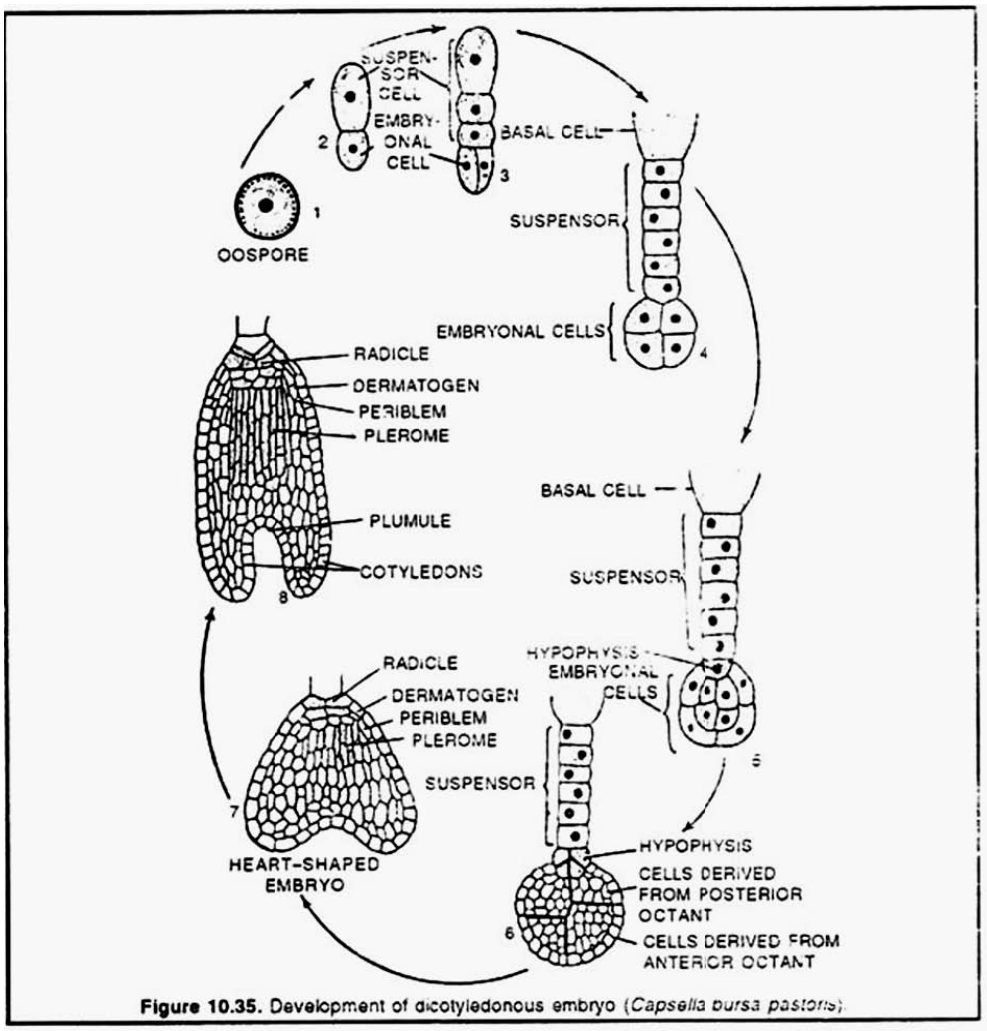


Figure 10.35. Development of dicotyledonous embryo (*Capsella bursa pastoris*)

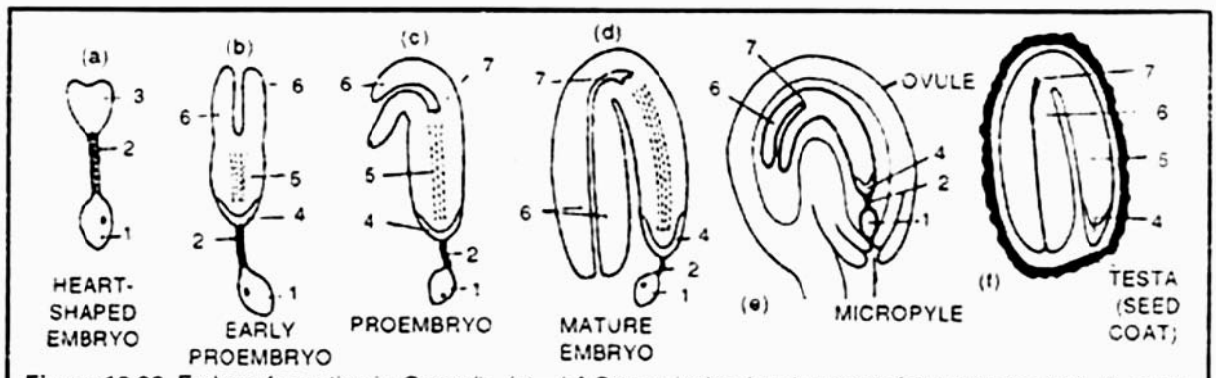


Figure 10.36. Embryo formation in *Capsella*. (*a*)—(*d*) Stages in the development of the embryo. (e) L. S. ovule showing developing embryo. (*f*) L. S. ripe seed. (1. end cell of suspensor; 2. suspensor; 3. embryo proper; 4. root cap; hypocotyl with vascular system; 6. cotyledon; 7. stem apex.)

The peripheral cells of the proembryo subsequently divide by anticlinal walls, thus differentiating a single-layered dermatogen (it forms epidermis). The middle-cells of the proembryo also divide periclinally and form plerome (which forms vascular tissue). The tissue in between dermatogen and plerome is known as periblem (it forms the cortex).

As the development further proceeds, the free end of the developing embryo becomes heart-shaped, The two lobes of the 'heart' are the primordia of the cotyledons. Plumule is situated in the depression in between two cotyledons. Unlike monocot embryo, here the cotyledons are lateral and the plumule is terminal.

As in Capsella bursa pastoris, the embryo enlarges rapidly consuming the surrounding endosperm. Thus the seed so formed becomes non-endospermic. The endosperm may not be completely exhausted and persists in the seed; such condition is found in endospermic seeds leg:, castor).

Development of Endosperm:

The endosperm, a food tissue of varying degree of importance in different species of angiosperm plants, is formed in most cases as a result of fusion of the two polar nuclei and one male gamete. Since all three of die fusing nuclei are haploid, the endosperm is triploid. In the families Orchidaceae and Podostemonaceae, the endosperm formation is completely or partly suppressed.

Types of endosperm formation:

Three types of endosperm formation has been reported in the angiosperms:

(1) Free nuclear type.

(2) Cellular type.

(3) Helobial type.

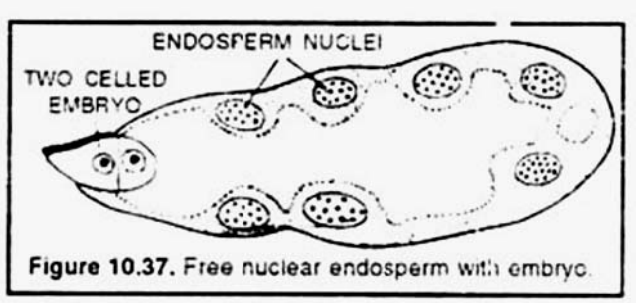


Figure 10.37. Free nuclear endosperm with embryo.

(1) Free nuclear type of endosperm:

The first and usually several of the following divisions of the primary endosperm nucleus are not accompanied by cell wall formation, The endosperm nuclei may either remain free or, in later stages, they may gel separated by cell-walls. The divisions of the endosperm nuclei are quite irregular and, in an endosperm, can be seen in different stages of divisions. As divisions progress, the nuclei are pushed more and more towards periphery that the centre is occupied by a large vacuole.

Quite frequently, the endosperm nuclei in the chalazal part of the embryo sac have been observed to be larger than those in the micropylar region. The number of free nuclear divisions varies in different species of plants, for example *in Primula, Mangifera, Malva, Mains Cannabis* etc. several hundred endosperm nuclei may be seen lining the wall of the embryo sac.

In *Asclepias, Rafflesia and Calatropis* etc., the cell wall formation starts at the very early stage when only 8 to 16 nuclei have been formed, and in Coffea cell wall formation occurs at the 4-nucleate stage. The cell wall formation starts from the periphery of the embryo sac. Formation of endosperm haustoria has also been reported in several plants e.g., members of family Proteaceae (Kaushik, 1938, 1942).

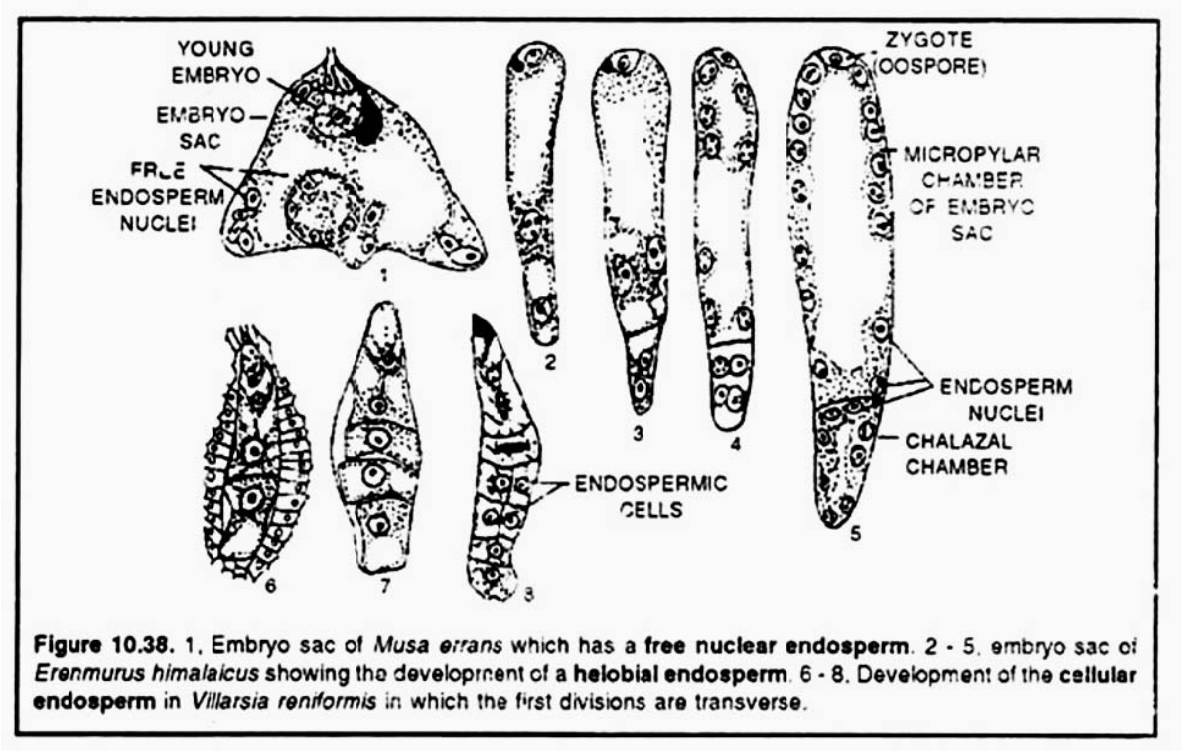


Figure 10.38. 1. Embryo sac of *Musa errans* which has a **free nuclear endosperm**. 2 - 5. embryo sac of *Eremurus himalaicus* showing the development of a **helobial endosperm**. 6 - 8. Development of the **cellular endosperm** in *Villarsia reniformis* in which the first divisions are transverse.

(2) Cellular type of endosperm:

Here the first division of the primary endosperm nucleus results in the partition of embryo sac into two chambers. The first wall is usually transverse (e.g., *Villarsia raniformis, Impatiens roylei, Ruellia* etc.,) but sometimes vertical (e.g., *Adoxa, Scabiosa, and Circaeastere* etc.,) or oblique (e.g., *Paperomia, Centranthus, and Helosise*), and in few cases the plane of division is variable (e.g., *Senecio*). Endosperm haustoria may also develop at the micropylar or chalazal ends. In the family Scrophulariaceae, both micropylar and chalazal haustoria are formed.

(3) Helobial type of endosperm:

It is an intermediate type between the nuclear and the cellular. Here the first division of the primary endosperm nucleus results in the chambering of the embryosac. The micropylar part of the sac is usually larger than the chalazal part. The endosperm nucleus in the micropylar chamber undergoes several free nuclear divisions, while nucleus in the chalazal region either remains undivided or undergoes only a few divisions, (e.g., *Eremurus*)

Structure of the endosperm:

The endosperm, as stated earlier, is a food tissue of varying degree of importance in different species of angiosperms. Its cells are mostly isodiametric in shape and store large amount of food materials. Although the cell-walls are usually thin and devoid of pits. In majority of the grasses and some other plants, an aleurone layer is present on the outside of the endosperm. Possibly, the aleurone layer secretes certain enzymes which transform the stored food into liquid form so that it may be consumed by the developing embryo.

In fully ripe seeds, the endosperm represents a physiologically dead tissue because of the degeneration of the nuclei of the endosperm cells. Dying of the nuclei in the endosperm cells, however, promotes the filling of the grain, and embryo can secure the food material more easily from a dead rather than a living tissue.

Endospermic and Non-Endospermic Seeds (The Final Fate of Endosperm):

1. Endospermic Seeds:

In some seeds the endosperm forms a permanent tissue (e.g., *Ricinus, Phoenix. Triticum*) which persists, enlarges and become a prominent tissue of the seed, rich in accumulated food in the form of oil, starch and proteins. The food stored in the endosperm is utilized by the embryo when the seed germinates. Such seeds are called endospermic or albumionous seeds.

2. Non-endospermic Seeds:

In other seeds (e.g., *Cucurbits, Pisutn, Arachis*) it is used up by the growing embryo and is no longer seen in the mature seed. Such seeds are called non-endospermic or ex-albuminous seeds. Of special interest is Symplocarpus in which the embryo consumes not only the endosperm but also integuments of the ovule so that the embryo lies naked inside the ovary wall. An even more extreme case is that of *Melocanna bambusiodes* in which embryo dissolves even the ovary wall and lies completely naked at maturity.

(3) Mosaic endosperm:

Occasionally there is a lack of uniformity in the tissue of endosperm. The patches of two different colours are often observed in *Zea mays* forming a sort of irregular pattern. Some part of the endosperm may be starchy while the **other** part may be sugary. It was observed by Webber in 1900.

(4) Ruminate Endosperm:

Mature endosperm showing irregularity or unevenness in its surface contour is called ruminate endosperm (e.g..*Passiflora calarata, Cocoloba*). Accoridng to Pariasamy (1963), ruminations arise due to in growth or infolding of the seed coat or by unequal radial elongations of the seed coat cells.

Symmetry in plants:

Symmetry in biology is the balanced distribution of duplicate body parts or shapes within the body of an organism. In nature and biology, symmetry is always approximate. For example, plant leaves –

while considered symmetrical – rarely match up exactly when folded in half. Symmetry creates a class of patterns in nature, where the near-repetition of the pattern element is by reflection or rotation.

The body plans of most multicellular organisms exhibit some form of symmetry, whether radial, bilateral, or spherical. A small minority, notably among the sponges, exhibit no symmetry (i.e., are asymmetric).

Radially symmetric organisms resemble a pie where several cutting planes produce roughly identical pieces. Such an organism exhibits no left or right sides. They have a top and a bottom surface, or a front and a back. Many flowers are radially symmetric or actinomorphic. Roughly identical flower parts – petals, sepals, and stamens – occur at regular intervals around the axis of the flower, which is often the female part, with the carpel, style and stigma. Many viruses have radial symmetries.

Spherical symmetry occurs in an organism if it is able to be cut into two identical halves through any cut that runs through the organism's center. Organisms which show approximate spherical symmetry include the freshwater green alga *Volvox*.

In **bilateral symmetry** (also called plane symmetry), only one plane, called the sagittal plane, divides an organism into roughly mirror image halves. Thus there is approximate reflection symmetry. Internal organs are however not necessarily symmetric. Flowers in some families of flowering plants, such as the orchid and pea families, and also most of the figwort family, are bilaterally symmetric (zygomorphic). It is believed that the evolution of specialized pollinators may play a part in the transition of radially symmetrical flowers to bilaterally symmetrical flowers.

Plants exhibit both radial symmetry and bilateral symmetry, often at the same time. In flowers, it has a lot to do with pollination strategy. A sunflower head has radial symmetry, allowing small insects to land on top, while a snapdragon flower has bilateral symmetry, inviting large bees to enter from the side.

Seed formation:

A **seed** is an embryonic plant enclosed in a protective outer covering. The formation of the seed is part of the process of reproduction in seed plants, the spermatophytes, including the gymnosperm and angiosperm plants.

Seeds are the product of the ripened ovule, after fertilization by pollen and some growth within the mother plant. The embryo is developed from the zygote and the seed coat from the integuments of the ovule.

Seed production

Seeds are produced in several related groups of plants, and their manner of production distinguishes the angiosperms ("enclosed seeds") from the gymnosperms ("naked seeds"). Angiosperm seeds are produced in a hard or fleshy structure called a fruit that encloses the seeds for protection in order to secure healthy growth. Some fruits have layers of both hard and fleshy material. In gymnosperms, no special structure develops to enclose the seeds, which begin their development "naked" on the bracts of cones. However, the seeds do become covered by the cone scales as they develop in some species of conifer.

Development

Stages of seed development:

Angiosperm (flowering plants) seeds consist of three genetically distinct constituents: (1) the embryo formed from the zygote, (2) the endosperm, which is normally triploid, (3) the seed coat from tissue derived from the maternal tissue of the ovule. In angiosperms, the process of seed development begins with double fertilization, which involves the fusion of two male gametes with the egg cell and the central cell to form the primary endosperm and the zygote. Right after fertilization, the zygote is mostly inactive, but the primary endosperm divides rapidly to form the endosperm tissue. This tissue becomes the food the young plant will consume until the roots have developed after germination.

I Zygote

II Proembryo

III Globular

IV Heart

V Torpedo

VI Mature Embryo

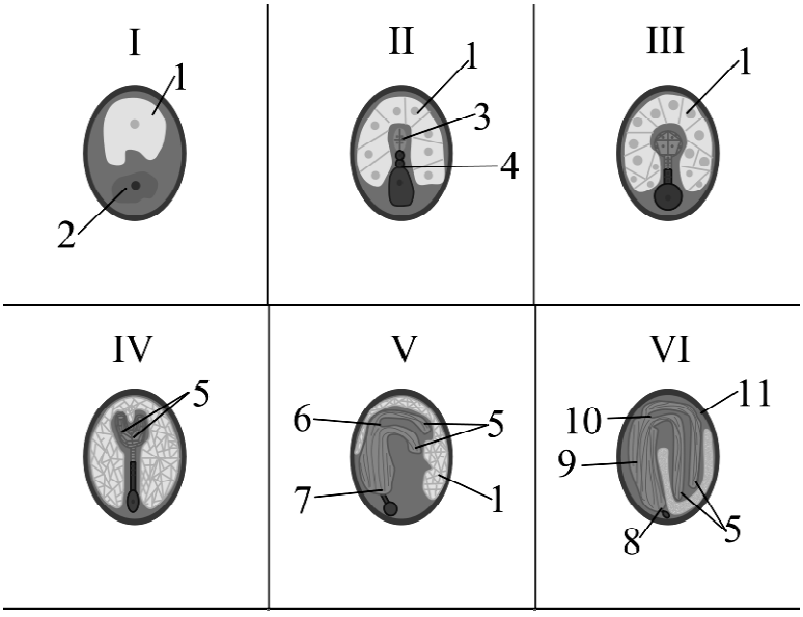
Ovule

After fertilization the ovules develop into the seeds. The ovule consists of a number of components:

The **funicle** (funiculus, funiculi) or seed stalk which attaches the ovule to the placenta and hence ovary or fruit wall, at the pericarp.

The **nucellus**, the remnant of the megasporangium and main region of the ovule where the megagametophyte develops.

The **micropyle**, a small pore or opening in the apex of the integument of the ovule where the pollen tube usually enters during the process of fertilization.



The **chalaza**, the base of the ovule opposite the micropyle, where integument and nucellus are joined together.

The shape of the ovules as they develop often affects the final shape of the seeds. Plants generally produce ovules of four shapes: the most common shape is called anatropous, with a curved shape. Orthotropous ovules are straight with all the parts of the ovule lined up in a long row producing an uncurved seed. Campylotropous ovules have a curved megagametophyte often giving the seed a tight

"C" shape. The last ovule shape is called amphitropous, where the ovule is partly inverted and turned back 90 degrees on its stalk (the funicle or funiculus).

In the majority of flowering plants, the zygote's first division is transversely oriented in regards to the long axis, and this establishes the polarity of the embryo. The upper or chalazal pole becomes the main area of growth of the embryo, while the lower or micropylar pole produces the stalk-like suspensor that attaches to the micropyle. The suspensor absorbs and manufactures nutrients from the endosperm that are used during the embryo's growth.[3]

Embryo

The inside of a Ginkgo seed, showing a well-developed embryo, nutritive tissue (megagametophyte), and a bit of the surrounding seed coat

The main components of the embryo are:

The **cotyledons**, the seed leaves, attached to the embryonic axis. There may be one (Monocotyledons), or two (Dicotyledons). The cotyledons are also the source of nutrients in the non-endospermic dicotyledons, in which case they replace the endosperm, and are thick and leathery. In endospermic seeds the cotyledons are thin and papery. Dicotyledons have the point of attachment opposite one another on the axis.

The **epicotyl**, the embryonic axis above the point of attachment of the cotyledon(s).

The **plumule**, the tip of the epicotyl, and has a feathery appearance due to the presence of young leaf primordia at the apex, and will become the shoot upon germination.

The **hypocotyl**, the embryonic axis below the point of attachment of the cotyledon(s), connecting the epicotyl and the radicle, being the stem-root transition zone.

The **radicle**, the basal tip of the hypocotyl, grows into the primary root.

Monocotyledonous plants have two additional structures in the form of sheaths. The plumule is covered with a coleoptile that forms the first leaf while the radicle is covered with a **coleorhiza** that connects to the primary root and adventitious roots form from the sides. Here the hypocotyl is a rudimentary axis between radicle and plumule. The seeds of corn are constructed with these structures; pericarp, **scutellum** (single large cotyledon) that absorbs nutrients from the endosperm, plumule, radicle, coleoptile and coleorhiza – these last two structures are sheath-like and enclose the plumule and radicle, acting as a protective covering.

7. Developmental routes of apomixis, polyembryony and its molecular aspects.

Apomixis:

Apomixis is the phenomenon where there is no normal fertilisation of the egg cell, hence no normal development of embryo from the egg cell. However, embryo may develop from an un-fertilised egg

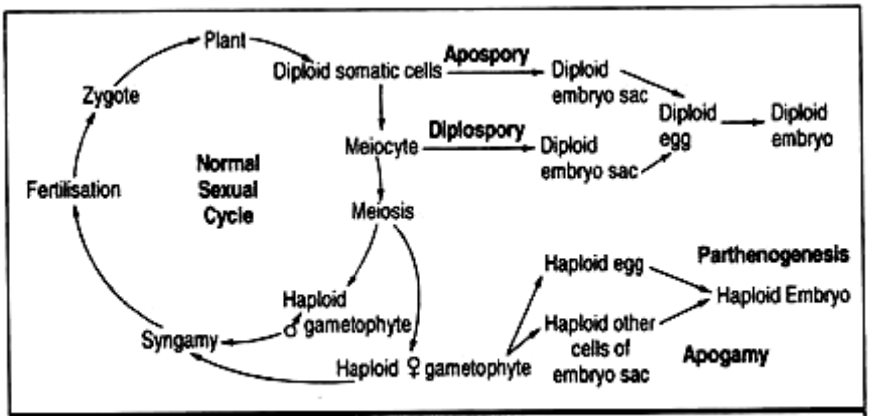


Fig. 2.1: Sexual and apomictic reproduction

cell or from a cell other than the egg cell within the embryo sac or from the cell outside the embryo sac.

Sexual Apomictic Reproduction

The plants produced by apomixis are called apomictic which are of two types: obligate, producing only apomictic embryos, and facultative, producing both apomictic and normal embryos. This phenomenon where the substitution of sexual process occurs by asexual methods is known as apomixis (apo = away from, mixis = act of mixing), the plants are called apomictic.

Types of Apomixis:

Non-Recurrent Apomixis (Haploid Seed/Plant Formation):

Here the seed/plant develops from the un-fertilised egg cell or any other un-fertilised haploid cell of embryo sac. In this type of apomixis the first event of normal sexual cycle, meiosis takes place, the embryo sac is formed normally, but due to various reasons the fertilisation does not take place.

The reasons may be:

(i) Absence of pollen tube;

(ii) Inability of the tube to discharge its contents;

(iii) No attraction between male and female nuclei;

(iv) Early degeneration of sperms;

(v) Maturation of egg and sperm is not synchronized. ,

(a) Haploid Parthenogenesis:

Here a haploid embryo develops from an un-fertilised egg cell of the embryo sac, e.g., in *Orchis maculata, Platenthera chlorantha, Cephalanthera damasonium* – the pollen tube enters but fails to fertilize**.**

(b) Haploid Apogamy:

It is the development of haploid embryo from any haploid cell of the embryo sac other than the egg cell. In *Lilium martagon, Erythraea centaurium* – two pro-embryos are formed; one is from fertilized egg (zygotic cell) and another from the synergid cell (haploid cell).

Recurrent Apomixis:

Here the meiotic event does not take place either in any somatic cell or the meiocyte cell fails to undergo meiosis, thereby produces the embryo sac with diploid cells only. These diploid cells directly give rise to diploid embryos, called apospory.

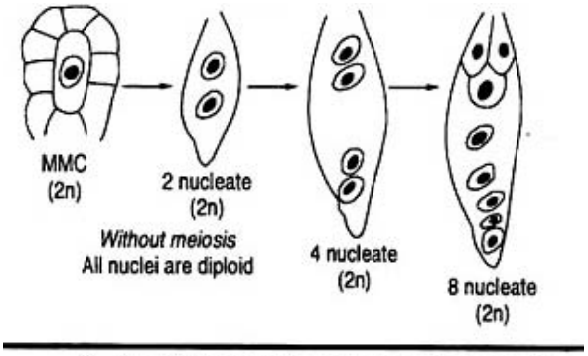


Fig. 2.2a: Aposporous embryo sac in *Eupatorium*

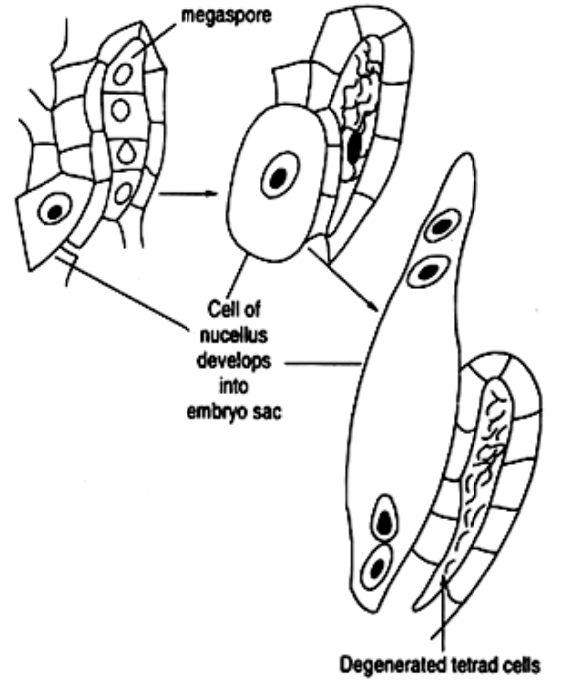


Fig. 2.2b: Aposporous embryo sac in *Hieracium*

(a) Generative Apospory:

The diploid egg cell develops into embryo without fertilisation, one particular variety of Parthenium argentatum exhibits.

(b) Somatic Apospory:

The diploid cell of nucellus or integument develops directly into diploid embryo sac and the diploid embryo is developed from the diploid un-fertilised egg cell. This phenomenon is found in *Allium, Meliss, Crepis* etc.

Agamospermy:

This term is used to categories the plants which has the seed habit for propagation but not through normal sexual cycle, either meiosis or syngamy or both are eliminated. This includes either the embryos which may develop from cell of the unreduced female gametophyte or directly from the diploid sporophytic cell of the ovule, such as nucellus or integuments. Diplospory, apospory and adventive embryony, all are included within this category.

Adventive Embryony:

In this case the gametophytic generation is completely eliminated. It is very much close to vegetative propagation, but the plants here retained the seed habit, i.e., the diploid embryo is developed from any

diploid cell outside the embryo sac but matures into embryo within the embryo sac, zygotic embryo degenerates or competes with apomictic embryo. This kind of embryos is found in *Citrus, mango*, etc.

Diplospory:

Here the MMC differentiates into sexual ovules, but it does not enter into meiosis, it produces the embryo sac directly by mitotic division, all the cells within the embryo sac are diploid, and the embryos are formed.

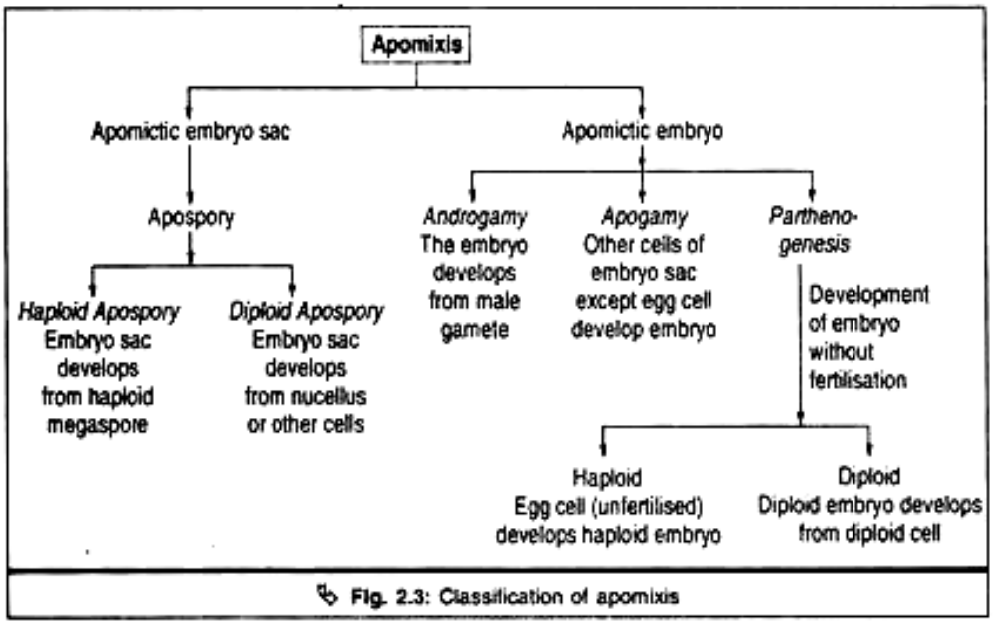


Fig. 2.3: Classification of apomixis

Significance:

Apomictic plants tend to conserve the genetic structure and are also capable of maintaining heterozygote advantages. Due to prohibition of fertilisation process, apomixis is the way for exploitation of maternal influence or perpetuation of maternal individuals or maternal properties.

The use of apomixis in plant breeding can be summarised:

1. Rapid multiplication of genetically, uniform individuals without any risk of segregation.
2. Heterosis or hybrid vigour can be utilised for recurring production of seeds of F1 hybrids.
3. From generation to generation the maternal effect can be exploited.

Apomixis Mechanisms

In contrast to sexual seed formation, apomixis can occur by various mechanisms that share three common developmental components:

(i) a bypass of meiosis during embryo sac formation (apomeiosis),

(ii) development of an embryo independent of fertilization (a process known as parthenogenesis), and

(iii) formation of viable endosperm either via fertilization-independent means or following fertilization with a sperm cell (Koltunow and Grossniklaus 2003).

Derivation of the egg from a diploid maternal cell without meiotic reduction, and its subsequent fertilization-independent development into an embryo, means that the progeny derived from apomictic development are clonal and therefore genetically identical to the maternal parent.

Apomixis mechanisms are historically subdivided into two categories and classified as either **gametophytic** or **sporophytic**, based on whether the embryo develops via a gametophyte (embryo sac) or directly from diploid somatic (sporophytic) cells within the ovule (Nogler 1984; Koltunow 1993). During sporophytic apomixis, development of an embryo sac following the typical angiosperm sexual pathway still occurs. However, during mitosis of the functional megaspore, diploid somatic ovule cells surrounding the embryo sac differentiate and have an embryogenic cell fate. These embryo initial cells begin mitosis forming multiple globular-shaped embryos that can develop to maturity only if the sexually derived embryo sac is fertilized, as the sexual and asexual embryos share the nutritive endosperm. Sporophytic apomixis can therefore lead to a seed containing multiple embryos and is common in *citrus*. The sexually derived embryo may or may not mature or germinate (Koltunow et al. 1995; Koltunow et al. 1996). Sporophytic apomixis has not been extensively studied at the molecular level; however, it appears genetically complex (García et al. 1999).

Gametophytic apomixis relates to mechanisms where an embryo sac is mitotically formed from a diploid cell in the ovule, bypassing meiosis. Another term for such mitotic embryo sac development is apomeiosis. Embryo development in gametophytic apomixis is fertilization independent whereas endosperm formation may or may not require fertilization. Apomeiotic embryo sac development is further subdivided into two types (diplospory and apospory) based upon the origin of the diploid precursor cell that ultimately gives rise to the mitotically derived embryo sac. In diplospory, the precursor is the megaspore mother cell (or a cell with an altered program that differentiates in the megaspore mother cell position). This cell may enter meiosis and abort the process or it may immediately begin mitosis. Diplospory has been observed in species including *Taraxacum officinale* (dandelion), *Boechera* spp., *Erigeron annuus*, and *Tripsacum dactyloides*. By contrast, apospory involves development of the embryo sac via mitosis not from the megaspore mother cell, but from a diploid somatic cell positioned adjacent to the megaspore mother cell. This cell, termed the aposporous initial cell, undergoes mitosis and the nuclei cellularize. The mitotic events of diplospory and apospory may or may not make a seven-nucleate *Polygonum*-type embryo sac; however, an egg, a central cell, and synergids are typically formed. Depending on the species, both sexually derived and aposporous embryo sacs can coexist within the one ovule, as occurs in *Brachiaria* species (Araujo et al. 2000). Alternatively, development of the aposporous embryo sac may lead to the demise of the sexually derived embryo sac or pathway as occurs in aposporous *Hieracium* and *Pennisetum* species (Peel et al. 1997; Koltunow et al. 2011). Endosperm development can occur without fertilization of the central cell, although this is rare, occurring predominantly in members of the daisy family (Asteraceae). Apomicts that require fertilization to produce endosperm have disturbed maternal and paternal genome contributions (m:p) in the endosperm. For example, in such apomicts, fertilization of a tetraploid central cell may lead to a 4m:1p endosperm genome ratio, in contrast to the typical 2m:1p ratio of fully sexually reproducing species. Those apomicts that require fertilization to develop endosperm have therefore developed multiple strategies to ensure seed viability and these have been discussed in previous reviews (Koltunow and Grossniklaus 2003; Curtis and Grossniklaus 2008).

Genetics and Inheritance of Apomixis

The presence of varied apomictic mechanisms and the phylogenetic positioning of apomictic species throughout many angiosperm families together imply that apomixis has evolved independently multiple times (Carman 1997; Van Dijk and Vijverberg 2005). Genetic analyses using apomicts as

pollen donors in crosses with sexual individuals as maternal parents have shown apomixis to be inherited as a dominant trait. For some species, it has since been revealed that many apomixis loci exhibit suppressed recombination. Studies involving genetic analyses searching for rare recombinants in suppressed regions and gamma deletion mutagenesis have revealed that in some species, the developmental components of apomixis (meiotic avoidance, parthenogenesis, and fertilization-independent endosperm development) are controlled by independent loci. For instance, in *Taraxacum* and *Erigeron* species, two independent loci have been identified that control diplospory and parthenogenesis (Van Dijk et al. 1999; Noyes and Rieseberg 2000). Similarly, apospory and parthenogenesis are controlled by two independent loci in *Hypericum, Poa, Hieracium,* and *Cenchrus* species (Albertini et al. 2001; Catanach et al. 2006; Schallau et al. 2010; Conner et al. 2013).

Interestingly, characterized gamma deletion mutants have revealed that sexual reproduction is the default pathway in apomictic *Hieracium praealtum*. A series of deletion mutants that lack the apospory locus (called LOSS OF APOMEIOSIS or LOA) and an additional locus responsible for fertilization-independent seed development (called LOSS OF PARTHENOGENESIS or LOP) were developed. Deletion of either LOA or LOP alone sees the return of the sexual pathway for that component. For example, plants with LOA deleted no longer produce diploid embryo sacs via apospory as they lack apomeiosis function. Instead, the megaspore mother cell undergoes meiosis and as aposporous initial cells are not formed, a functional haploid embryo sac develops, and the egg and central cell form an embryo and endosperm, respectively, in the absence of fertilization. Deletion of both the LOA and LOP loci results in complete reversion to sexual development (Catanach et al. 2006; Koltunow et al. 2011). Apomixis in aposporous *Hieracium* therefore seems to be superimposed on the sexual pathway, suggesting that apomixis may redirect the fate of cells with gametic potential, rather than being a detached and completely independent pathway (Koltunow et al. 2013). This theory is consistent with the known coexistence of apomixis and sex, suggesting that if the apomixis pathway is not initiated in an ovule, the sexual pathway remains functional. One interpretation is that apomixis may be thought of as an alteration to the spatial and temporal expression of sexual reproductive processes, where the sexual pathway is initiated at a different time and/or in a different cell. For diplospory, the megaspore mother cell appears to be specified with gametic potential; however, the sexual pathway is altered so that the events of meiosis do not initiate, or meiosis is altered so that it is not successfully completed. For apospory, an altered sexual pathway appears to be expressed in additional cells within the ovule, which results in diploid somatic cells mitotically forming an embryo sac, which is usually the role of the haploid functional megaspore in sexual reproduction. During the development of the diploid diplosporous and aposporous embryo sacs, the egg cell and central cell differentiate with a capability to directly transition into an embryo and endosperm, respectively, if seed formation is completely fertilization independent. Studies using various reproductive markers support these hypotheses (Tucker et al. 2003; Rodrigues et al. 2010).

Most apomicts are polyploid, and apomixis has previously been proposed to be a consequence of hybridization and/or genome doubling, i.e., the events of polyploidization (Carman 1997). However, polyploidization alone is not sufficient to induce apomixis, as not all polyploids are apomicts.

Polyembryony:

Presence of more than one embryo inside the seed is known as polyembryony. It was first reported by A.V. Leuwenhoek (1719) in Citrusi Fam-Rutaceae).This phenomenon is very common in gymnosperm than angiosperm. Besides normal embryoe (develops from Zygote), other embryos are formed inside seed maybe as haploid (n) or Diploid (2n).

Types of polyembryony:

Polyembryony can be broadly categorized in to two groups.

1.True polyembryony: Many embryos are developed inside single embryo sac.



Fig. 2.35. A-C. Cleavage polyembryony : **A.** Embryonic mass formed by the basal cell of the zygote in *Erythronium americanum*, **B-C.** Differentiation of embryos from the cells of the embryonic mass.

2. **False polyembryony**: If the ovule carries more then one embryo sac & embryos develop in each embryo sac.

True polyembryony may be subdivided into two types. :

a) Cleavage polyembryony

b) Adventive polyembryony

a) Cleavage polyembryony :-

Where the embryos arise within an embryo sac either by a cleavage of the egg or from the synergids antipodals or endosperms.

b) Adventives polyembryony :-

Where the embryos arise from the tissue lieing outside the embryos sac. The cells of the nucellus or the integuments but generally they come to lie within the embryo sac.

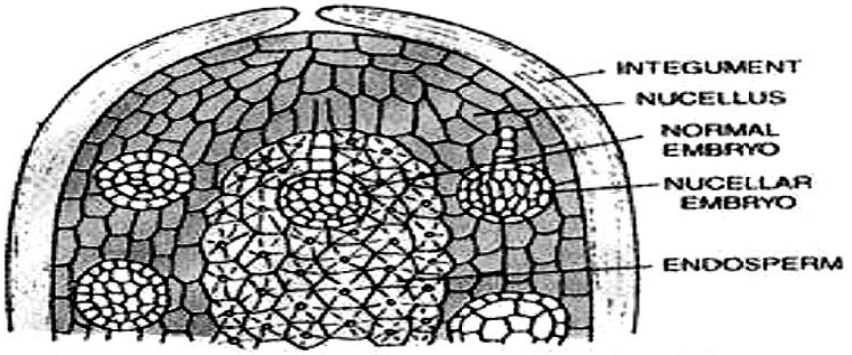


Fig. 2.33. *Citrus* ovule (Young seed) in section showing normal and nucellar (adventive) embryos.

Cause of polyembryony in angiosperm:

Many theories have been proposed to explain the occurence of polyembryony by different workers different times.

Some of the important theories are as follows :

A) Necrohormone theory:

This theory indicates that the degenerating cells of the nucellus act as source of stimulus for the adjacent cells to divide and form adventive embryos.

B) Hybridization theory :

According to this theory the occurrence of multiple embryo is due to hybridization.

The recombination of genes takes place during the process of hybridization, forming a single unit that gives rise to the multiple embryos.

Different Ways of Polyembryony

The development of additional embryo may occur by different ways. In plants polyembryonic cases arise from maternal tissue(adventitious polyembryony) is the most common followed by cleavageof the fertilized embryo (Bhatnagar and Bhojwani 1999; Ganeshaiah et al. 1991) whereas formation of additional embryo sac and participation ofhaploid cells in embryo formation is less common. The different ways ofpolyembryony are;

i. Formation of embryo by sporophytic/maternal tissue (2n) of ovule

ii. Formation of embryos by cells of embryo sac other than egg cell

iii. Development of more than one embryo sac within the same ovule

iv. Cleavage of proembryos

Significance of polyembryony

- The adventives embryos develop from nucellar embryo provide uniform seedlings for root stocks which yield consistent results in fruit production.
- The nucellar seedling as a root stock has a tap root and, therefore develops a better root system.
- The nucellar seedlings show a restoration of the vigour lost after repeat vegetative propagation.
- Polyembryony has ecological significance as it increases the probability of survival under varied conditions.
- Nucellar polyembryony is the only practical approach to raise virus-free clones of polyembryonatic citrus species in nature.
- Disease-free plants can also be obtained through nucellar embryo culture.

In spite of significance in plant propagation, polyembryony has a drawback as it interferes with the hybridization programme. In hybridization, zygotic embryo is the outcome, but polyembryony hampers the growth of zygotic embryo and thus affects the hybridization in plants.

Molecular aspects:

Molecular techniques by using molecular markers (RAPD, RFLP, SSR, ISSR, QTL, etc.) are the recent, advanced, effective, expeditious and reliable tools to study gene expression and to identify zygotic and nucellar seedlings at an early stage as results are not infl uenced by external factors. Genes show conspicuous difference in expression between polyembryonic (apomictic) and monoembryonic (nonapomictic) genotypes. The msg-2 gene was highly expressed in the late stage of somatic embryogenesis in monoembryonic cultivars of *Citrus*, whereas, msg-2 was not expressed in the initiation stage of embryogenesis in polyembryonic cultivar suggesting the suppressing role in initial cell formation of somatic embryos (Nakano 2013). Similarly, genes like Ig1 in maize (Evans

2007), OsCem (Yang and Hwa 2008) and OsPE in rice (Puri et al. 2009) associated to polyembryony have been reported. Somatic Embryogenesis Receptor Kinase (SERK) gene, a leucine-rich repeat trans-membrane protein kinase, enhances the ability of the apical meristem to form somatic embryos. SERK genes have been linked to somatic embryogenesis (SE) in a number of species including *Dactylis glomerata* (Somleva et al. 2000), *Arabidopsis thaliana* (Hecht et al. 2001), *Medicago truncatula* (Nolan et al. 2003), *Helianthus annuus* (Thomas et al. 2004), Ocotea catharinensis (Santa-Catarina et al. 2004), Citrus unshiu (Shimada et al. 2005), and Theobroma cacao (de Oliveira Santos et al. 2005). SERK genes have also been described in relation to apomixis in Hieracium (Tucker et al. 2003) and Poa pratensis (Albertini et al. 2005) as well as zygotic embryogenesis in carrot, Arabidopsis, and wheat (Schmidt et al. 1997; Hecht et al. 2001; Singla et al. 2008). The best defined SERK gene in relation to SE is the Arabidopsis SERK1 (AtSERK1) and over expression of this SERK was shown to enhance embryogenic competence in *Arabidopsis* cultures (Hecht et al. 2001). Das et al. (2007) identifi ed the zygotic seedlings through RAPD markers and reported that zygotic seedlings (twin or triplets) usually had one or more extra band than those of the nucellar seedlings they also observed the variability in mandarins of north east by RAPD profi ling. Maria et al. (2006) identifi ed the nucellar seedlings of mango through RAPD and also determined the position of zygotic and nucellar embryos in an ovule.

13. Let’s sum up

- Plant development is an umbrella term for a broad spectrum of processes that include: the formation of a complete embryo from a zygote; seed germination; the elaboration of a mature vegetative plant from the embryo; the formation of flowers, fruits, and seeds; and many of the plant's responses to its environment.
- Differentiation is the process in which generalized cells specialize into the morphologically and physiologically different cells.
- The summary of ABC model is: class A genes together and class C gene alone are responsible for the development of sepals and carpel respectively. The class B genes and class A genes function cooperatively to determine the development of petals. The class B genes and class C gene act together to induce the development of stamens.
- Transgenic plants are developed by transferring or modifying genes from another organism by a diverse technique like physical, chemical and biological methods.
- Within the anther, male sporogenous cells differentiate and undergo meiosis to produce microspores, which give rise to pollen grains, whereas other cell types contribute to pollen maturation, protection, or release.
- Female gametophyte development occurs over two phases referred to as megasporogenesis and megagametogenesis.
- Self-incompatibility is a well-designed genetic mechanism by which certain plants recognize and reject their own pollen thus forcing outbreeding. It is defined as inability of the plant producing functional gametes to set seed upon self-pollination.

- Fertilization is angiosperms, is unique. It is called double fertilisation. This is because there are two sperms per pollen tube. One of the sperms fertilises the egg cell to form zygote, while remaining sperm fertilises two polar nuclei, resulting in the formation of a triploid endosperm cell.
- In monocotyledons, the fertilized egg greatly enlarges in size and divides transversely to form a 3-celled proembryo, the basal cell forms major portion of the suspensor, the middle cell differentiating few suspensor cells, radicle, plumule and hypocotyl and the terminal cell forms a single cotyledon.
- Development of embryo in dicotyledonous plant basically follows a uniform pattern except for slight variations. The fertilized egg or oospore greatly enlarges in size, elongates and divides transversely into a suspensor cell and an embryonal cell.
- The endosperm, a food tissue formed as a result of fusion of the two polar nuclei and one male gamete and Three types of endosperm formation has been reported in the angiosperms- (1) Free nuclear type (2) Cellular type (3) Helobial type.
- Apomixis is the phenomenon where there is no normal fertilisation of the egg cell, hence no normal development of embryo from the egg cell.
- Presence of more than one embryo inside the seed is known as polyembryony. It was first reported by A.V. Leuwenhoek (1719).

14. Suggested Reading

1. Raghavan, V. Molecular Embryology of Flowering Plants, 1997, Camb. University Press.
2. Maheswari, P. An Introduction to Embryology of Angiosperm, Latest Ed., Tata McGraw Hill.
3. Raghavan, V. Embryogenesis in Angiosperms: A Development & Experimental Study, 1986, Cambridge University Press.
4. Bhojwan, S.S. & Bhatnagar, S.D. The Embryology of Angiosperms(4th ed.), 1989, Vikas Publishing House.
5. John, B.M.(ed.). Experimental Embryology of Vascular Plants, 1982, Springer, Heidelberg.
6. Westhaff, P. et al. Molecular Plant Development; From Gene to plant, 1998, Oxford University Press.:
7. http://www.biologyreference.com/
8. https://en.wikipedia.org/wiki/

15. Assignments

1) What are the unique features of developments of plant?
2) What is Protogyny?
3) Explain ABC model of floral part development in *Arabidopsis*.
4) Short note on a) Double fertilization. b) establishment of symmetry c) Anther development, d) Molecular aspect of male gametophyte

5) Distinguish between endosperm development in gymnosperms and angiosperms.
6) Write down molecular and genetic basis of self incompatibility..
7) Describe Steps Involved In Production of Transgenic Plants.
8) How transition from vegetative to reproductive phase take place.
9) What is meant by polyembryony? Write down its significance.
10) Write a short note on embryo development in dicot plant.
11) Distinguish between megasporogenesis and megagametogenesis.
12) Elucidate the gametophytic self-incompatibility with suitable examples.
13) What is meant by apomixes?
14) f. How endosperm is formed?
15) What is meant by androgenesis?
16) Mention the flower characteristics of ‘apetala 3’ mutant.
17) Define tetrasporic type of megasporogenesis.
18) What is meant by MADS-box gene family?

COURSE – BOHCT2.3

Emryology, Plant Breeding & Biometry (Course – II)

Hard Core Theory Paper **Credit: (Groups A+B) = 3**

Group – Group B (Plant Breeding & Biometry)

Content Structure

---

1. Introduction
2. Course Objectives
3. Breeding methods: Introduction and conservation of germplasm, mass selection, pure line selection, clonal selection, hybridization, selection after hybridization (bulk, pedigree, recurrent), heterosis & inbreeding depression.
4. An outline of mutation and polyploid breeding.
5. Cytogenetics & breeding practices of crop plants: rice, maize, potato.
6. An outline of breeding methods for development of disease resistance.
7. Molecular marker assisted breeding – an outline
8. Population samples, sampling methods.
9. Frequency distribution, histogram, normal curve, mean, median, mode, variance, standard deviation, standard error.
10. Probability & test of significance (Student & paired t) $\chi 2$ test (detection of segregation ratio & linkage, test of independence & heterogeneity), analysis of variance (F test) CD, genetic advance, heritability estimation.
11. Correlation & regression.
12. Design of experiments: general principles of field trails, randomized blocks, latin square, split plot designs, layout of breeding experiment..
13. Let's sum up
14. Suggested Reading
15. Assignment

---

1. Introduction

Plant breeding is an art and science, which tells us ways and means to change the genetic architecture of plants so as to attain a particular objective. Plant breeding can be accomplished through many

different techniques ranging from simply selecting plants with desirable characteristics for propagation, to more complex molecular techniques Plant breeding has been practiced for thousands of years, since near the beginning of human civilization. It is now practiced worldwide by individuals such as gardeners and farmers, or by professional plant breeders employed by organizations such as government institutions, universities, crop-specific industry associations or research centers. International development agencies believe that breeding new crops is important for ensuring food security by developing new varieties that are higher-yielding, resistant to pests and diseases, drought-resistant or regionally adapted to different environments and growing conditions.

From this course, you will get the detail knowledge about various breeding approaches and biostatistical experiments relating to estimation and hypothesis testing.

2. Course Objectives

You should gather knowledge after studying the course:

1. To know achievements about various breeding methodologies
2. Details explanation about Mass Selection Breeding
3. Gathering knowledge about disease resistance of plant

In this course you will learn how to effectively collect data, describe data, and use data to make inferences and conclusions about real world phenomena. After finishing this course, you should be able to:

1. Recognize the importance of data collection and its role in determining scope of inference.
2. Choose and apply appropriate statistical methods for analyzing one or two variables
3. Interpret statistical results correctly, effectively, and in context.
4. Understand and critique data-based claims.

3. Breeding methods: Introduction and conservation of germplasm, mass selection, pure line selection, clonal selection, hybridization, selection after hybridization (bulk, pedigree, recurrent), heterosis & inbreeding depression.

What is plant breeding?

Plant breeding is the genetic improvement of the crop in order to create desired plant types that are better suited for cultivation, give better yields and are disease resistant. Conventional plant breeding is in practice from 9,000-11,000 years ago. Most of our major food crops are derived from the domesticated varieties.

But now due to advancements in genetics, molecular biology and tissue culture, plant breeding is being carried out by using molecular genetics tools. Classical plant breeding includes hybridization (crossing) of pure lines, artificial selection to produce plants with desirable characters of higher yield, nutrition and resistance to diseases.

When the breeders wish to incorporate desired characters (traits) into the crop plants, they should increase yield and improve the quality. Increased tolerance to salinity, extreme temperatures, drought, resistance to viruses, fungi, bacteria and increased tolerance to insect pests should also be the desired traits in these crop plants.

Historical Background:

1. R. Camerarius produced the first artificial hybrid plant of maize in 1694.

2. Kolreuter (1733-1806) produced successful hybrids through artificial crosses in many plants.

3. The discipline of plant breeding witnessed great advances with the increased knowledge in the field of genetics.

4. Shull (1908, 1909) while investigating effect of inbreeding and cross-breeding in maize gave the concept of heterosis which has resulted in manifold increase in agricultural production.

5. Male Sterility in plants was reported by Kolreuter in 1763 which led to the economic exploitation of heterosis.

6. Alphonse de Condolle in 1882 was the first to give an account of the history and origin of cultivated plants.

7. N.I. Vavilov in 1925 proposed eight centres of origin of crops. These centres provided the regions of immense genetic resources of cultivated plants which existed there.

8. Monkambu Sambasivan Swaminathan (M.S. Swaminathan) initi-ated collaboration with Dr. Borlaug which reached the highest point into the “Green Revo-lution” through introduction of Mexican varieties of wheat in India.

Institutes Engaged in Plant Breeding at National and International Level:

1. International Rice Research Institute (IRRI), Philippines.

2. International Maize and Wheat Improvement Centre (CIMMYT), Mexico.

3. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad.

4. International Potato Centre (CIP), Peru.

5. International Board of Plant Genetic resources (now International Plant Genetic Resources Institute; IBPGR, now IPGRI).

6. Central Potato Research Institute (CPRI), Kufri (Shimla).

7. Indian Agricultural Research Institute (IARI), New Delhi.

8. Sugarcane Breeding Institute (SBI), Coimbatore.

9. Jute Agriculture Research Institute (JARI), Barackpore.

10. Indian Grassland and Fodder Research Institute (IGFRI), Jhansi.

11. Forest Research Institute, Dehradun.

12. Central Arid Zone Research Institute (CAZRI), Jodhpur.

Why breed plants?

The reasons for manipulating plant attributes or performance change according to the needs of society. Plants provide food, feed, fiber, pharmaceuticals, and shelter for humans. Furthermore, plants are used for aesthetic and other functional purposes in the landscape and indoors.

- Addressing world food and feed quality needs
- Addressing food supply needs for a growing world population
- Need to adapt plants to environmental stresses
- Need to adapt crops to specific production systems
- Developing new horticultural plant varieties
- Satisfying industrial and other end-use requirements

Germplasm

It is impossible to improve plants or develop new cultivars without genetic variability. Once the objectives have been determined, the breeder then assembles the germplasm to be used to initiate the breeding program. Sometimes, new variability is created through crossing of selected parents, inducing mutations, or using biotechnological techniques. Whether used as such or recombined through crossing, the base population used to initiate a breeding program must of necessity include the gene(s) of interest. That is, you cannot breed for disease resistance, if the gene conferring resistance to the disease of interest does not occur in the base population.

Plant introduction:

Plant introduction is a process of introducing plants (a genotype or a group of genotypes) from their own environment to a new environment.

The process of introduction may involve new varieties of crop or the wild relatives of crop species or totally a new crop species for the area.

Introduction may be classified into two categories:

Primary Introduction:

When the introduced variety is well suited to new environ-ment then it is released for commercial cultivation without any alteration of genotype. For example, dwarf wheat varieties like ‘Sonora-64’, ‘Lerma rojo’ and dwarf rice varieties like ‘Taichung Native 1’, ‘IR-8’ are the examples of primary introduction.

Secondary Introduction:

When the introduced variety is subjected to Selection or used in hybridization programme with local varieties to get the improved varieties with some new characters introduced called secondary introduction. For example, the varieties like ‘Kalyan Sona’ and ‘Sonalika’ of wheat have been selected from material introduced from CIMMYT, Mexico.

The process of plant introduction is the successful compliance of two important aspects, viz., domestication and acclimatization. Domestication is the process of bring-ing of a wild species under cultivation by making them changed in behaviour suitable for new environment.

Acclimatization is the ability of a crop to become adapted to a new climatic and edaphic condition.

The process of acclimatization leads to increase the frequency of most adapted genotypes which depends on three factors:

(a) Breeding system,

(b) Genetic variation,

(c) Duration of the crop.

Purpose of Plant Introduction:

(a) Use in Agriculture, Forestry and Industry:

New varieties of plants or crops are introduced from various places for use as food, fibre, wood or medicinal purposes as well as the breeding material for hybridisation work.

(b) For Aesthetic Interest:

Various ornamental plants are introduced for beautification.

(c) For Germplasm Conservation:

The spread of high yielding varieties causes a danger to old varieties to get lost from an area. But germplasm collection and conservation help to maintain lines, clones, mutants, cultivars, etc. from as many sources as possible.

(d) For Studying Origin and Distribution:

The distribution of crop plants and their various forms in different parts of the world gives an idea of their origin and evolution.

Agencies of Plant Introduction in India:

The central plant introduction agency in India is National Bureau of Plant Genetic Resources (NBPGR), which has its headquarters at New Delhi, but has substations for testing the plant materials. NBPGR has the gene bank for long term storage and future use; it helps to assess the introduced plant material, coordinates the work of other agencies and imparts training in plant collection, introduction and maintenance in India.

The Substations under NBPGR are:

Shimla:

It represents the temperate zone of approx. 2300m above sea level, place for germplasm collection in northern hills, station for acclimatization of material introduced from temperate zone and high altitude.

Jodhpur:

Exclusively meant for exploring and acclimatizing plant material for arid zone, this is under Central Arid Zone Research Institute.

Shillong:

This centre has been created for collection of germplasm from North-East India.

Other agencies engaged for this purpose are:

Botanical Survey of India:

This body is meant for introduction of medicinal plants and also plants for botanical importance.

Achievements of Plant Introduction:

The process of introduction from the pre-historic times helped the whole world to achieve newer crop species to new places as well as the new varieties of crop plants.

The examples of achievements are listed below:

(A) New Crop Species:

The crops like potato, maize, groundnut, chillies; coffee, rubber, guava, grape, pineapple, many ornamentals like Gulmohor, Phlox, Salvia, Aster-all have been introduced in India from outside.

(B) New Crop Varieties:

(a) Direct Multiplication and Released as New Varieties:

Dwarf wheat varieties like 'Sonora 64', 'Lerma Rojo' and dwarf rice varieties like TN-1′, 'IR-8' are introduced in India and used directly. There are many more examples of direct release of varieties in other crop plants like oat, soya-bean, tomato, cauliflower, onion, etc.

(b) Selection of Desirable Varieties:

Many varieties have come up after selection from introduced varieties, e.g., 'Kafyan Sona', 'Sonalika' were selected after introduction of Mexican varieties.

Mass selection:

Here a large number of plants having similar phenotype are selected and their seeds are mixed together to constitute a new variety. Thus the population obtained-from selected plants will be more uniform than the original population. However they are genotypically different.

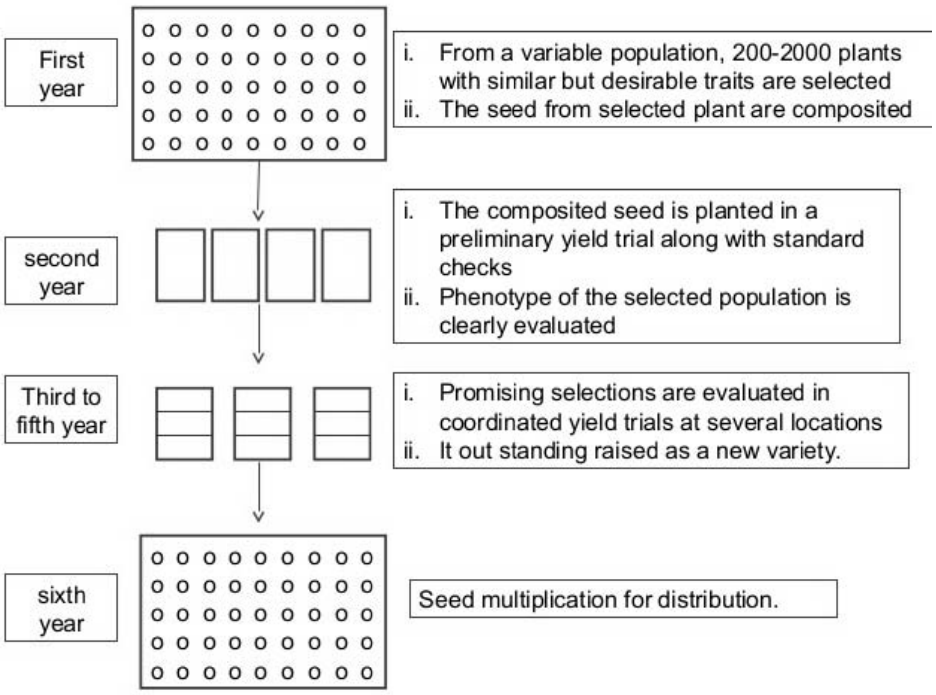
Steps

1st year: From the base population select phenotypically similar plants, which may be 200 2000. Harvest the selected plants as a bulk.

2nd year: The bulk seed is divided into smaller lots and grown in preliminary yield trial along with control variety. Dissimilar phenotypes are rejected. Higher yielding plots are selected.

3rd to 6th years: With the selected lots conduct yield trials along with appropriate check or control. Select the best one and release it as a variety.

Fig. Mass selection steps



Merits of Mass Selection

1. Since a large number plant is selected, the variety is more stable in perfor-mance over different environments as it is more adapted than a single pure line. So the varieties developed through mass selection are more widely ac-cepted than pure line.

2. Extensive and prolonged field trials are not necessary. This reduces the time and cost needed for developing a new variety. This method is also less labour consuming.

3. Mass selection retains considerable genetic variability, so another mass selec-tion after few years improves the variety.

4. This method can be applied to cross pollinated crops.

Demerits of Mass Selection

1. The varieties developed through mass selection show variation and are not uniform as pure line varieties, and strict selection for uniformity would lead to inbreeding depression.

2. The improvement of variety through mass selection is poorer than through pure line selection.

3. When the mass selection is done without progeny test, the homozygosity of the selected plants is not assured. In the self-pollinating species as there are chances for cross pollination to some extent, some plants must be heterozy-gous. In this method the genetic superiority either may be due to environment or due to genotype.

4. Pure line selection is more used than mass selection, only improvements of local old varieties are done through this method.

5. In seed certification programme, it is more difficult to identify the mass se-lected variety than the pure line.

6. This method utilizes only the variability already exists in the population, im-provement is done only through selection. So the limitation is that it cannot generate new genetic variability.

Achievements:

Mass selection is effective when the population has the following characters:

(a) High genetic variability for different traits.

(b) The characters should be highly heritable in nature.

(c) The crop is grown under low population density.

(d) If only one particular character is chosen.

At present use of mass selection is limited to purification of pure line varieties of self-pollinated crops, because the superiority of the pure lines would be lost quickly if their purity is not maintained through mass selection.

Although no proper records are available, but the following varieties in different crops have been developed by mass selection in India:

Maize — Jaunpur local, Tinpakhia, Basri

Pearl Millet — Bajra-207, Bajri-28-15, Bichpuri local, Pusa moti

Mustard — Gurgaon brown sarson

Sorghum — RSI, T22

Cotton — Dodahatti local, Dharwar American, Combodian cotton

Pureline selection:

A large number of plants are selected from a self-pollinated crop. The selected plants are harvested individually. The selected individual plants are grown in individual rows and evaluated and best progeny is selected, yield tested and released as a variety.

Characteristics of purelines

i. All plants within a pure line have the same genotype.
ii. The variation with in a pureline is environmental and nonheritable.
iii. Purelines become genetically variable with time due to natural hybridization, mutation and mechanical mixtures.

General steps for making a pureline selection

1st year: From the base population select best looking plants having the desirable characters. Harvest them on single plant basis.

2nd year: The selected single plants are grown in progeny rows and estimate the performance. Reject unwanted progenies.

3rd year: Repeat the process of second.

4th year: Grow the selected single plants in replicated preliminary yield trial along with suitable check or controI variety.

5th year: Conduct regular comparative yield trial along with check variety and select the best culture.

6th year: Conduct multilocation trial in different research stations along with local check.

7th year: Conduct Adaptive Research Trial in farmer's field. Fix the best yielder and release it as a variety thro' Variety Release committee.

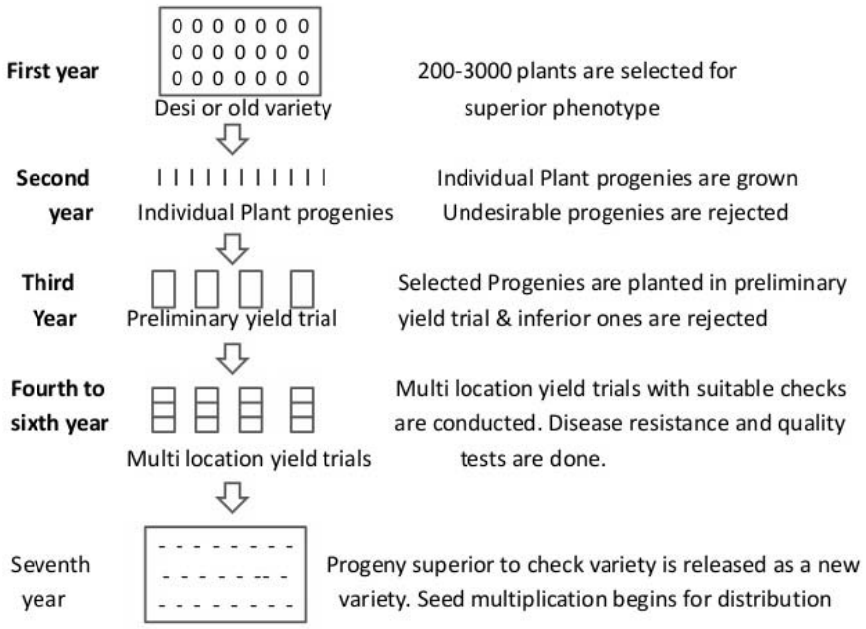


Fig. Pure line selection steps

Merits:

1. Pure line selection can achieve maximum possible improvement over the original variety. This is because the variety is the best pure line present in the population.

2. The pure line variety is extremely uniform since all the plants in the variety have the same genotype. Such uniformity is liked both by the farmers and the consumers.

3. Due to extreme uniformity, the variety is easily identified in seed certification programme.

Demerits:

1. As the pure line variety is genetically more homogeneous, the adaptability to various environments is less.

2. This procedure requires more time, space, labour and more expensive yield trials.

3. The upper limit of improvement is set by the genetic variation present in the original population.

4. Once a pure line is developed, the variability is frozen and further selection becomes less effective.

Achievements:

Pure line selection is the most extensively used breeding method in early days of crop improvement in India because at that time many genetically variable ‘desi’ varieties were available.

A large number of improved varieties were developed by this method in many self-pollinated crops:

Wheat — NP4, NP6, NP12, Pb-8, CI3, K46

Rice — M-351, Vidisa 60-1, Patni 6, Aispuri, BP 53

Cotton — Coimbatore 2, Gadag 1, MCU1

Barley — C-251, C-50, K-12

Tobacco — NP28, NP63, NP70

Comparison between pure line and mass selection

Pureline selection	Mass selection
The new variety is a pureline	The new variety is a mixture of purelines.
The new variety is highly uniform. In fact, the variation within a pureline variety is purely environmental.	The variety has genetic variation of quantitative characters, although it is relatively uniform in general appearance
The selected plants are subjected to progeny test	Progeny test is generally not carried out
The variety is generally the best pureline present in the original population. The pure line selection brings about the greatest improvement over the original variety	The variety is inferior to the best pureline because most of the purelines included in it will be inferior to the best pure line
Generally, a pure line variety is expected to have narrower adaptation and lower stability in performance than a mixture of pure lines	Usually the variety has a wider adaptation and greater stability than a pureline variety
The plants are selected for the desirability. It is not necessary they should have a similar phenotype	The selected plants have to be similar in phenotype since their seeds are mixed to make up the new variety
It is more demanding because careful progeny tests and yield trials have to be conducted.	If a large number of plants are selected, expensive yield trials are not necessary. Thus it is less demanding on the breeder.

Clonal selection:

A clone may be defined as a group of plants derived from a single plant by vegeta-tive propagation.

The clone can be characterised by the following characters:

(a) Clone is homogeneous,

(b) Individuals of a clone are either homozygous or heterozygous,

(c) Clones are stable in nature,

(d) Variability can be induced through mutation,

(e) Clone is propagated vegetatively.

Selection Procedure

In view of these considerations, in the earlier stages of clonal selection, when selection is based on single plants or single plots, the emphasis is on the elimination of weak and undesirable plants or clones. The breeder cannot reasonably hope to identify superior' genotypes at this stage.

First Year

From a mixed variable population, few hundreds to few thousand desirable plants are selected. A rigid selection can be done for simply inherited characters with high heritability.

Plants with obvious weaknesses are eliminated.

Second Year

Clones from the selected plants are grown separately, generally, without replication This is done in view of the limited supply of the propagating material for each clone, and because of the large number of clones involved. The characteristics of clones will be clearer now than in the previous generation when the observations were based on individual plants. The number of clones is drastically reduced and inferior clones eliminated.

Third Year

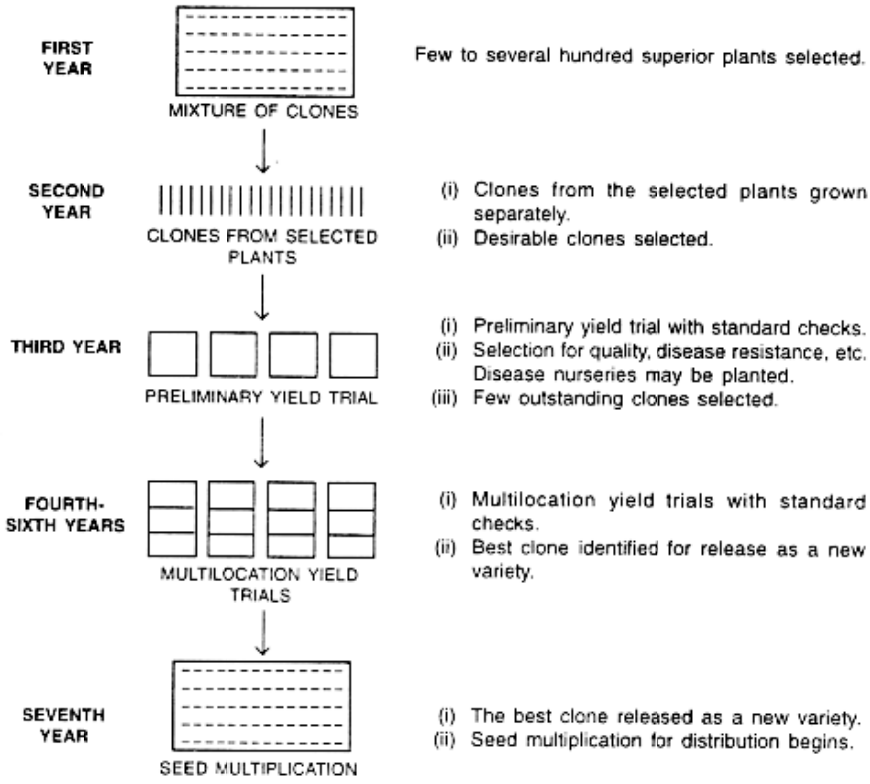
Replicated preliminary yield trial is conducted. Suitable, checks included for comparison. Few superior performing clones with desirable characteristics selected for multilocation trials. At this stage, selection for quality is also done.

Fourth to Sixth Years

Replicated yield trials are conducted at several locations along with a suitable check. The yielding ability, quality and disease resistance, etc. of the clones are rigidly evaluated. The best clone that is superior to the check in one or more characteristics is identified for release as a new variety.

Seventh Year

The superior clone is multiplied released as a new variety.



Merits of Clonal Selection

[1] It is the only method of selection applicable to clonal crops. It avoids inbreeding depression, and preserves the gene combinations present in the clones.

[2] Clonal selection, without any substantial modification, can be combined with hybridization to generate the variability necessary for selection.

[3] The selection scheme is useful in maintaining the purity of clones.

Demerits of Clonal Selection

[1] This selection method utilizes the natural variability already present in the population; it has not been devised to generate variability.

[2] Sexual reproduction is a prerequisite for the creation of variability through hybridization.

Hybridization

Individual produced as a result of cross between two genetically different parents is known as hybrid. The natural or artificial process that results in the formation of hybrid is known as hybridization.

The production of a hybrid by crossing two individuals of unlike genetical constitution is known as hybridization. Hybridization is an important method of combining characters of different plants. Hybridization does not change genetic contents of organisms but it produces new combination of genes.

The first natural hybridization was recorded by Cotton Mather (1716) in corn. The first artificial interspecific plant hybrid was produced by Thomas Fairchild in 1717. It is commonly known as 'Fairchild Mule'.

Hybridization was first of all practically utilized in crop improvement by German botanist Joseph Koerauter in 1760. Mendel onward, the hybridization had become the key method of crop improvement.

Objectives of Hybridization:

I. To artificially create a variable population for the selection of types with desired combination of characters.

II. To combine the desired characters into a single individual, and

III. To exploit and utilize the hybrid varieties.

Types of Hybridization:

Hybridization may be of following types:

(i) Intra-varietal hybridization:

The crosses are made between the plants of the same variety.

(ii) Inter-varietal or Intraspecific hybridization:

The crosses are made between the plants belonging to two different varieties.

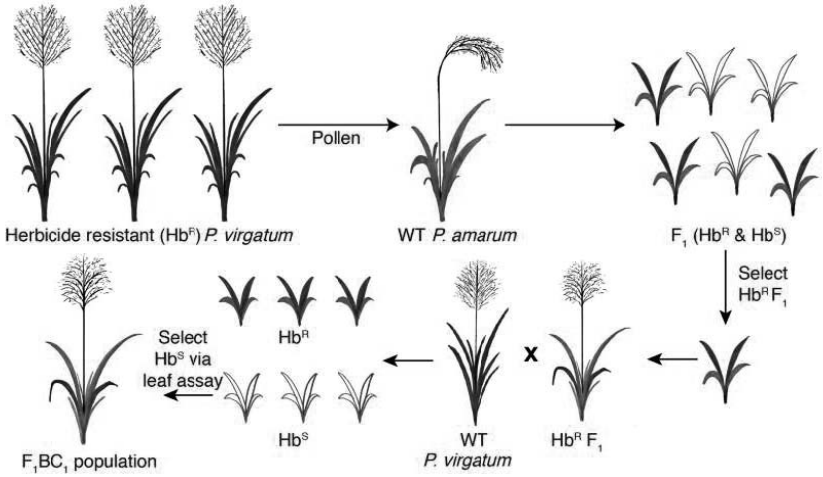
(iv) Interspecific hybridization or intragenric hybridization:

The crosses are made between two different species of the same genus.

(v) Introgressive hybridization:

Transfer of some genes from one species into the genome of the other species is known as introgressive hybridization. The crosses between different species of the same genus or different

genera of the same family are also known as distant hybridization or wide crossing. Such crosses are called distant crosses.



Procedure of Hybridization:

It involves the following steps:

(i) Selection of parents:

The selection of parents depends upon the aims and objectives of breeding. Parental plants must be selected from the local areas and are supposed to be the best suited to the existing conditions.

(ii) Selfing of parents or artificial self-pollination:

It is essential for inducing homozygosity for eliminating the undesirable characters and obtaining inbreeds.

(iii) Emasculation:

It is the third step in hybridization. Inbreeds are grown under normal conditions and are emasculated. Emasculation is the removal of stamens from female parent before they burst and shed their pollens.

It can be defined as the removal of stamens or anthers or the killing of the pollen grains of a flower without affecting in any way the female reproductive organs. Emasculation is not required in unisexual plants but it is essential in bisexual or self-pollinated plants.

Various methods used for emasculation are:

(a) Hand Emasculation or Forceps or Scissor Method:

This method is generally used in those plants which have large flowers. In this method the corolla of the selected flowers is opened and the anthers carefully removed with the help of fine-tip forceps.

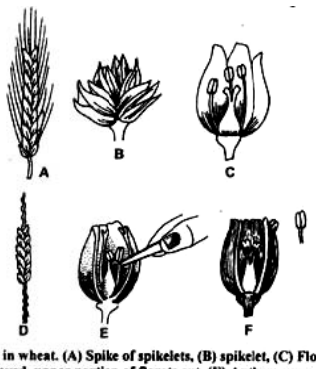


Fig. 6. (A-F) Emasculation in wheat. (A) Spike of spikelets, (B) spikelet, (C) Floret, (D) Upper and lower spikelets removed, awns removed, upper portion of florets cut, (E) Anthers removed with the help of fine-tip forceps. (F) Removed anther.

Following are the important precautions while performing this method:

i. Flowers should be selected at proper stage.

ii. Stigma should be receptive and anthers should not have dehisced.

iii. All the anthers should be removed from the flowers without breaking.

iv. Stigma and ovary of the flower should not be damaged.

(b) Hot Water Treatment:

Removal of stamens with the help of forceps is very difficult in minute flowers. In such small hermaphrodite flowers (e.g., Bajra, Jowar) emasculation is done by dipping the flowers in hot water for certain duration (1-10 minutes) of time. This method is based on the fact that gynoecia can withstand the hot temperature at which the anthers are killed. In this method an equipment is used which is placed on a simple heavy stand.

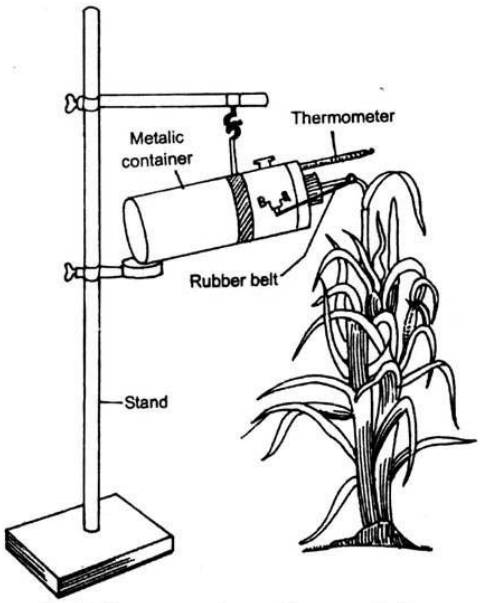


Fig. 7. Hot water equipment for emasculation.

It consists of a cylindrical metallic container of 60 cm length, with one hole of 5 cm to 16 cm diameter on one end to pass over a bajra or jowar head. After inserting the panicle inside the container a cork is fitted in the hole to close it. A 35 cm long rubber tube or belt is stretched over the side of the container, and when in use this tube is tied around the peduncle of the head. To measure the temperature, in the upper side of the container a thermometer is placed. In the field water is carried in a thermos jug.

The panicle is inserted in the container prior to blooming for a particular duration of time. It has been observed that pollen grains of rice are killed by immersing the inflorescence for 5 to 10 minutes in the hot water maintained at 40-44°C in a thermos flask.

(c) Cold Water Treatment:

Like hot water cold water also kills pollen grains without damaging the gynoecium. In rice 0-6°C temperature is maintained to kill the pollen grains. This method is less effective than hot water treatment.

(d) Alcohol Treatment Method:

This method is not commonly used for emasculation because duration of treatment is an important factor since a very short duration is required failing which even the gynoecium may be damaged. Flowers or inflorescences are immersed in alcohol of a suitable concentration for a brief period. In alfa-alfa, a treatment of even 10 seconds with 57 % alcohol is sufficient to kill the pollen grains.

(e) Suction Method:

It is a mechanical method and is suitable for the crops having minute flowers. In this method the amount of pressure is applied in such a way that only anthers are sucked out and other parts of the flower like gynoecium remain intact. However, in this method 10-15% self-pollination takes place. It is one of the major drawback of this method.

(f) Male Sterility or Self-incompatibility Method:

Emasculation option can be eliminated by the use of male-sterile plants, In some self-pollinated plants for example, Sorghum, Onion, Barley etc. anthers are sterile and do not produce any viable pollens! Similarly self-incompatibility may also be used to avoid emasculation.

(g) Chemical Gametocides:

Certain chemicals are capable of causing male sterility, when sprayed before flowering e.g., 2, 4-D, naphthalene acetic acid (NAA), maleic-hydrazide (MA), tribenzoic acid etc. FW450 in cotton may be used for bringing about emasculation.

(iv) Bagging:

It is the fourth step and completed with emasculation. The emasculated flower or inflorescence is immediately bagged to avoid pollination by any foreign pollen. The bags may be made of paper, butter paper, glassine or fine cloth. Butter paper or vegetable parchment bags are most commonly used.



Fig. 8. Different methods of Bagging.

The bags are tied to the base of the inflorescence or to the stalk of the flower with the help of thread, wire or pins. The bagging is done with the emasculation in bisexual plants and before the stigma receptivity and dehiscence of the anthers in unisexual plants. Both male and female flowers are bagged separately to prevent contamination in male flowers and cross-pollination in female flowers.

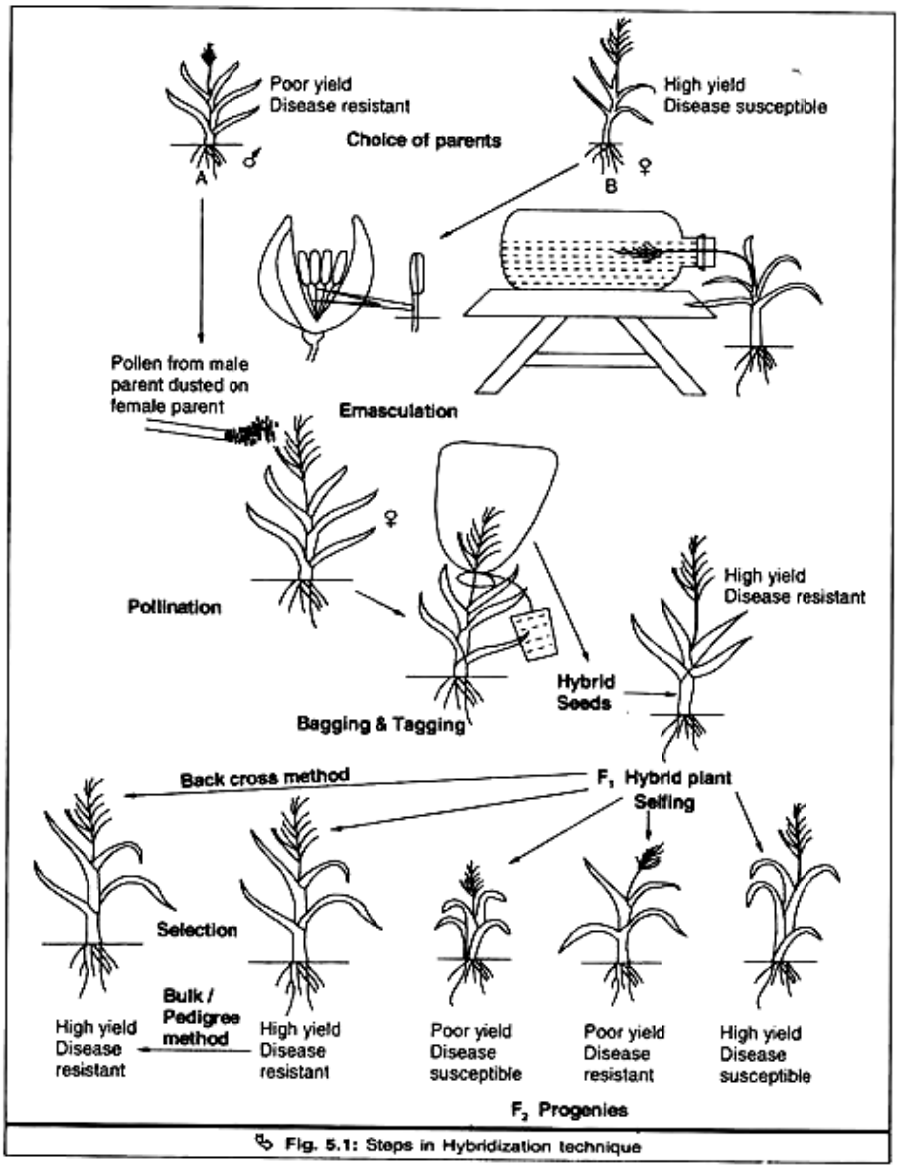


Fig. 5.1: Steps in Hybridization technique

(v)Tagging:

The emasculated flowers are tagged just after bagging. Generally circular tags of about 3 cm or rectangular tags of about 3 x 2 cm are used. The tags are attached to the base of flower or inflorescence with the help of thread.

The information on tag must be as brief as possible but complete bearing the following information:

i. Number referring to the field record
ii. Date of emasculation
iii. Date of crossing
iv. Name of the female parent is written first followed by a cross sign (x) and then the male parent, e.g., C x D denotes that C is the female parent and D is the male parent.

(vi) Crossing:

It is the sixth step. It can be defined as the artificial cross-pollination between the genetically unlike plants. In this method mature, fertile and viable pollens from the male parent are placed on the receptive stigma of emasculated flowers to bring about fertilization.

Pollen grains are collected in petridishs (e.g., Wheat, cotton etc.) or in paper bags {e.g., maize) and applied to the receptive stigmas with the help of a camel hair brush, piece of paper, tooth pick or forceps. In some crops (e.g., Jowar, Bajra) the inflorescences of both the parents are enclosed in the same bag.

(vii) Harvesting and Storing the F_1 Seeds:

Crossed heads or pods of desirable plants are harvested and after complete drying they are threshed. Seeds are stored properly with original tags.

(viii) Raising the F_1 generation:

In the coming season, the stored seeds are sown separately to raise the F_1 generation. The plants of F_1 generation are progenies of cross seeds and therefore are hybrids.

Selection after hybridization:

There are several methods of improvement of self-fertilized crops by hybridization. These are:

a. Pedigree method
b. Bulk method
c. Recurrent

Pedigree Method:

Record of the ancestry of an individual selected plant for various generations is known as pedigree. A selection method, which is used in segregating population of self-pollinated species and keeps proper record of plants and progeny selected in each generation is known as pedigree breeding. This method is widely used for the development of varieties in self-pollinated crops.

In this method individual plants are selected till the progenies become homozygous. Selection for plants in the desired combination of characters is started in the F_2 generation and continued in succeeding generations until genetic purity is reached.

I Year:

Plants are chosen for hybridization and F1 seeds are produced.

II Year (F1 generation):

F1 plants are space planted to produce maximum number of F2 seeds (see Fig. 9).

III Year (F2 generation):

2000-10000 F2 plants are space planted. About 200-500 desirable superior plants are selected.

IV Year (F3 generation):

Selected superior plants in III year are space planted to study the individual plant. 3 to 5 best plants in these rows are selected and harvested (F4)

V Year and VI Year (F4, F5 generation):

Process is continued as in F3 generation. Normally 20-50 families may be retained at the end of F5 generation.

VII Year (F6 generation):

Due to successive self-pollination most of the lines become homozygous and uniform. The plants uniform in desired characters are harvested and the seed, bulked together to constitute the variety.

VIII Year (F7 generation):

Preliminary yield trials are conducted.

IX to XI year (F8 – F10 generation):

Trials of superior lines are confirmed. During the testing period observations are made on height, tendency to lodge, maturity, disease resistance and quality.

XII to XIII Year (F10, F11 generation):

Seeds are multiplied and distributed to the farmers.

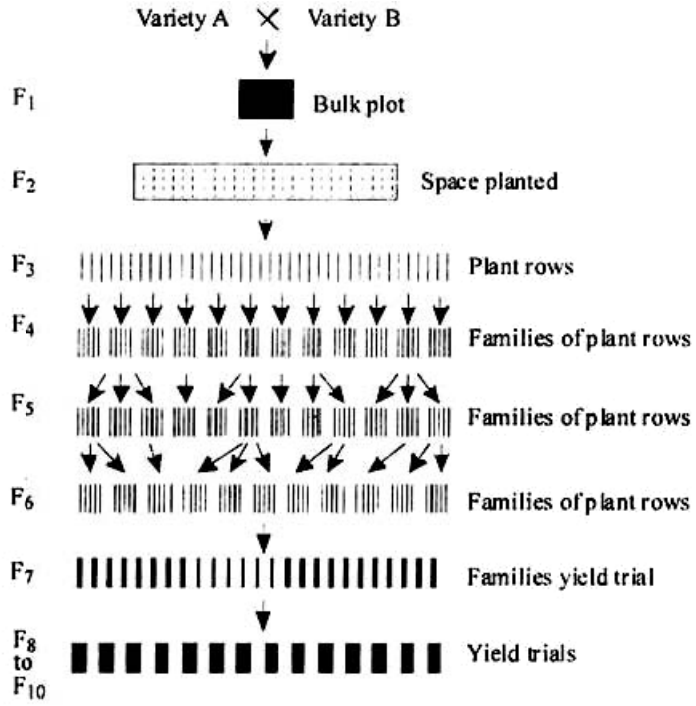


Fig. 9. Different steps involved in pedigree method.

Merits:

1. It is the quickest method.
2. Plant breeders can also obtain the genetic information.
3. There are chances of recovering transgenic segregation by this method.

Demerits:

1. Maintenance of accurate pedigree record is not easy. It takes much time.
2. Selected material becomes so large that handling of the same becomes very difficult.
3. Success of this method depends upon the skill of the breeder.

Bulk Method

A selection procedure which is used in segregating population of self-.pollinated species in which material is grown in bulk plot from F2to F5 with or without selection, next generation is grown from bulk seed and individual plant selection is practiced in F6 or later generations is called bulk method or breeding.

This method is also known as the mass or population method. Nilsson-Eule of Sweden was first to use the bulk method and it is in use ever since. This method differs from the pedigree method in that no selection is practiced in F_2-F_5 generations.

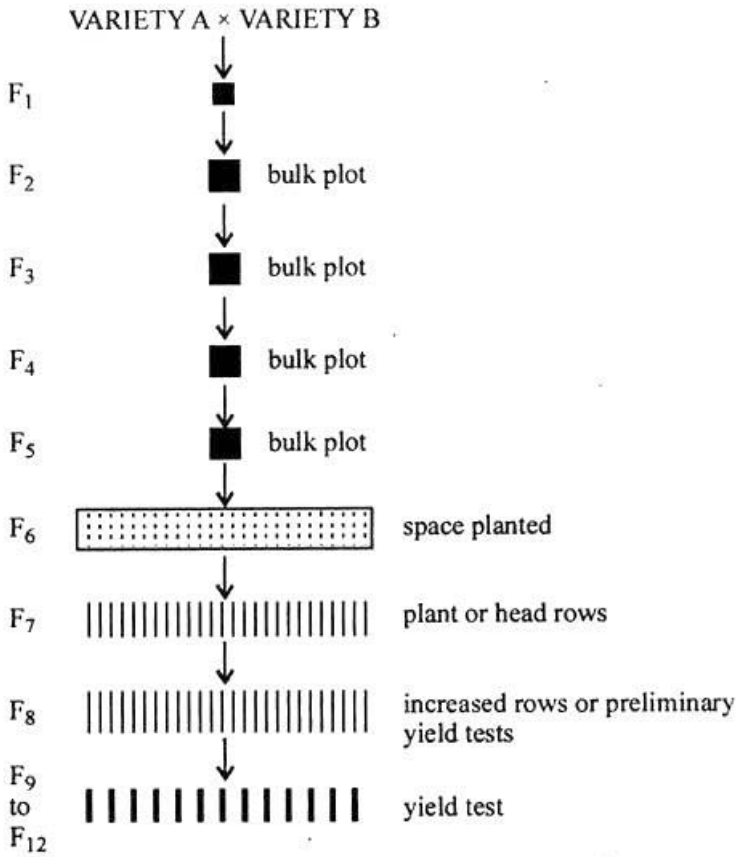


Fig. 10. Procedure of Bulk breeding method.

The method is as follows:

I Year:

Plants are chosen for hybridization and F1 seeds are produced.

II Year (F1 generation):

50-100 F1 plants are grown and their F2 seeds are harvested in bulk,

III Year (F2 generation):

F2 plants are grown and their F2 seeds are harvested in bulk.

IV Year (F3 generation):

F3 plants are grown and their F4 seeds are harvested in bulk.

V Year (F4 generation):

FA plants are grown and their F5 seeds are harvested in bulk.

VI Year (F5 generation):

F5 plants are grown and their F6 seeds are harvested in bulk. (The process may be repeated until the desired period of homozygosity is achieved. In general bulk period is allowed up to F5 generation)

VII Year (F6 generation):

Seeds are space planted and single plant selection is done (F7generation).

VIII Year (F7 generation):

The progeny of each single plant is grown separately and superior progeny are selected and isolated (F8).

IX Year (F8 generation):

Preliminary yield test are conducted (F9).

X-XII Year (F9-F12 generations):

Multi-locations field trials are carried out, best performing strain is multiplied for seed distribution.

Merits:

(i) The bulk method is simple, convenient, inexpensive and less labour consuming (no pedigree record is to be kept).

(ii) During early segregating generations, very little work and attention is needed, which gives the breeder more time to concentrate on other breeding projects.

(iii) Selection is done by nature only and it increases the frequency of superior types in the population.

(iv) This method is suitable for studies on the survival of genes and genotypes in populations.

Demerits:

(i) This method takes much longer time to develop a new variety.

(ii) The breeder is enabling to exercise his skill and judgement in selection and therefore the method is less satisfying to him.

(iii) Information on the inheritance of characters cannot be obtained.

(iv) This method is totally dependent on natural selection to select the superior types. These types may not be necessarily the best yielding types.

Recurrent selection

Recurrent selection is defined as reselection generation after generation, with intermating of selected plant to produce the population for the next cycle of selection. The idea of this method was to ensure the isolation of superior inbreds from the population subjected to recurrent selection. The isolation of an outstanding inbred line depends on two factors:

1) The proportion of superior genotypes present in the base population from which lines are isolated and
2) The effectiveness of selection during the inbreeding of desirable genes.

Types of Recurrent Selection:

These are four types of recurrent selection.

i) Simple Recurrent Selection (SRS):

A type of recurrent selection that does not include tester is referred as simple recurrent selection. It is also known as phenotypic recurrent selection.

Procedure of Simple Recurrent Selection:

1) A number of plants with desirable phenotype are selected and self-pollinated in the 1st year.

2) In the second year separate progeny rows are grown from selfed seed of the selected plants.

3) The progenies are inter crossed in all possible combination by hand.

4) Equal amount of seed from each cross is composited to produce the next generation. This complete the original selection cycle.

5) In the third year, bulked seeds are grown and superior plants are selected and selfed, like first year.

6) In the fourth year, progeny of selected plants are grown from selfed seed and intermating is done like first year.

7) The crossed seed is composited in equal quantity for use in the next cycle of selection. This complete first cycle of simple recurrent selection. Thus selection cycles may be repeated till the desired improvement is achieved.

Recurrent Selection for General Combining Ability (GCA):

Recurrent Selection for SCA:

It was originally proposed by Hull in 1945, a form of recurrent selection that is used to improve the SCA of a population for a character by using homozygous tester is referred as (RSSCA) recurrent selection for specific combining ability. It is also known as half site recurrent selection with homozygous tester.

Reciprocal Recurrent Selection (RRS):

A form of recurrent selection used to improve both GCA and SCA of a population for a character using two heterozygous testers is known as RRS. It is also known as recurrent reciprocal half sib selection. Comstock et al in 1949, proposed this method.

Main Features of these Methods:

1) It is used for improvement of polygenic characters.

2) Selection is made on the basis of test cross performance.

3) Two heterozygous tester are used as a source of population.

4) It is used for improving population for GCA and SCA for specific characters.

5) It is equally effective with incomplete, complete and over dominance.

6) It is used for improvement of those characters, which are governed by both additive and non-additive gene action.

7) This method also requires three seasons for completion of each cycle of selection.

Merits:

1) Recurrent selection is an efficient breeding method for increasing the frequency of superior genes for various economic characters.

2) It helps in breaking repulsion phase of linkage.

3) It helps in maintaining high genetic variability due to repeated intermating of heterozygous population.

Demerits:

1) It is not directly used for the development of new varieties.

2) This method involves lot of selection crossing and selfing work.

3) It permits selfing, which leads to loss of genetic variability.Heterosis and Inbreeding Depression

Heterosis

When two homozygous inbreeds (a true breeding line obtained by continuous inbreeding) of genetically unlike constituents are crossed together, the resulting hybrids obtained from the crossed seeds are usually robust, vigorous, productive and taller than the either parents.

This increased productivity or superiority over the parents is known as heterosis or hybrid vigour. Heterosis can be defined as the superiority of F1 hybrid over both the parents in terms of yield or some other character.

History of Heterosis:

Heterosis has been known since the art of hybridization came into existence. Koelreuter (1763) was the first to report hybrid vigour in the hybrids of tobacco, *Datura* etc. Mendel (1865) observed this in pea crosses. While discussing the work on maize during a lecture at Gottingen (West Germany), Dr. G.H. ShuII (1914) proposed the term heterosis (Gr. heteros different and osis = condition). Poweri (1944, 45) reported that the crossing, however, may result in either weak or vigorous hybrids as compared to parental inbreeds.

Hybrid vigour is used as synonym of heterosis. It is generally agreed that hybrid vigour describes only superiority of the hybrid over the parents while heterosis describes the other situation as well i.e., crossing over may result in weak hybrids e.g., many hybrids in tomato are earlier (vegetative phase is replaced by reproductive phase). However, Whaley (1944) was of the opinion that it would be more appropriate to term the developed superiority of the hybrids as hybrid vigour and to refer to the mechanism by which the superiority is developed as heterosis. Smith (1955) opined that the use of heterosis and hybrid vigour as synonyms is highly desirable on the basis of their long usage.

Types of Heterosis:

Heterosis is of two types:

1. True heterosis:

It is inherited.

It can be further divided into two types:

(a) Mutational true heterosis:

It is the sheltering or shadowing of the deleterious, un-favourable, often lethal, recessive mutant genes by their adaptively superior dominant alleles.

(b) Balanced true heterosis:

It arises out of balanced gene combinations with better adaptive value and agricultural usefulness.

2. Pseudo-heterosis:

Crossing of the two parental forms brings in an accidental, excessive and un-adaptable expression of temporary vigour and vegetative overgrowth. It is also called luxuriance.

Manifestation of Heterosis:

1. Increased Yield:

Increase in yield which may be measured in terms of grain, fruit, seed, leaf, tuber or the whole plant is one of the most important manifes-tations of heterosis.

2. Increase in Size and General Vigour:

Heterosis results in more vigorous growth which ultimately leads to healthier and faster growing plants with increase in size than the parents.

3. Better Quality:

In many cases heterosis yields better quality which may be accompanied with higher yield.

4. Greater Adaptability:

Hybrids are generally more adapted to environmental changes than the inbred lines due to heterozygosity.

5. More Disease Resistant:

Heterosis sometimes results into development of more disease resistant character in the hybrids.

6. Increased Reproductive Ability:

Hybrids exhibit heterosis by expressing high fertility rate or reproductive ability, which is ultimately expressed in yield character.

7. Increase in Growth Rate:

In many cases the hybrids show faster growth rate than the parents, but that does not always produce larger plant size than the parents.

8. Early Flowering and Maturity:

In many cases the hybrids may show early-ness in flowering and maturity than the parents, for some crops these are the desir-able characters for crop improvement. All these manifestations of heterosis can be traced at all levels of hybrid plant organisation.

Molecular Level:

Heterosis is manifested in increased rate of DNA reduplication, transcription and translation influencing the formation of genetic information, enzymatic activity, other regulatory mechanisms and also hybrid protein molecule formation.

Functional Level:

Heterosis is expressed as an effective regulation in metabolic pro-cesses and morphogenesis in hybrid organism.

Cellular Level:

Due to change in electro-kinetic properties of hybrid cell nuclei, the heterosis is manifested by increased mitosis.

Organism Level:

Heterosis is expressed as increased growth and differentiation of vegetative organs, synthesis and accumulation of nutritional substances and utilisation of metabolic process for yield formation.

Genetic Basis of Heterosis:

There are two main theories to explain the genetic cause of heterosis.

(A) Dominance Hypothesis:

This hypothesis was proposed by Davenport and further expanded by others. This hypothesis suggests that at each locus dominant allele has the favourable character, whereas the recessive allele has the unfavourable character.

When they are combined together; i.e., in heterozygous condition in the hybrids, the favourable characters get expressed whereas the unfavourable characters are masked. So the heterosis results from the masking of harmful effects of recessive alleles by their dominant alleles.

Dominance Hypothesis has Assumptions:

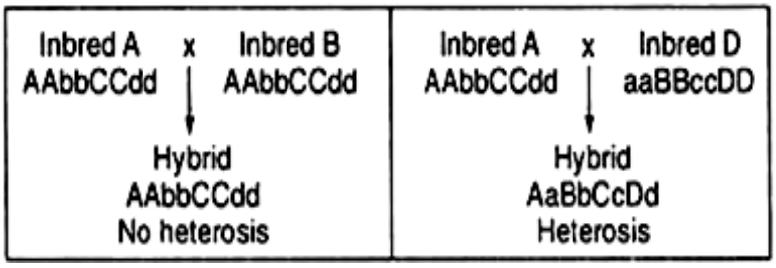
(a) Dominant genes are beneficial and recessive genes are deleterious.

(b) The loci show addition effects, non-allelic interactions are absent.

(c) No recombination barrier between the genes.

With the help of following example heterosis can be explained:

In a cross between Inbred A (AAbbCCdd) with Inbred B (AAbbCCdd), there will be no heterosis in F1 hybrid, there is no masking of recessive gene in hybrid. But in another cross, Inbred A (AAbbCCdd) is crossed with Inbred D (aaBBccDD), where the F1 hybrid is (AaBbCcDd) with all the genes having dominant allele.



As a result the harmful effects of a, b, c, d are hidden by the dominant alleles A, B, C and D. Thus some parents produce heterotic progeny while others do not. Generally parents of diverse or different origin are more likely to produce heterotic progeny than those of similar origin.

Objection:

1. Failure in Isolation of Inbreds as Vigorous as Hybrids:

According to dominance hypothesis it should be possible to get the inbred line with all the dominant genes. Such inbreds should be as vigorous as the F1 hybrids, but such inbreds have not been isolated.

2. Symmetrical Distribution in F2:

According to dominance hypothesis, the quan-titative characters should not show symmetrical distribution as because dominant and recessive alleles should segregate in the proportion of 3: 1, but generally the F2 shows symmetrical distribution.

Above two objections can be explained by linked genes. Many of the quantitative characters are governed by linked genes together, so to get the inbred line with all domi-nant genes require several precisely placed crossovers. In another explanation it can be showed that if the number of genes governing the quantitative characters is large, sym-metrical distribution would be obtained even without linkage.

(B) Over-dominance Hypothesis:

This hypothesis was independently proposed by East and Shull. This is sometimes known as single gene heterosis, super-dominance, cumulative action of divergent alleles and stimulation of divergent alleles. According to this hypothesis, heterozygotes are supe-rior to both the homozygotes.

So the heterozygote Aa would be superior to both the homozygotes AA and aa. Consequently, heterozygosity is essential for the cause of heterosis. In case of maize, the gene ma affects maturity. The heterozygote Ma/ma is more vigorous with late maturity than the homozygotes Ma/Ma or ma/ma.

Another proposal by East was that there are several alleles, e.g., a1, a2, a3, a4…….. etc. with increasingly different functions. Heterozygotes between more divergent alleles would be more heterotic than those involving less divergent genes, e.g., a1a4 is more heterotic than a1a2, a2a3, a3a4, etc. In these cases due to presence of divergent alleles the hybrids have the capacity to perform different functions which is not possible by any of the heterozygotes.

Objection:

1. There are many examples where the superiority is due to the epistatic affect of several non-allelic genes, not due to over-dominance (which is the interaction between allelic genes).

2. There is another objection against over-dominance hypothesis that there are many examples where the homozygotes are superior to the heterozygotes.

Physiological Basis of Heterosis:

Hybrid vigour, the product of heterotic mechanism, is essentially a physiological manifestation.

This better physiological efficiency of hybrids is derived chiefly from:

1. Better initial growth.

2. Greater uptake followed by better utilisation of nutrients by hybrids.

The initial growth activities include the different physiological processes during ger-mination:\

(a) Efficient water absorption,

(b) Better activity of enzymes,

(c) Rapid mobilization and utilization of stored food matter,

(d) Transformation and building up of active protoplasmic synthesis.

Applications of Heterosis in Plant Breeding:

Heterosis is observed in almost every crop species studied, the application of this phenomenon for its commercial exploitation depends on the expression of the degree of heterosis. This phenomenon is commercially used to produce hybrid or synthetic variet-ies, which needs the maximisation of its expression and also fixation.

Maximisation of hybrid vigour (HF1) can be achieved by increasing either ‘d’ (direc-tional dominance) or ‘y’ (initial differences in gene frequency between parents), i.e., choosing genetically divergent parents, HF1 = ∑dy2.

Fixation of hybrid vigour is needed for its commercial application which can be done by vegetative propagation, or by stable apomictic reproduction, or by transferring heterozygosity to polyploid or fixation by obtaining structural heterozygotes.

Fixation of heterosis in crops like potato, sweet potato, sugarcane, sugarbeet and many ornamental plants can be achieved by vegetative propagation as there seeds are not essential. Incorporation of genes conditioning vegetative apospory or diploid partheno-genesis in hybrid seed crops may lead to a permanent heterozygote advantage.

Heterozygosity can be maintained or saved from being lost due to segregation by converting dip-loid heterozygotes into tetraploid or hexaploid. Incorporation of gaudensvelans combi-nation helps to survive only the heterozygotes not the homozygous combination — such mechanism may be introduced in crop plants, thereby hybrid vigour can be fixed with great success.

Heterosis or hybrid vigour have been commercially utilised in both cross pollinated and in some self-pollinated species. In most of the cases the utilisation of this heterosis phenomenon is not successful because of difficulty in production of large quantities of hybrid seeds. This is particularly difficult in self-pollinated species.

Few examples where the heterosis has been utilised for improvement of the crop plant are:

Crop Species:

Asexually propagated species and also cross pollinated species like maize, jowar, bajra, sunflower, legume, cotton, etc.

Vegetable Crops:

Tomato, brinjal, onions, cucurbits, etc.

Fruits:

In almost all the fruit trees.

Inbreeding Depression

Inbreeding depression is the reduced biological fitness in a given population as a result of inbreeding, or breeding of related individuals. Population biological fitness refers to an organism's ability to survive and perpetuate its genetic material. Inbreeding depression is often the result of a population bottleneck. In general, the higher the genetic variation or gene pool within a breeding population, the less likely it is to suffer from inbreeding depression.

Inbreeding depression seems to be present in most groups of organisms, but varies across mating systems. Hermaphroditic species often exhibit lower degrees of inbreeding depression than outcrossing species, as repeated generations of selfing is thought to purge deleterious alleles from populations. For example, the outcrossing nematode (roundworm) Caenorhabditis remanei has been demonstrated to suffer severely from inbreeding depression, unlike its hermaphroditic relative C. elegans, which experiences outbreeding depression.

It is mating between individuals related by descent or having common ancestry. (Brother - Sister mating or sib mating). The highest degree of inbreeding is obtained by selfing.

4. An outline of mutation and polyploid breeding

Mutation breeding

Mutation is a sudden heritable change in a characteristic of an organisam. This definition requires that the change in the characteristic be heritable, but it does not state the genetic basis of the heritable change. Clearly, a mutation (as defined above) may be the result of a change in a gene, a change in chromosome(s) that involves several genes or a change in a plasmagene (genes present in the cytoplasm, e.g., in chloroplasts, mitochondria, etc., which have circular naked DNA as chromosomes). Mutations produced by changes in the base sequences of genes (as a result of base pair transition or transversion, deletion, duplication or inversion, etc.) are known as gene or point mutations.

Gene mutations can be easily and clearly shown by fine genetic analysis techniques available with microorganisms. Some mutations may be produced by changes in chromosome structure, or even in chromosome number; they are termed as chromosomal mutations. Gross chromosomal changes, e.g., changes in chromosome number, translocations, inversions, large deletions and duplications

are detectable cytologically under the microscope. But small deletions and duplications can rarely be detected, and would be considered as gene mutations.

This is particularly so in higher organisms where the techniques of genetic analysis are not yet as refined as those in the case of microorganisms. Thus what we refer to as gene mutation in plants is likely to include a fair number of small chromosomal changes. In clonal crops, mutations may even include gross changes in chromosome structure, sometimes even in number, unless cytological analyses are performed.

Spontaneous and Induced Mutations

Mutations occur in natural populations (without any treatment by man) at a low rate; these are known as spontaneous mutations. The frequency of spontaneous mutations is generally one in 10 lacs,i.e., 10^{-6} but different genes may show considerably different mutation rates. For example, R locus in maize mutates at a frequency of 4.92×10^{-4}, Su at 2.4×10^{-6}, while Wx appears to be highly stable. Spontaneous mutation rates of genes may be considerably affected by the genetic background; some mutator genes may promote mutation of other genes. Mutations may be artificially induced by a treatment with certain physical or chemica1 agents; such mutations are known as induced mutations, and the agents used for producing them are termed as mutagens. The utilization of induced mutations for crop improvement is known as mutation breeding.

Mutation induction rarely produces new alleles; it produces alleles, which are already known to occur spontaneously or may be discovered if an extensive search were made. It is reasonable to say that induced mutations are comparable to spontaneous mutations in their effects and in the variability they produce. But the induced mutations have a great advantage over the spontaneous ones; they occur at a relatively higher frequency so that it is practical to work with them. Mutations have certain general characteristics; those that concern us the most are summarized below:

- Mutations are generally recessive but dominant mutations also occur.
- Mutations are generally harmful to the organism. Most of the mutations have deleterious effects, but a small proportion (Ca0.1%} of them are beneficial.
- Mutations are random. i,e., they may occur in any gene. However, some genes show higher mutation rates than others.
- Mutations are recurrent that is, the same mutation may occur again and again.
- Induced mutations commonly show pleiotropy, often due to mutations in c1osely linked genes.

Types of mutation:

Mutations called **macro- and micro- mutations** depending on the magnitude of phenotypic effect produced by them.

Macromutations:

- Produces a large phenotypic effect
- Easily recognizable on individual plant basis

- Oligogenic in nature
- Can be easily selected in M_2 generation

Micromutations:

- Produces small phenotypic effect
- Cannot be recognizable on individual plant basis
- Detected only in group of plants and need treatment of statistical data
- Polygenic nature and selection delays till M_3

Mutagens

Agents that induce mutations are known as mutagens. Mutagens may be different kinds of radiation (physical mutagens) or certain chemicals (chemical mutagens). The different mutagens may be grouped as follows.

A. Physical mutagens (all of them are various kinds of radiation)

1. Ionizing radiation

a. Particulate radiation. Eg. α–rays (DI), β-rays (SI), fast neutrons* (DI), and thermal neutrons (DI)

b. Non Particulate radiation (electromagnetic radiation), eg., X-rays* (SI), and γ-rays (SI).

2. Non Ionizing radiation. Eg. UV radiation.

B. Chemical mutagens

1. Alkylating agents, e.g., sulphur mustards, nitrogen mustards, epoxides, imines, (e.g., ethylene imine or EI)*, sulphates and sulphonates, diazoalkanes, nitroso compounds, e.g., N-methyl- N- nitro- N-nitroso-guanidine or MNNG).

2. Acridine dyes, e.g., acriflavine, proflavine, acridine orange, acridine yellow, ethidium bromide.

3. Base analogues, e.g., 5- bromouracil, 5-chlorouracil.

4. Others, e.g., nitrous acid, hydroxyl amine, sodium azide*.

(*denotes that these agents are commonly used in mutation breeding. DI denotes densely ionizing and SI denotes sparsely ionizing radiations).

Beta-Rays

Beta rays are high energy electrons produced by the decay of radioactive isotopes, e.g., ^{3}H, ^{32}P, ^{35}S, etc. High energy electrons are slowed down by positively charged molecules in and they have very

little penetrating power as compared to X-rays. Beta-rays transfer energy to electrons of the atoms in their path causing these electrons to fly away from their orbit leaving the nucleus positively charged; this is known as ionisation. When the amount of energy transferred to an electron is not sufficient to cause ionisation, the electron is pushed to an outer orbit representing a higher level of energy, thus producing excitation. Electrons are easily deflected by atoms in their path, hence they move in a zig-zag line. After energy is spent, electrons attach to an atom making it negatively charged. Beta-rays may interact with the nuclei of atoms to produce electromagnetic radiation similar to X-rays. Electrons liberated as a result of ionisation also produce ionisation and excitation, that is, they behave like beta-rays.

Alpha-Rays

The alpha particles making up alpha-rays have two protons and two neutrons each; thus the alpha-particles have double positive charge. Alpha-particles are produced by fission of radioactive isotopes of heavier elements. Since they are heavy particles, they move in a straight line. Alpha-particles have a strong attraction for electrons and pull them away from the nuclei of atoms in their path. Alpha-rays produce both ionisation and excitation. After energy, each alpha-particle captures two electrons and produces an atom of helium. As -particles move away from their source, they slow down and produce dense ionisation. Alpha particles are much less penetrating than neutrons and even beta-rays.

Fast and Thermal Neutrons

Fast Neutrons are produced in cyclotrons or atomic reactors as a result of radioactive decay of heavier elements. The velocity of fast neutrons is reduced by graphite or heavy water to generate thermal or slow neutrons. Neutrons are uncharged particles, and are highly penetrating in biological tissues. They are not repel1ed by nuclei of atoms, and move in a straight line. They do not cause ionisation directly. Ionisation is produced by (1) elastic scatering, in which nuclei, of atoms are kicked away by the neutron; these nuclei then cause ionisation, and (2) production of gamma-rays as thermal neutrons are captured by atomic nuclei, which then become unstable and give off gamma-rays. Fast and thermal neutrons are densely ionising radiations.

X-rays and Gamma Rays

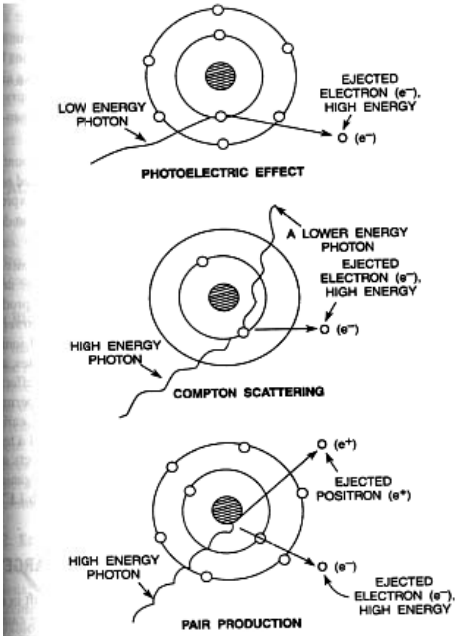
X-rays and gamma-rays are nonparticulate electromagnetic radiation with a wavelength of 10^{-11} to 10^{-7} cm. These are high energy radiation and consist of photons, i.e., small packets of energy. The physical properties and the biological effects of X-rays and gamma-rays are similar, but they differ in the source of their origin. X-rays are produced by X-ray tubes, while gamma-rays are produced by radioactive decay of certain elements, e.g., radium, ^{14}C, ^{60}Co, etc.^{60}Co is the common Source of gamma-rays used for biological studies. X-rays are often referred to as hard (0.1-0.001A˚) or soft (10-1A˚) depending upon their wavelength. X-rays and gamma-rays are highly penetrating and sparsely ionising. The electromagnetic radiations produce the following effects.

Photoelectric Effects

Low energy photons transfer all their energy to individual electrons, which are kicked off as high energy electrons (e-) from their orbit producing ionisation. These high energy e- produce secondary ionisations, which are of greater significance than the primary ones.

Ultraviolet Radiation

Ultraviolet (UV) rays have a wavelength of 100 to 3,900A˚ (10-390 nm). UV is present is solar radiation and is produced by mercury vapour lamps or tubes. UV is a low energy radiation; it does not cause ionisation and has a very limited penetrating capacity (usual1y limited to one or two cel1layers). UV rays generally produce dimers of thymine, uracil and, sometimes, cytosine present in the same strand of DNA. It also produces addition of a molecule of water to the 5, 6 double bond of uracil and cytosine, which promotes deamination of cytosine. The mutagenic action of UV is most likely due to dimer formation and deamination. The most effective wavelength of UV is 2,540A˚ since DNA bases show the maximum absorption at this wavelength. UV is commonly used in microorganisms since penetration presents no problem in that system. But in higher organisms, poor penetration of UV has limited its use to irradiation of pollen grains (in plants) and of small eggs (e.g., in Drosophila). In plants, pollen grains may be irradiated and used for pollination. But the difficulty in collecting large quantities of pollen grains in most of the crop species, except in maize and similar crops, and the limited duration of pollen viability have prevented the use of UV in crop improvement.

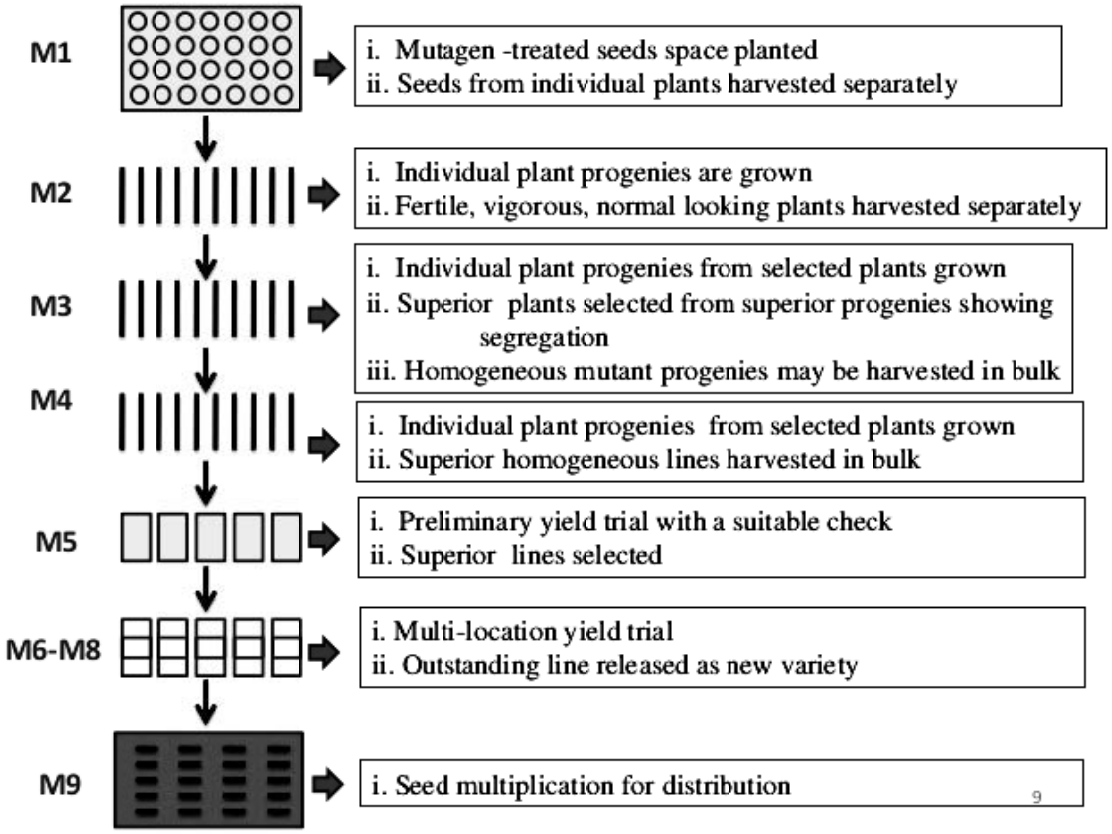


Ion production by X-ray and gamma-ray photons. The large central solid circle represents the nucleus of an atom, the small open circles on the periphery denote electrons (e^-), and the particle e^+ is a positron. The atom pictured here is an oxygen atom.

Procedure for Mutation Breeding

Treating a biological material with a mutagen in order to induce mutations is known as mutagenesis. Exposure of a biological material to a radiation like X rays,_gamma - rays, etc. is known as irradiation. When mutations are induced for crop improvement, the entire operation of the induction and isolation, etc. of mutants is termed as mutation breeding. A mutation breeding programme should be clearly planned and should be large enough with sufficient facilities to permit an effective

screening of large populations. The various steps involved in mutation breeding are briefly discussed below:



Objectives of the Programme

A mutation breeding programme should have well defined and clear-cut objectives. If the experimenter starts a mutagenesis programme just with the hope that he will discover something useful, he is most likely wasting his time and resources. This is because the ratio of beneficial to useless mutations is very small (1 in 800 mutations, that is, about 0.1 % of the mutations), and identifying desirable mutations from among the undesirable ones is a very difficult task indeed. Further, if a character governed by oligogenes is to be improved, the procedure for the handling of treated populations would be different from that when a polygenic trait is the target for improvement.

Selection of the Variety for Mutagen Treatment

Generally, the variety selected for mutagenesis should be the best variety available in the crop. This is particularly so when polygenic traits are to be improved. It serves no purpose to isolate desirable mutants in a less adapted inferior variety only to discover that the mutant lines have no agricultural worth, or that the mutants have to be used in a hybridization programme for transferring the mutant characteristics to a superior variety. In certain situations; however, it may be desirable to isolate

mutants in varieties other than the best one. For example, an extensive search is being made for alternative dwarfing genes in cereals, particularly in wheat and rice (*O. sativa*). In this situation, dwarf and semi dwarf mutants would have to be isolated from tall varieties, which obviously would not be the best varieties of these crops.

Part of the plant to be treated

Seeds, pollen grains or vegetative propagules (buds and cuttings) or even complete plants may be used for mutagenesis. Which plant part should be used for mutagen treatment depends primarily on whether the crop is sexually or asexually propagated and on the mutagen to be used. In sexually propagated crops, seeds are the most commonly used plant part. Dry dormant seeds are biologically almost inert and they can stand a range of extreme environmental conditions, such as, soaking, desiccation, heating, freezing, oxic or anoxic regimes, etc. Mutagenic treatment of seeds is essentially a treatment of embryo meristems.

Since mutation is a single cell event, the M1 plants will carry an induced mutation only in parts of the shoot, i.e., they will be chimaeras. Pollen grains may be used, but they are infrequently used because (1) it is difficult to collect large quantities of pollen grains in most crop species, (2) hand pollination (with treated pollen) is difficult, and (3) pollen al is relatively short. Pollen grains are the only plant part, which can be successfully treated with UV radiation.

Radiation (except UV) is suitable for use with all the three plant parts and even with the plants. Whole plants are generally irradiated during the flowering stage so that it is equivalent to the irradiation of pollen grains and egg cellsChemical mutagens are best used with seeds, but some workers have used them with vegetative propagules as well.

Dose of the mutagen

The usefulness of a mutagen and the type of treatment required to obtain a high efficiency pendent upon specific properties of the mutagenic agent employed (its effectiveness, effect relationship and mode of application) as well as on specific characteristics of the biological system to be treated (the sensitivity of the treated tissues depending upon anatomical, physiological, biochemical and genetic peculiarities). The most appropriate plant or stage to be treated requires a thorough knowledge of the organisms and a clear definition of experimental objectives.

Mutagen treatments reduce germination, growth rate, vigour and fertility (pollen as well lie). There is considerable killing of plants during the various stages of development after mutagen treatment; thus survival is reduced considerably. Mutagens generally induce a high frequency of chromosomal changes and mitotic and meiotic irregularities. Usually, the damage increases with the mutagen dose, but it may not necessarily be proportional. An optimum dose is the one, which produces the maximum frequency of mutations and causes the minimum killing.

The dose required for high mutagenic efficiency depends on the properties of the mutagenic agent, of the solvent medium and of the biological system. Many workers that a dose close to LDso should be the optimum. LDso is that dose of a mutagen, which would kill 50 per cent of the treated individuals. LDso varies with the crop species and with mutagen used. A preliminary

experiment is generally conducted to determine the suitable mutagen dose. In general, an overdose is likely to kill too many treated individuals, while an underdose would produce too few mutations. Dose of the mutagen may be varied by varying the intensity or the treatment duration. In case of radiation, intensity may be varied by changing the radiation source or by changing the nee from the radiation source of the material being irradiated. Intensity in the case of chemical mutagens may be varied by changing the concentration of mutagens.

Giving Mutagen Treatment

The selected plant part is exposed to the desired mutagen dose. In case of irradiation, the plant parts are immediately planted to raise M1 plants from them (pollen grains are used for pollination). In case of chemical mutagens, seeds are usually presoaked for a few hours to initiate metabolic activities, exposed to the desired mutagen and then washed in running tap water to remove the mutagen present in them. The treated seeds are, usually, immediately planted in the field to raise the M1 generation. M1 is the generation produced directly from the mutagen-treated plant parts without a recourse to sexual or asexual reproduction. But when pollen grains are treated, the generation resulting from the seeds that were produced by pollination with the treated pollen grains would be the M1 generation. M2, M3, M4, etc. are the subsequent generations derived from M1, M2, M3, etc. plants through selfing or clonal propagation.

Handling of the Mutagen-Treated Population

Treatment of seeds and vegetative propagules commonly produces chimaeras. A chimera is an individual with one genotype in some of its parts and another genotype in the others. Shoot-tip meristem usually has three functional layers as follows: (1) L1 gives rise to epidermis, (2) L2 produces a part of leaf mesophyll and gametes, and (3) L3 yields the rest of plant body. When the whole of Ll, L2 or L3 layer is affected, the chimaera is known as periclimal chimaera, while in a sectorial chimaera only a part of Ll, L2 or L3 layer is affected.

In sexually reproducing species, only the L2 chimaera (periclinal or sectorial) will be transmitted to the next generation; other chimaeras will not be recovered since these layers do not contribute to the production of gametes. In clonal crops, however, all chimaeras can be utilized either as periclinal chimaeras or by producing homogeneous individuals through sexual reproduction (only if the L2 layer is affected), tissue culture or certain other horticultural manipulations, e.g., wounding, etc., which induce production of adventitious shoot buds (all chimaeras are utilized). Sectorial chimaeras are unstable in clonal crops and have to be made periclinal through successive clonal propagation and selection for stability.

Mutations usually occur in small sectors of the meristem and, as a result, only a part of the plant is affected. One or more sexual or clonal generations coupled with selection are necessary to obtain a stable mutant phenotype. Mutant alleles are generally recessive, but some dominant mutations may also occur. In case of sexually reproducing crops, mutation breeding utilizes both recessive and dominant mutations and, in addition, excellent opportunities exist for mutation

breeding for polygenic traits. Mutation breeding in clonal crops, however, primarily depends on dominant mutations; recessive mutations may also he utilized provided the clone used for mutagen treatment was heterozygous for the gene in question. For example, if recessive mutant allele a is to be useful in a clonal crop, the clone used for mutagenesis has to have the genotype Aa. Such situations are, however, rare; more frequently, the mutants useful in the improvement of clonal crops contain dominant mutations, and they may even include changes in chromosome structure or even number.

Mutations are called macro or micro-mutations depending on the magnitude of phenotypic effect produced by them. A macromutation produces a large phenotypic effect recognizable on individual plant basis; obviously, such mutations are oligogenic in nature and can be easily selected in the M2 generation. In contrast, a micromutation has a small phenotypic effect that cannot be recognised on individual plant basis; it can be detected only in a group of plants and often statistical treatment of data may be necessary. Obviously, macromutations are polygenic in nature and selection for them is delayed till M3 or a later generation.

Merits of mutation breeding

1. De Novo (New) Creation of Genetic Variability:

When the desired character does not exist, mutations are used to get the new character.

2. Breaking Undesirable Linkage:

When two undesirable characters are linked together, mutagen treatment may be used to break the linkage.

3. Production of Haploids:

Haploids may be produced using X-rayed pollen for pollination; un-fertilised egg develops into haploid which are variously used.

4. Increase or Decrease of Chiasma Frequency:

Mutation may change the chiasma frequency which is directly related with recombination and segregation of characters.

5. Production of Transitory Sexuality in Apomicts:

Apomictic plants breed like asexually propagated plants and breeding is difficult. Transitory sexuality can be induced so the better apomictic type can be selected after crossing with various sexual types.

6. Reduction of Incompatibility in Wide Crosses:

Though actual mechanism is not known but irradiation induces better pollen tube growth in few genera which enables inter-specific crosses with the irradiated pollen.

7. Variation in F1 Hybrids:

F1 hybrids from inter-varietal crosses may be treated with mutagens in order to increase genetic variability by inducing mutation and to facilitate recombination of linked genes.

8. Production of Distant Hybrid with Translocation:

Irradiation of inter-spe-cific (distant) hybrid causes chromosomal segment translocation carrying some desirable genes which may help in transfer of character from one species to another.**Limitations of mutation breeding**

[1] The frequency of desirable mutations is very low, about 0.1 per cent of the total mutations. Therefore, large M2 and subsequent populations have to be grown and carefully studied. This involves considerable time, labour and other resources.

[2] The breeder has to screen large populations to select desirable mutations. Therefore, efficient, quick and inexpensive selection techniques are required to screen large populations. Mutation breeding is more easily applied to such characters where quick screening techniques are available, e.g., disease resistance. But in the case of characters where elaborate tests are required, e.g., quality characteristics, mutation breeding is virtually impractical. For this reason, mutation breeding has been more successful with those characteristics where the mutant phenotype is distinct and easily detectable.

[3] Desirable mutations are commonly associated with undesirable side effects due to other mutations, chromosomal aberrations, etc. The mutant lines often have to be backcrossed to the respective parent varieties to remove these defects. This increases the time requirement of mutation breeding programmes and involves additional labour, time and expenditure.

[4] Often mutations produce pleiotropic effects. The chief procedure for reducing or eliminating pleiotropic effects is to transfer the gene into different genetic backgrounds by hybridizing the mutant with a randomly selected range of elite varieties. Alternatively, when the pleiotropic {effect is on a specific trait, e.g., delayed flowering, appropriate genes for correction of the defect, e.g., genes for early flowering, can be introgressed into the mutant strain.

[5] Mutations in quantitative traits are usually in the direction away from the selection history of the parent variety; this conclusion was reached by Brock in 1965 and is generally regarded as valid. This may tend to limit the degree of improvement attainable in a quantitative trait that has been the object of selection for a long period of time, e.g., yield.

[6] There may be problems in the registration of a mutant variety since it may be difficult to convincingly demonstrate the new variety to be distinct from the parent variety; the PBR laws, where they exist, require a new variety to be distinct, uniform and stable (the DOS requirement). Such a variety may also attract a royalty liability in case the PBR title of the parent variety was held by another breeder/organization.

[7] Most of the mutations are recessive; detection of recessive mutations is almost impossible in clonal crops and is difficult in polyploidy species. Consequently, in polyploidy species, larger population have to be grown and larger doses of mutagens have to be applied. Mutagenesis has been most commonly applied to diploid species that reproduce sexually, more particularly to self-pollinated species.

Achievements

The year 1969 is widely regarded as the year of transition from mainly fundamental investigations to practical mutation breeding; upto this time, only 77 varieties had been developed through mutagenesis. In 1983, this number rose to 337, e number would be well over 2,000 by now. Of the 1542 varieties (up to 1990), 1019 varieties were in seed propagated crops (609 direct releases of mutants, and 410 varieties were derived from crosses involving mutants as, at least, one of the parents). the varities (1029) were due to direct release of mutants and only a small proportion (Ca. 23%) were obtained by using mutants in hybridization programmes. Point mutation as well as chromosomal mutation both has been used to produce many improved varieties which represent the improvement in oligogenic as well as poly-genic characteristics.

'Sonora 64', a good variety of wheat had red colour grain, ϒ-radiation produced 'Sharbati Sonora' with white colour grain. Chromosome segments of Aegilops, Secale, Agropyron carrying rust resistance genes have been trans-located to wheat by using X-rays.

High yielding groundnut variety NC4 X modified into NC4 by micro mutation which looks like the parent.

Point mutations have produced superior sugarcane varieties TS-1 and TS-2 which are the mutants of CO 419. In sugarcane, CO 8152 is a ϒ-ray induced mutant from CO- 527 which gives 40% higher yield, but CO 8152 has two chromosomes less than CO-527.

Duplication in a segment of chromosome 6 of barley carrying gene 'orange lemma' associated with a-amylase activity improves the matting quality of barley.

'Jagannath' rice variety is a ϒ-ray induced semi-dwarf mutant from tall cultivar T- 141, which has the improved resistance to lodging, high yield, responsive to fertiliser.

JRO-3690, a high yielding jute variety has been developed by hybridisation between two low yield mutants of jute.

Polyploidy breeding:

Aneuploidy

Aneuploidy is defined as the loss or gain of one or few chromosomes compared to its somatic chromosome number of a species.

a) **Additions to chromosome number:**

i. **Trisomy [2n + 1]:** When somatic cells of an organism contain an extra chromosome (2n+1). The number of possible trisomies in an organism is equal to the haploid chromosome number.

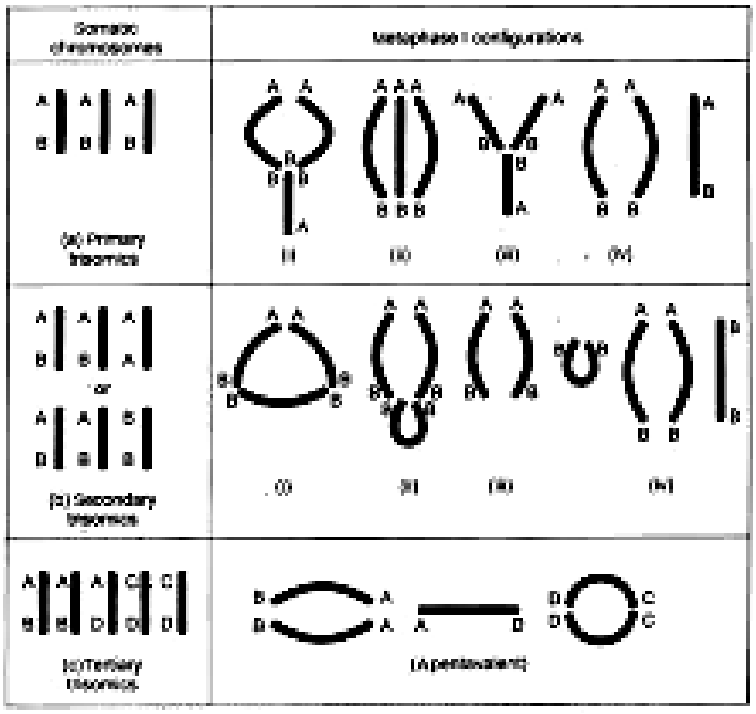
Trisomics are of different types —

primary trisomics where extra chromosome is identical to two homologues.

secondary trisomics where the extra chromosome is an isochromosome with two genetically identical arms.

tertiary trisomies are the products of translocation. Double trisomies (2n + 1 + 1) are also available in nature.

The origin of the trisomics may be from the production of n + 1 types of gametes due to rare non-disjunction of a bivalent in a diploid or may also be produced by triploids through irregular meiosis.



In primary and secondary trisomics, the three homologues of the concerned chromosome may pair with each other to form a trivalent of different configurations at MI, or two of the chromosomes may form a bivalent leaving the additional chromosome as a univalent. In tertiary trisomic, some PMCs would show a dumbbell shaped figure containing five chromosomes at MI.

Gain of a complete chromosome causes a major imbalance, but its effects are not as deleterious as those due to the loss of a full chromosome. Therefore, trisomics are viable in diploid species, e.g. maize, tomato, pea, barley etc.

i. **Tetrasomy [2n + 2]:** When somatic cells contain one pair of a chromosome in excess of the normal somatic compliment, i.e.,, chromosome compliment is present in four copies. Tetrasomics may be originated by selfing of trisomics. During meiosis, the four homologues of the tetrasomic set tend to form a quadrivalent.

The four homologues of the concerned chromosome may form one quardivalent, a trivalent and a univalent, or two bivalents at MI. Quadrivalents usually disjoin 2:2 at AI, but 3:1 disjunction may also take place.

b) **Reductions in chromosome number:**

(i) **Monosomy [2n − 1]:** When one chromosome is missing from the somatic complement. Monosomics of diploid species is not tolerated due to genetic imbalance but are fully viable in polyploidy species e.g., wheat, cotton, oats and tobacco. Double monosomics (2n - 1 - 1) as well as triple monosomics (2n - 1 – 1 - 1) may be obtained in polyploidy species.

The one chromosome remains unpaired during meiosis The remaining chromosomes pair normally and produce of n – 1 types of gametes due to rare nondisjunction of a bivalent. In progeny of a monosomic, a mixture of disomic (2n), monosomies (2n – 1) and nuilisomics (2n – 2) is obtained.

(ii) **Nullisomy [2n − 2]:** When one pair of chromosomes is missing from the somatic complement. Nullisomic has a strong deleterious effect on the organism, only viable in the highly polyploidy species e.g. hexaploid or bread wheat (*Triticum aestivum*) and oats (*Avena sativa*).

Cytologically, chromosome pairing is normal and at MI n-1 bivalents can be seen. These bivalents disjoin normally to produce all normal n-1 gametes.

Importance of Aneuploidy in Plants:

Aneuploids have played a role in evolution and have importance in plant breeding in addi-tion to genetic analysis.

(a) Detecting linkage group:

The aneu-ploids have played an important role in locating a linkage group and a gene in a particular chromosome. Particularly nullisomics, mono-somies and trisomies have been used to deter-mine linkage groups in tobacco, wheat, etc.

The study of aneuploidy has shown homoeology between A, B and D genomes of wheat. Identification of the chromosome involved in translocation has also been done with the help of aneuploids.

(b) Chromosome substitution in plant breeding:

The major contribution of aneuploids has been in the field of plant breeding. The substitu-tion of whole chromosome or part of the chro-mosome using aneuploids has been done. These substitutions resulted in significant modification of yield, resistance, lodging, etc.

(c) Speciation:

Aneuploidy can generate variation and source of speciation in vegetatively propagating species. In *Crepis,* aneuploid variations form a series X = 3, 4, 5, 6 and 7 among species. A very extensive aneuploid series has been observed in *Carex* (n = 6 to 56).

Limitations of Aneuploid Analyses

It is necessary to produce and maintain fl complete set of aneuploids. Production, identification and maintenance of aneuploids require elaborate cytogenetic analysis, which is difficult, time consuming and requires considerable skill.

Maintenance of aneuploids is complicated by the phenomenon of univalent shift. Univalent shift denotes that some of the progeny of an aneuploid plant would become aneuploid for a different chromosome as compared to the parent plant. Univalent shift generally occurs in monosomic .lines and is a result of univalent formation in a chromosome other than that for which they are monosomic. Therefore, cytological analysis and testing must form an integral part of the aneuploid programmes.

During aneuploid analysis and chromosome substitution, cytological analysis must be carried out for accuracy. This involves a considerable cytological work and makes aneuploid analysis a time consuming and tedious task.

Monoploids and haploids

Monoploids and haploids are weaker than diploids and are of little agricultural value directly. But they are of great interest because they offer certain unique opportunities in crop improvement. (1) They are used for developing homozygous diploid lines, following chromosome doubling in two years. This greatly reduces the time and labor required for the isolation of inbreds and purelines. (2) They may be useful in the isolation of mutants because the mutant allele (even if it is recessive) expresses itself in M, due to a single dose of the gene in somatic tissues. Chromosome number of mutants may be doubled to produce homozygous mutant lines in a single generation. (3) Since desirable gametes are more frequent (=p, that is, the frequency of desirable allele in the population) than desirable zygotes (=p2), selection based on haploids or doubled haploids may be expected to be more efficient than that based on diploid (zygote-derived) plants. There is some evidence that this may be so. And (4) in autotetraploids like potato, breeding is relatively much easier at the haploid (2x) level than at the tetraploid level (4x). For comparison, consider segregation in an autotetraploid and in a diploid. There is an increasing tendency to breed potato varieties at the haploid level and then double their chromosome number to obtain tetraploid varieties. In monoploids and most haploids, the chromosomes do not pair and their distribution at anaphase I is random leading to an almost complete sterility. Some functional gametes with n chromosomes may be produced, which may give rise to 2n progeny. Monoploids and haploids occur spontaneously in low frequencies, may be induced from pollen grains or haploid cells of unfertilized ovaries through callus formation or embryo production and by chromosome elimination in certain interspecific crosses, e.g., Hordeum bulbosum X H. vulgare . In the first method, the recovery of haploids is generally very low (1 in 1,000 plants or lower). But the latter two methods produce a relatively high frequency of haploids in case of those species for which appropriate techniques are available. It may be pointed out that the latter two methods are not applicable to many crop species.

Polyploids (three or more genomes):

The organisms having more than two sets of chromosomes by the addition of another chromosome is called polyploidy. It may arise as a result of abnormal mitosis where chromosomes divide by cytoplasm fails to divide during cytokinesis. The basic set of chromosomes undergoes multiplications. For example, in *Chrysanthemum* basic set is x = 9. Its species and hybrids show multiple of 9, such as 18, 27, 36, 45. In *Nicotiana* and *Solanum* basic set is x = 12 and multiple of somatic chromosome numbers are 24, 48 and 72 and in *Triticum* it is x = 7 and multiples are 14, 21, 42.

Origin of Polyploidy:

i) Polyploids are originated by failure of normal mitotic division in somatic cells.

ii) Polyploids may originate due to abnormal reduction divisions resulting

iii) It may naturally occur due to fertilization of egg by more than one male gamete.

iv) It may originate by artificial induction using colchicines or may be originated by cross hybridization between haploid and diploids.

Types of Polyploidy:

There are mainly four different types of Polyploidy, namely:

i) Auto-Polyploidy,

ii) Allopolyploids,

iii) Segmental allopolyploids and

iv) Auto-allopolyploids.

(i) Auto-Polyploidy:

When more than two genomes developed by the multiplication of chromosome number of some individual is called Autopolyploids. On the other hand autopolyploids are the individuals of which body cells contains more than two identical set of chromosomes derived by self-duplication. It arises due to failure of anaphase.

Examples:

Autotriploids (3n) – *Oenothera, Datura, Dahlia,* Rose etc. autotriploids are more vigorous, shows more perenation of the organs of vegetative propagation and are highly sterile.

Autotetraploids: (4n) – it contain four identical genomes and arise by fusion of two diploid gametes. It may results from duplication of somatic chromosomes. Autotetraploids show great adaptability, disease resistant, larger seeds, high vitamin-C content and low fertility. These are found in Apples, Grapes, Marigolds.

Origin and Production of Doubled Chromosome Numbers

Cells/individuals having doubled chromosome numbers may originate in one of the following several ways: (1) spontaneous, (2) due to treatment with physical agents, (3) regeneration in vitro, (4) colchicine treatment, and (5) other chemical agents.

Spontaneous

Chromosome doubling occurs occasionally in somatic tissues and unreduced gametes are also produced in low frequencies. Production of unreduced gametes is promoted by certain genes, e.g., genes causing complete asynapsis or desynapsis and, more particularly, Such mutant genes as those producing parallel spindle (ps) and omission of second division (os) in potato.

Production of Adventitious Buds

Decapitation in some plants leads to callus development at the cut end of stem. Such a callus has some polyploid cells, and some of the Shoot buds regenerated from the callus may be polyploid. This is of common occurrence in Solanaceae where 6-36 per cent of adventitious shoot buds are reported to be tetraploid. The frequency of polyploidy buds may be increased by the application of 1% IAA at the cut ends as it promotes callus development.

Physical Agents

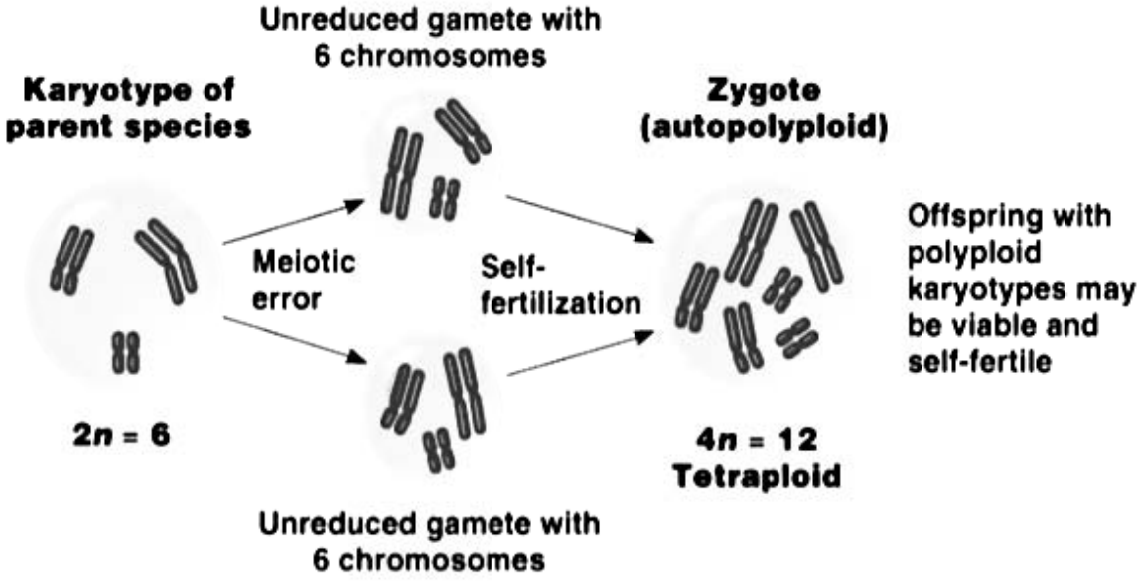
Heat or cold treatments, centrifugation and X-ray or gamma ray irradiation may produce polyploids in low frequencies. Tetraploid branches were produced in Datura in response to cold treatment. Exposure of maize plants or ears to a temperature of 38-45°C at the time of the first division of zygote produces 2-5 per cent, tetraploid progeny. Heat treatment has been successfully used in barley, wheat, rye and some other crop species.

Regeneration *in Vitro*

Polyploidy is a common feature of the cells cultured in vitro. Some of the plants regenerated from callus and suspension cultures may be polyploids. Plants of various ploidy have been regenerated from callus cultures of Nicotiana, Datura, rice (*O. sativa*) and several other species.

Colchicine Treatment

Colchicine treatment is the most effective and the most widely used treatment for chromosome doubling. It has been used with great success in a large number of crop species belonging to both dicot and monocot groups. Colchicine is a poisonous chemical isolated from seeds (0.2-0.8%) and bulbs (0.1-0.5%) of autumn crocus (*Colchicum autumnale*). It is readily soluble in alcohol, chloroform or cold water, but is relatively less soluble in hot water. Pure colchicine is $C_{22}H_{25}0_6N$. It blocks spindle formation and thus inhibits the movement of sister chromatids to the opposite poles. The resulting restitution nucleus includes all the chromatids; as a result, the chromosome number of the cell is doubled. Since colchicine affects only dividing cells, it should be applied to a shoot-tip meristem only when its cells are actively dividing. At any given time, only a small proportion of the cells would be in division; therefore, repeated treatments should be given at brief intervals to double the chromosome number in a large number of cells of the shoot apex. The polyploid and diploid cells present in a shoot-tip compete with each other and diploid cells may often out compete the polyploid ones. The degree of competition varies from species to species and even among varieties within species.



Meiosis in an Autopolyploid:

Meiotic behaviour in an autopolyploid such as autotetraploid is different than in a diploid. This is due to the presence of four homologous chromosomes of each kind.

Assuming that the primary material is a diploid species with 14 chromosomes (AA), these will form seven pairs (bivalents) at meiosis . In the tetraploid (AAAA) there will be four chromosomes of each type, and at meiosis, these seven groups of four chromosomes may form seven quadrivalents.

A quadrivalent is an association of four homologous chromosomes. Quadrivalents may be of different appearances. Sometimes, the homologous chro-mosomes are represented by an association of three chromosomes, called a trivalent and a univalent or by two bivalents.

As a rule, the average number of quadrivalents per cell is, therefore, lower than the medium possible num-ber. Autotetraploids of different species behave differently in this respect. Some of them have a very high frequency of quadrivalents as in *A. tuberosum*, in some cases bivalents are formed.

The occurrence of trivaients and univalents at meiosis in an autotetraploid leads to distur-bances in chromosome distribution and to the formation of gametes with deviating chromo-some numbers. This is the principal cause for the high degree of sterility in an autotetraploid.

Segregation of Genes in Autopolyploids:

The number of alleles of each gene is represented according to the ploidy level of the Polyploidy individual and gametes containing more than one allele of each gene (homo- or heterozygotic) may be produced.

According to the number of dominant and recessive alleles at a particular locus, the genotype of an autotetraploid may be quadriplex (AAAA or A_4), triplex (AAAa or A_3a), duplex (AAaa or A_2a_2), monoplex or simplex (Aaaa or Aa_3) and nulliplex (aaaa or a_4).

Auto-Polyploidy such as tetraploids show the so called tetrasomic inheritance. The segregation of genes in auto-Polyploidy is affected by factors which play no essential role in diploid.

Among such factors are the number and position of chiasmata in the multivalents, the distance between particular locus and centromere, the behaviour of homologues in multivalent associations during anaphase I and the presence of univalents.

In auto-tetraploids, if it is assumed that the four homologous chromosomes are distributed to poles in 2:2 during anaphase I, theoretical seg-regation ratios for various autotetraploid genotypes of a locus may be calculated.

Table 11.3: Frequencies of the gamete types and zygote types of autotetraploid genotypes

Parent	Gametes				Zygotes					
Genotype	AA	Aa	aa	divisor	A^4	A^3a	A^2a^2	Aa^3	a^4	divisor
Quadriplex (AAAA)	1	–	–	1	1	–	–	–	–	1
Triplex (AAAa)	1	1	–	2	1	2	1	–	–	4
Duplex (AAaa)	1		1	6	1	8	18	8	1	36
Monoplex (Aaaa)	–	1	1	2	–	–	1	2	1	4
Nulliplex (aaaa)	–	–	1	1	–	–	–	–	1	1

Applications of Autopolyploidy in Crop Improvement

Autopolyploidy has found some valuable applications in crop improvement. These are briefly summarized below:

Triploids –

Triploids are produced by hybridization between tetraploid and diploid strains. They are generally highly sterile, except in a few cases. This feature is useful in the production of seedless watermelons. In certain species, they may be more vigorous than the normal diploids, e.g., in sugarbeets. These two examples are described in some detail.

Seedless watermelons are grown commercially in Japan. They are produced by crossing tetraploid (4x, used as female) and diploid (2x, used as male) lines, since the reciprocal cross (2x x 4x) is not successful. The triploid plants do not produce true seeds; almost all the seeds are small, white

rudimentary structures like cucumber (*Cucumis sativus*) seeds. But a few normal sized seeds may occur, which are generally empty. For good fruit setting, pollination is essential. For this purpose, diploid lines are planted in the ratio 1 diploid: 5 triploid plants. There are several, problems, viz., genetic instability of 4x lines, irregular fruit shape, a tendency towards hollowness of fruits, production of empty seeds and the labour involved in triploid seed production.

Triploid sugarbeet produce larger roots and more sugar per unit area than do diploids, while tetraploids produce smaller roots and lower yields than diploids. Apparently, 3x is the optimum level of ploidy in sugarbeets. Triploid sugarbeet varieties have been grown commercially in Europe and Japan, but their popularity is declining. rapidly. The triploid varieties are mixtures of triploid, diploid and other ploidy level plants. Seed production of triploid sugarbeet is difficult because the beet flower is small. Triploid seed may by produced in one of the following two ways: (1) using 4x plants as female and 2x as male or (2) using 4x as male and 2x as female.

Tetraploids –

Autotetraploids have been produced in a large number of crop species and have been extensively studied in several cases. Tetraploids may be useful in one of the following ways: (1) in breeding, (2) improving quality, (3) overcoming self incompatibility, (4) making distant crosses and (5) used directly as varieties.

In banana (*M. sapientum*), autotetraploids are inferior to triploids in that they have weaker leaves and increased fertility. But they offer the only available chance of adding disease resistance to commercially successful varieties. In banana, autoteraploids are produced by chance fertilization of an unreduced triploid egg (AAA) by a haploid pollen from a disease resistant diploid parent. A large number of such tetraploids have been produced, but they have not yet gained any commercial success. This is an unusual case where auto tetraploidy is the only practical approach to breeding an otherwise successful triploid crop species.

Autotetraploidy is able to overcome self-incompatibility in certain cases, e.g., some genotypes of tobacco and white clover (*Trifolium repens*), Petunia, etc. Certain distant crosses are not successful at the diploid level, but are relatively successful at the autotetraploid level, e.g., 4x Brassica oleracea x B. chinensis is successful, but when B. oleracea is diploid it is unsuccessful. Similarly, autotetraploids of certain Solanum species produce hybrids with *S. tuberosum*, while the diploids do not.

Autotetraploids are larger in size and are more vigorous than diploids. Autotetraploid varieties of forage crops have been considerably successful. The most successful examples are, tetraploid red clover (*Trifolium pratense*) and ryegrass (*Lolium perenne*). Other examples are tetraploids of alsika clover (Trifolium hybridum, Variety Tetra) and berseem (*Trifolium alexandrium*, variety Pusa Giant Berseem). Autotetraploid red clover and ryegrass are more vigorous, more digestible and palatable, and have greater resistance to nematodes as compared to the diploids. Autotetraploid turnips (*B. rapa*) and cabbage (*B. oleracea*) are larger in size, but they also have more water content than the diploids; thus they are not commercially attractive. Many ornamentals are autotetraploids. In cases of ornamentals, increased flower size, and longer flowering duration of the tetraploids are desirable. Pusa Giant Berseem is the first autopolyploid variety released for

general cultivation in India. In case of crops where seed is the commercial product, autotetraploidy has been much less successful. The chief difficulty is the high sterility and genetic instability of autotetraploids. Fertility can be improved through breeding and selection, but the progress is slow. Autotetraploids have been explored in several crop species but the most successful case that of rye (*S. cereale*) where tetraploid varieties have been released for cultivation (e.g., Double Steel, Tetra Petkus). Other extensive programmes on autotetraploidy are on crops like barley (H. vulgare) and jowar (S. *bicolor*) where larger grains, increased protein content and larger yields are the objective. After many years of extensive breeding, some success in achieving these goals has been realized. Based on the experience so far, the following generalizations may be made about autopolyploidy.

- Autopolyploidy is more likely to succeed in species with lower chromosome numbers than in those with higher chromosome numbers.
- Cross-pollinating species are generally more responsive than self pollinating species.
- Crops grown for vegetative parts are more likely to succeed as polyploids than those grown for seeds.

Limitations of Autopolyploidy

The larger size of autopolyploids is generally accompanied with a higher water content. As a result, autopolyploids of the crop species grown for vegetative parts do not always produce more dry matter than the respective diploids. For example, tetraploid turnip (*B. rapa*) and cabbage (*B. oleracea*) out yield the diploids in fresh weight, but are comparable, or even inferior, to them in terms of dry matter production.

In crop species grown for seed, autopolyploids show high sterility accompanied with poor seed set. Consequently, the larger seed size of autotetraploids does not generally lead to an increased seed yield per unit area. Fertility in autotetraploids can be increased by hybridization and selection at thetetraploid level. But due to the complex segregation in autotetraploids, progress under selection is slow. It would take many years to raise the fertility to acceptable levels.

Triploids cannot be maintained except through clonal propagation. The progeny of triploids and tetraploids are variable in chromosome number since they produce aneuploid gametes as well. Triploids have to be regularly produced by crossing 4n x 2n plants. Maintenance of tetraploids is somewhat less difficult. Thus genetic instability of autotriploids and autotetraploids makes their maintenance difficult, and commercial seed production presents many problems.

The hope that polyploidy would help to create new agricultural types at will was entirely misplaced. New polyploids (raw polyploids) are always characterized by a few or more undesirable features, e.g., poor strength of stem in grapes, irregular fruit size in watermelons, etc. Thus new polyploids can rarely be used directly in crop production. A considerable improvement through hybridization and selection is essential to remove these defects.

Allopolyploids:

Polyploidy may also result from doubling of chromosome number in hybrid which is derived from two or more distinctly different species. This brings two (or more) different sets of chromosome in hybrid. The doubling of chromo-somes in the hybrid, which gives rise to a Polyploidy, is called an allopolyploid.

An allopolyploid in which a sterile hybrid (AB) originating out of the combination of two different species, undergoes duplication of chromosome set, is known as **amphidiploid** (AABB).

Raphanobrassica is a classical example of amphidiploidy. In 1927, Karpechenko, a Russian scientist, reported a cross between *Raphanus sativus* (2n = 18) and *Brassica oleracea* (2n = 18) to produce F2 hybrids which were completely sterile.

This sterility was due to lack of chromosome pairing, since there is no homology between genomes from *Raphanus sativus* and *Brassica oleracea*. Among these ste-rile hybrids certain fertile plants were found. On cytological examination, these fertile plants were found to have 2n = 36 chromosomes, which showed normal pairing into 18 bivalents.

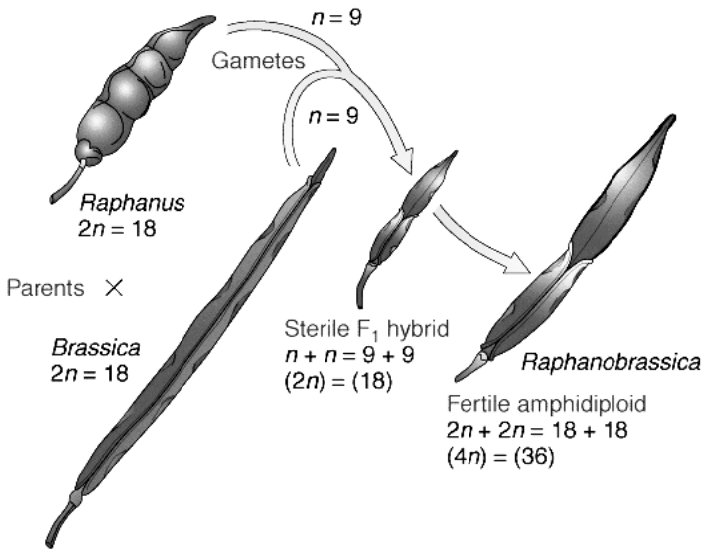


Fig. *Raphanobrassica*. Source: http://theelusiveguanaco.blogspot.com/2011/06/joy-of-chromosomes-part-2_16.html

Thus in allopolyploids the paring is of autosyndesis type (paternal-paternal or maternal- maternal pairing) in contrast to allosyndesis (paternal-maternal pairing) in diploids and autopolyploid.

Of the allopolyploids, amphidiploid hybrids containing two sets of each species are of special importance because they are usually fertile, occur rather widely among angiosperms in nature, afford clues to the relationship of certain species, and open a new path to the improvement of cultivated plants.

Triticale is the most successful synthetic allopolyploid produced by crossing wheat (tetraploid or hexaploid) with rye. *Triticales* derived from tetraploid wheats have been the most successful, but those from hexaploid wheats may also become a successful crop species. The breeding strategy involves (l) production of a large number of triticale strains using different combinations (varieties as well as species) of wheat and, rye, (2) hybridization of these triticale strains among themselves, and (3) improvement of the defects of the triticale through selection.

One of the earliest known amphidiploid hybrids was the fertile *Primula kewensis*, with 36 somatic chromosomes. A cross between *P. floribunda* (2n = 18) and *P. verticillata* (2n = 18) had yielded the sterile diploid *P. kewensis* (2n = 18) with one genome from each parent species.

Amphidiploids sometimes arise in ways other than by somatic chromosome doubling. Diploid spores, and, therefore, diploid gametes may appear on failure of meiosis and union of two diploid gametes gives rise to tetraploid. Although the chance of obtaining such plants in this manner seems to be relatively small.

(iii) Segmental Allopolyploids:

In some allopolyploids the different genomes that are present are not quite different from one another, i.e.,, having partial homology with each other (616,8282). Consequently, in these Polyploidy, chromosomes from different genomes do pair together to some extent and multivalents are formed. This means that segments of chromosomes and not the whole chromosome is homologous.

Such allopolyploids are called segmental allopolyploids (Stebbins). These chromosomes which are partially homologous and not completely homologous with each other are sometimes also described as homologous chromosomes. It is also believed that most of the naturally occurring Polyploidy are neither true auto-Polyploidy nor true allopolyploids.

Solanum tuberosum is the best example of segmental allopolyploid.

(iv) Auto-Allopolyploids:

When autopolyploidy is combined with allopolyploidy, autoallopolyploids are produced (AAAA6B). Polyploidy of this type are possible from hexaploid level upward as observed in *Nicotiana tabacum* and *Solanum nigrum.* Autoallopolyploids have importance in the evolution of certain plant species.

Applications of Allopolyploidy in Crop Improvement

Allopolyploidy has three major applications in crop improvement: (1) as bridging species in the transfer of character from one species into another. (2) In the production of new crop species, and (3) for widening the genetic base of existing allopolyploid crop species.

Utilization as a Bridging Species

Amphidiploids serve as a bridge in the transfer of characters from one species to a related species, generally from wild species to cultivated species. The use of an amphidiploid as a bridging species becomes necessary when the hybrid between the cultivated species (recipient species) and the wild species (donor species) is sterile. The sterility of F1 hybrid makes it impossible to cross the recipient species with the F1 and this does not permit the transfer of characters from the donor to the recipient species. In such cases, chromosome number of the F1 interspecific hybrid is doubled to produce an amphidiploid, which is a novel species; it will be generally reasonably fertile and can be crossed to the recipient species. Progeny from the cross between the recipient species and the amphidiploid would have the somatic (2n) chromosome complement of the recipient species and one genome from the donor species. As a result, they would be sufficiently fertile to be used in backcross with the recipient species. From such a programme,

alien addition and alien substitution lines are recovered, which are used in the transfer of genes, groups of genes or of small chromosome segments to the recipient species. An example of the use of an amphidiploid as a bridging species is the use of synthetic *N. digluta* (allohexaploid) for the transfer of resistance to tobacco mosaic virus from *N. sylvestris* to *N. tabacum*. The F1 obtained from the cross *N. tabacum* x *N. sylvestris* is sterile. Chromosome doubling of the F J hybrid produces the synthetic allohexaploid *N. digluta*, which is reasonably fertile. *N. digluta* is backcrossed to the recipient species (*N. tabacum*) to produce a pentaploid having the complete somatic (2n) chromosome complement of *N. tabacum* and one genome of *N. sylvestris*. The pentaploid is sufficiently fertile to be backcrossed to N. tabacum. In the progeny, *N. tabacum* like plants resistant to tobacco mosaic are ,:r\lreselected and cytologically analysed. From among the backcross progeny;, both alien <llddition and alien substitution lines can be recovered. Other examples of the use of an amphidiploid as a bridging species are in the cases of transfer of genes from G. thurberi to G. hirsutum and of chromosomes from Haynaldia villosa to *T. aestivum*.

Role of Polyploidy in Plant Breeding:

When the techniques for artificial chromosome doubling became established, investigations on the origin of many of our economic plants were resumed. Many important crop plants like wheat, oat, sugarcane, cotton, tobacco as well as many fruits and vegetables are the Polyploidy of various degrees.

One of the important effects of polyploidy is the changes in the blooming season of the induced Polyploidy. As such, interspecific hybrids can be obtained of such species which otherwise remain isolated by seasonal isolation and different blooming season.

By artificial polyploidy induction, disease resistance and other desirable characters have been incorporated into some commercial crop plants. For example, *Nicotiana tabacum* is susceptible to TMV whereas *N. glutinosa* appears to be resistant.

The two tobacco species when crossed, the hybrids were found to be resistant but totally sterile. When the chromosomes were doubled it was possible to secure a fertile Polyploidy resistant to the virus. Many Polyploidy are selected and culti-vated because of their larger size, vigour and ornamental values. Several varieties of apples, pears and grapes have produced giant fruits which are of much economic value.

Role of Polyploidy in Evolution:

Polyploidy combined with interspecific hybridization provides a mechanism by which new species may arise in nature and play a role in evolution. Allopolyploidy can produce new species by combining new characters and stable in evolution. It has already been discussed under amphidiploidy how different types of new species may be evolved.

Among the inter-specific hybridization, the most important are *Primula kewensis* (n = 18) obtained by crossing *P. floribunda* (n = 9) and *P. verticillata* (n = 9), *Digitalis mertqnensis* (n = 56) obtained by crossing *D. pur*purea (n = 28) and *D. ambigua* (n = 28) and *Spartina townsendii* (n = 63) obtained from cross of *S. stricta* (n = 28) and *S. alterniflora* (n = 35).

Origin of some of the economically important plants like rice, wheat, cotton, tobacco is important in this aspect. The chromosome number of rice (*Oryza sativa*) is 2n = 24. It is an example of typical secondary allopolyploids with basic chromosome number x = 5. The present cultivated variety of rice is actually produced by hybridization followed by aneuploidy and euploidy. The origin of wheat, cotton, Mustard, etc. are given below:

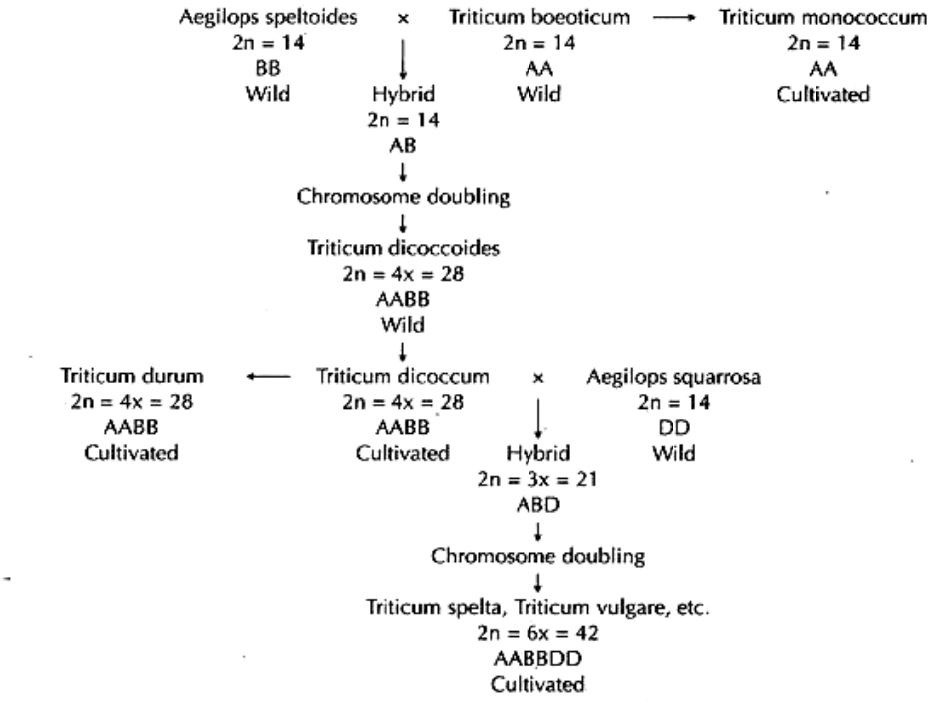


Fig. 11.12: Diagrammatic representation of the origin of tetraploid and hexaploid cultivated wheat from their wild ancestors

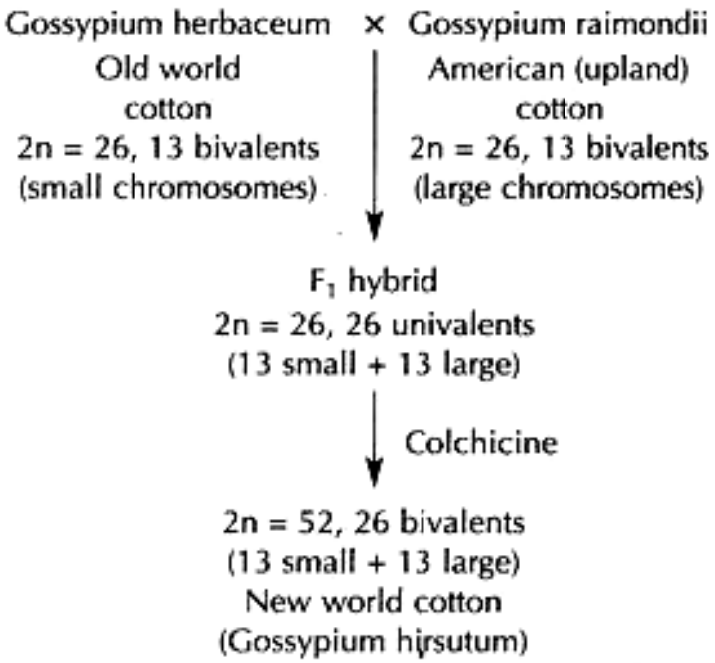


Fig. 11.13: Origin of new world cotton

Source: http://www.biologydiscussion.com/cell/notes-on-polyploidy-cell/38312

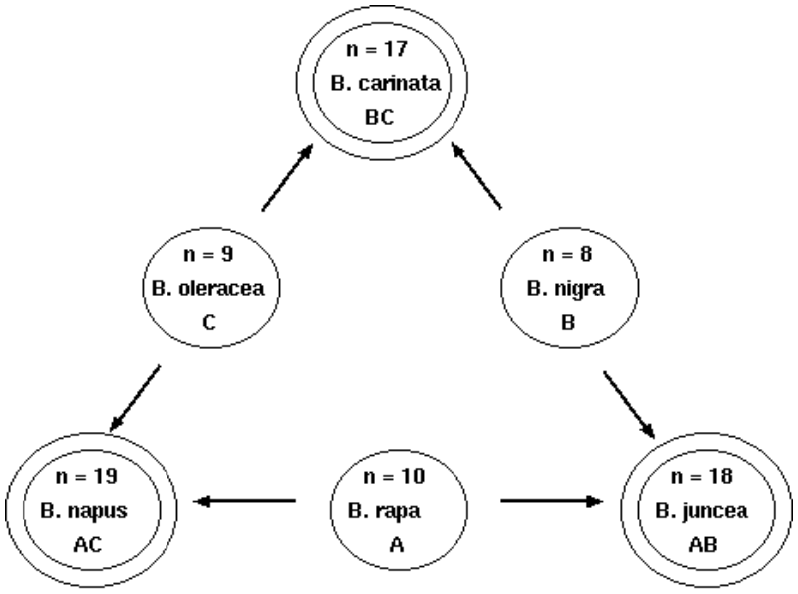


Fig. Origin of *Brassica* (triangle)

Source: http://www.biologydiscussion.com/genetics/cytoplasmic-inheritance

Media of Conservation of Characters:

Polyploidy plays an important role in conserving the characters. A recessive mutation in order to be expressed in an autotetraploid, all four genes must be in recessive condition which is a time requiring process. Thus the characters in a Polyploidy plant could be conserved.

Polyploidy and Geographical Distribution:

The Polyploidy plants can cope with diverse geographical areas than a diploid. Hence, the geographical distributions of Polyploidy plants are greater than diploids. Auto-polyploidy cannot produce new species, but they can colonize a new environment easily. As allopolyploids contain different genomes, they can withstand different environmental condition.

Both these power of colonization and coping with a diverse environment of the Polyploidy plants, help their wide geographical distribution.

Widening the genetic base of existing allopolyploids

The genetic base of some natural allopolyploids may be narrow, and it may be useful to introduce variability in such cases by producing the allopolyploids afresh from their parental species. B. napus is a case in point; the genetic variability of this species is narrow and the only recourse available is to synthesize new allopolyploid *B. napus* to widen its genetic base. This is being done by crossing *B. campestris* (n = 10, AA) with *B. oleracea* (n = 9, CC), the parental diploid species, to produce the amphidiploid *B. napus* (n = 19, AACC). The two species, *B. campestris* and *B. oleracea*, have to be crossed as autotetraploids the cross is very difficult and embryo culture has to be used; somatic hybridization is being used to get around these problems.

Limitations of Allopolyploidy

Limitation of Polyploidy:

Polyploidy has several limitations. Some important limitations of polyploidy in crop improvement are briefly presented below:

1. Limited use: The single species polyploidy has limited applications. It is generally useful in those crop species which propagate asexually like banana, potato, sugarcane, grapes etc.

2. Difficulty in maintenance: The maintenance of monoploids and triploids is not possible in case of sexually propagating crop species.

3. Undesirable characters: In bispecies or multispecies polyploids characters are contributed by each of the parental species. These characters may be sometimes undesirable as in case of *Raphanobrassica*.

4. Some other defects: Induced polyploids have several defects such as low fertility, genetic instability, low growth rate, late maturity, etc.

5. Chances of developing new species through allopolyploidy are extremely low.

5. Cytogenetics & breeding practices of crop plants: rice, maize, potato

RICE (2n=2x=24):

***ORYZA SATIVA* L.:**

Asian rice was first domesticated from the wild rice *Oryza rufipogon* in China between 8,200 and 13,500 years ago, then spread to South and Southeast Asia. Today it is cultivated on every continent save Antarctica. Worldwide there are more than 40,000 different varieties of *Oryza sativa*, classified into four major categories: *indica, japonica, aromatic and glutinous.*

Oryza sativa contains two major subspecies: the sticky, short grained *japonica* or *sinica*, and the non-sticky, long-grained *indica*. *Japonica* varieties are usually cultivated in dry fields, in temperate East Asia, upland areas of Southeast Asia and high elevations in South Asia, while indica varieties are mainly lowland rices, grown mostly submerged, throughout tropical Asia. Recent genetic evidence show that all forms of Asian rice, both indica and japonica, come from a single domestication event that occurred 8,200–13,500 years ago in the Pearl River valley region of China.Types:

1) India Type:

It is tropical type rice. It is extensively grown in India, Srilankha, Thailand, Malaysia and adjacent countries. These are tall plants, weak stem, photoperiod sensitivity, easy shattering, and broad droopy leaves and having grain dormancy.

2) *Japonica* Type:

These are temperate type extensively grown Yangtze river valley of china, Korea, and Japan, these are tolerant low temperature, have more leaves, few tillers, relatively resistant to shattering. The grains are short, broad low in amylase content. Rice becomes sticky on cooking. *Japonica* is a group of rice varieties from northern and eastern China grown extensively in some areas of the world. It is found in the cooler zones of the subtropics and in the temperate zones.

It is a relatively short plant with narrow, dark green leaves and medium-height tillers. *Japonica* grains are short and round, do not shatter easily and have low amylose content, making them moist and sticky when cooked.

1.1 *Javanica* or tropical *Japonica*

Once thought to be a third subspecies, javanica is now known as tropical *japonica*.

Examples of this variety include the medium grain 'Tinawon' and 'Unoy' cultivars, which are grown in the high-elevation rice terraces of the Cordillera Mountains of northern Luzon, Philippines.

Javanica plants are tall with broad, stiff, light green leaves. The grains are long, broad, and thick, do not shatter easily, and have low amylose content.

1.2. *Indica*

Indica rice is the major type of rice grown in the tropics and subtropics, including the Philippines, India, Pakistan, Java, Sri Lanka, Indonesia, central and southern China, and in some African countries.

Indica plants are tall with broad to narrow, light green leaves. The grains are long to short, slender, somewhat flat, tend to shatter more easily and have high amylose content, making them drier and flakier when cooked than *japonica* varieties.

3) *Javanica* Type:

These are intermediate type. These are cultivated in Indonesia, Phillippines, Taiwan and Japan. These are tall, thick stemmed, low to tillering and resistant to shattering. These varieties have broad stiff leaves, long awns and large bold grains.

Oryza glaberrima

Oryza glaberrima, commonly known as African rice, is a domesticated rice species. African rice is believed to have been domesticated 2,000–3,000 years ago in the inland delta of the Upper Niger river, in what is now Mali. Its ancestor, which still grows wild in Africa, is *Oryza barthii*.

This species is grown in West Africa. *O. glaberrima* shows several characteristics that make it less suitable for cultivation compared to *O. sativa*, such as brittle grains and poor milling quality. African rice also has lower yields than *O. sativa*, but it often shows more tolerance to fluctuations in water depth, iron toxicity, infertile soils, severe climatic conditions and human neglect. It also exhibits better resistance to various pests and diseases, such as nematodes, midges, viruses and the parasitic plants Striga.

Scientists from the Africa Rice Center managed to cross-breed African rice with Asian rice varieties to produce a rice variety called NERICA, which is an acronym for New Rice for Africa.

Origin:

Oryza sativa originated from India and China. The greatest diversity is observed in Assam and Meghalaya area and southwest china. The several authors considered annual type was likely to be the progenitor. Cultivated spp2, and wild up -16.

Breeding Methods:

1) Introduction:

The seeds of released varieties in other countries are imported tested, evaluatd along with native varieties and if found superior, released for commercial cultivation. The variety Taichung native-1 (TN-1) is the cross between

Dee-geo-woo-gene and Tsai Taichung is imported in 1960 for seed multiplication and distribution. Another variety IR-8, a tall and heavy tillering disease resistant developed from cross between Indica variety of Indonesia and dee-geo-gene imported in 1966.

2) Selection:

From land races out standing lines are selected and evaluated along with existing varieties and superior types are multiplied and released for commercial cultivation. The varieties developed by selection are Terna, Tuljapur-1, Ambika, Ambemohor pussa -33, etc.

3) Pedigree Method:

It is the most common method of rice breeding. Crosses made to combine genes for desirable characteristics from two or more parents. Seeds of such crosses sown in pedigree nursery. Selections are made in F3 and onwards generations and superior progenies are carried forward and tested with existing check variety and if found superior released for commercial cultivation.

Bulk selection method is not followed in rice but single seed decent known as rapid generation advance is in use in IARI. Two to three generation in green house can be grown in a year.

4) Breeding for Disease and Pest Resistance:

Name of Disease	**Donor**
Bacterial blight	Xa-4, B-J-1 and TKM-6
Blast	Ram-tulsi, Tetep and M-302.
Tungro	Ambemohor-157, IR-20 and Kamod
Grassy Stunt	IR-28, IR-29, IR-30,and IR-34.
Name of Pest	**Donor**
Brown plant hopper	Babawee, Rathu Henati
Gall midge	PTB-10, PTB-18, PTB-21
Stem Borer	TKM-6, MTU-15, TKM-3, and Ratna

Basmati Rice:

Basmati rice is a good source of foreign exchange earning for us. Traditional basmati rice varieties are tall prone to logging later and poor in yield. The incorporation of Basmati quality in high yielding semi dwarf backgrounds has been quite challenging ne. it is likely due to and complicated inheritance of quality components. Basmati quality is characterized by pleasant aroma, linear kernel elongation on cooking and soft texture.

Basmati Varieties:

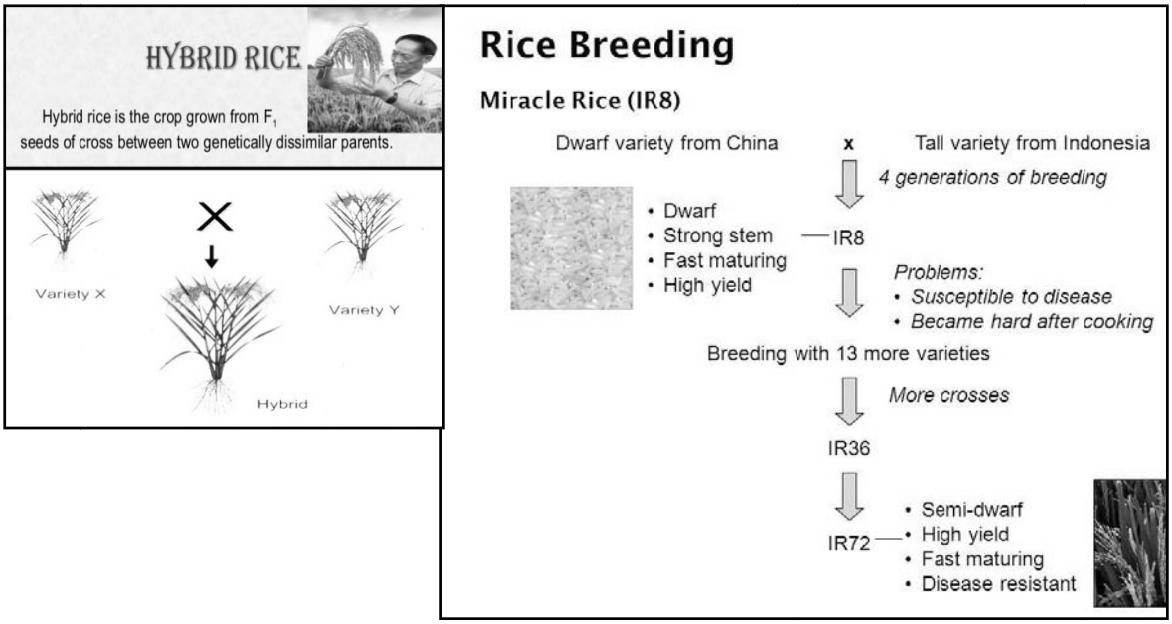
Basmati – 370, Pusa 150, Pusa-523, Kasturi , DRR-Hyderabd, Pusa basmati.

Hybrid Rice:

CGMS lines are used in production of hybrid seed china is the only country in the world where farmers use hybrid rice technology on commercial scale. To produce hybrid seed male and female parents are planted alternatively in specific row rations. The most common rations are 1:6 to 1:8 the technology includes.

1. Planting across the wind direction to increase pollen dispersal on female plants.

2. Clipping of flag leaves of male sterile and restores lines at booting to facilitate pollen circulation.



3. Applying 20-30 ppm GA3 to male sterile and Restore lines at heading to promote emergence of panicle from sheath.

4. Supplying pollination by rope pulling during flowering. Chinese now getting hybrid seed yield more than 1000 kg/ha. The seed rate used is only 25% of the conventional varieties and the country has sufficient manpower for hybrid seed production so the hybrid rice is popular in china. The countries where manpower is expensive and higher seedling rate is necessary. This production technique is not economically acceptable. Generally the hybrids must yield 25% higher than the commercial varieties to become technology commercially reliable. E.g. Karats Hy.1-(Saihydri).

Maize (*Zea mays* L. 2n=20):

Maize is second leading crop after wheat. United States produces 50% of the world production. In India the total cropped area under maize is 6 million hectares with 1250 kg productivity. The status growing maize on large scale are Uttar Pradesh. Bihar, Rajastan, Madhya Pradesh, Panjab and Himachal Pradesh. The maize has C4 photosynthetic pathway and has high seed multiplication ratio.

Origin:

Maize is native of America, with two possible centres of origins.

a) Peru,Ecuador,Bolivia,

b) Southeren Mexico and Central America.

Based on excavations Mexico is accepted as centre of origin for maize.

Species:

1) *Zea mays* L. (2n=2x=20); corn

2) *Zea maxicana* (2n=2x=20) Annual teosinte

3) *Zea perinnis* (2n=40) Perennianl tetraploid teosinte

4) *Zea diploperinnis* (2n=2x=20) Perennial diploid teosinte

Similarly, the genus *Tripsicum* is closely related to *Zea* and has 11 species. Crosses between maize and sugarcane are successful.

Breeding Objective:

1) To develop high yielding hybrids and composite having various maturity duration.

2) To breed varieties for high grain and fodder yield.

3) To breed fertilize responsive varieties.

4) Breeding for wider adaptability to soil and climate.

5) Breeding for resistance to disease and logging.

6) Breeding for industrial quality (Starch or oil content etc)

Emasculation:

The tassels of the female plants are removed immediately as soon as appeared. The process is called as detasseling. It is always done in the morning. Ear shoot which emerging from the leaf sheath is bagged 1 to 2 days below the tip of the preveious day of pollination.

The tassels of selected male parents is also covered with bag on following day in the morning between 9.00 to 10.00 a.m. pollens from tassel bag is dusted over the silk of the female cob/eat. The bag covered ear shoot is torn and bag from the male parent may be placed over the cob. Care should be taken to avoid contamination of silk with foreign pollens.

Breeding Methods:

I) Mass Selection:

This is oldest method of selection of cross pollinated crops. It consists of selection of ears on the basis of plant and ear characteristics and bulking seed of harvested ears to grow next cycle of mass

selection. During mass selection rigorous selection is avoided as it may create the problems of inbreeding depression. Varieties developed 1) KT-41. Basi and Jaunpuri.

II) Hybrid Breeding:

The Term hybrids used to designate F1 populations obtained by crossing genetically unlike parents.

Types of Hybrids:

A) Conventional Hybrids:

1) Single Crosses:

It is hybrid progeny from crosses between two unrelated inbreeds (A XB) X C. Popular hybrids are Ganga 5- (CM-202 X CM-111) X CM- 500 ; Ganga 11- (CM -202 X CM -111) X CM-501 ; high starch – (CM -400 X CM -300) X CM-601.

2) Double Crosses:

It is the hybrid progeny from crosses between two single crosses (AX B) X (C X D)

Popular Hybrids:

1) Deccan double hybrid: (CM-104 X CM -105) X (CM-202 X CM -201)

2) Deccan 101- (CM-202 X CM -206) X (CM -115 X CM-114)

3) Deccan 103- (CM-120 X CM-118) X (CM -208 X CM-119)

B) Non Conventional Hybrids:

1. International Hybrid:

It is the hybrid progeny cross between two varieties. It is similar to synthetic and composites. E.g. Variety X Variety.

2. Top Crosses (Inbreed Variety Cross):

It is the hybrid progeny from crosses between inbreed and variety E.g. A X variety.

3. Double Top Crosses:

It is the progeny of the cross between single cross of inbreds and variety. E.g. (AXB) X Variety.

Hybrid Seed Production:

Deta selling is common method used for producing hybrid seed in India. In USA detasseling and use of CMS line both are used. Male and female lines grown alternatively in the ratio 2:6. The female

rows are detasseled before inflorescence shades the pollens and seed produced on detasseled lines is the F1 hybrid seed.

III) Pedigree Method:

In this method the pair of elite lines that complement to one another are crossed to produce F2 generation and selection is practised in F2 and on word generations.

IV) Back Cross Breeding:

In this method two inbreeds A and B are crossed, F1 is backcrossed with A followed by selection if desirable traits of B and F1 is also backcrossed with B where selection for desirable traits for A are made. After 3 backcrosses and selections selfing is done to fix the selected genes. This method is used to transfer characters like pest resistance, disease resistance and lodging resistance etc.

V) Population Improvement:

A) Synthetic Variety:

The term used to designate the variety that is maintained from open pollinated seed following synthesis by hybridization in all combinations amongst the genotypes selected for high general combining ability. Now a day no variety is popular developed by this method in Maharashtra.

B) Composite Varieties:

Composite varieties in maize are derived from varietal crosses and advanced generations, which are not tested for GCA. The components are selected on the basis of phenotype i.e disease and pest resitance, early maturity, resistance to lodging, ear characteristics etc. the equal quantity of seed of these phenotypically out standing lines is mixed sown in isolated field to obtain crosses in all possible combinations and mild selection is operated to maintain uniformity and homogeneity in the population. The selected bulk is evaluated in multilicational testing before release.

Popular Varieties:

Manjri Composite, Hunis, African tall, Vijay, Kisan, Panchganga, Ambar, Jwahar, etc.

Potato (*Solanum tuberosum* L. 2n = 4x = 48):

Potato is a very popular vegetable grown all over the world and important food crop of grown more than 100 countries in world. Potato is a native of South America. In India, potato has been cultivated since its introduction in early part of 17th century. Potato popularly known as ‘The king of vegetables’, has emerged as fourth most important food crop in India after rice, wheat and maize. India is the third largest producer of Potato.

Cultivated species:

Solanum has about 2000 species. This species is divided into two sub-species; viz., ssp. *tuberosum* (cultivated throughout the world) and ssp *andigena* (confined to the hills of South America). In addition, diploid (2n=24), triploid (2n=36) and *pentaploid* (2n=60) forms are also cultivated in Peru and Bolovia.

Origin

The potato is a native of tropical south American region. It is believed that the cultivated potato originated from its wild ancestors near the lake Tritica basin in Peru Bolivian region in high mountains. The potato was introduced in India from Europe in early 17th century.

Cytology:

- Potato has basic chromosome number 12. Form diploid to hexaploid species are available. About 75% species are diploid while about 15% species are tetraploids.
- Triploids and pentaploids are highly sterile and maintained by vegetative propagation.
- *S.stenotomum* , *S.phureja* & *S.ajanhuioi* are cultivated diploids of which former too are sexually fertile while later one is less fertile and yet bred true.
- S.chaucha & S.Juzepezukii are cultivated triploid, more or less sterile.
- The cultivated tetraploid species *S.tuberosum* spp *tuberosum* &*S.tuberosum* spp andigena are fertile.
- S.curtilobum is pentaploid.
- Regular meiosis has been observed in diploid, allotetraploid & allohexaploids.
- Nearly all the diploid species are self-incompatible while all the tetraploids & hexaploids are self-compatible.

Genetics of traits:

Trait	No. of Genes	Gene Action
Skin colour	Digenic	Complementary
Flesh colour	Monogenic	Deep yellow/White
Stolen length	Monogenic	Long/short
Tuber shape		Long/short
Position of eyes	Monogenic	Plant/deep

Breeding methods

Potato is a self-pollinated crop but is vegetatively propagated. The cultivated tetrapliod varieties are highly heterozygous. Most of them are also pollen sterile. selfing or inbreeding in potato leads to loss of vigor of the progeny and non-flowering.hence the conventional method or pure line method of breeding is generally not practiced.

Hybridization and selection: In hybridization, crosses are made between selected parents. Hybridization can be between varieties (intervarietal) or between specific (interspecific).since yield and most of the desirable characters are polygenic in nature, the parents for hybridization are generally selected on the basis of their combining ability. Being vegetatively propagated, breeders

take advantage of selecting and multiplying genetically identical individuals in the succeeding generations.

Back cross method: Cultivated potato does not posses resistance to most of the diseases and pests. Resistance genes are mostly found scattered in wild and semi-cultivated species available in centre of origin and diversity in south America. In this method the hybridization is done between cultivated and wild or semi cultivated species with the aim of transferring specific characters like resistance to diseases and pests. It is followed by repeated back crossing keeping cultivated type as recurrent parent. Selection is practiced in successive back cross generation for the character to be retained from the wild species. However, transfer of the resistant genes from wild species into cultivated potato is a difficult task.

Hybridization technique: potato naturally flowers under cool climate and long-day condition of more than 15hrs light. Such conditions are available during long-summer days when potatoes are grown in hills. Hills are therefore,ideal for hybridization work. Potato flowers are hermaphrodite (bisexual) and therefore emasculation is done in selected female parents mostly in the evening.

Breeding Objective

- High tuber yield
- Earliness
- Photoperiod insensitivity
- Responsiveness to fertilizer
- Better keeping quality (resistance/tolerance against shrinkage,rottage etc)
- Better quality tubers
- Resistance to

  i. Late blight

  ii. Early blight

  iii.Charcoal rot

  iv.Common scab

  v. Bacterial wilt

Commercial Varieties and Hybrid

Maturity	Varieties	Resistant
Early-maturing	Kufri chandramukhi	Moderately Resistant to late & early blight warmer climate variety
	Kufri lauvkar, Kufri kuber	-
Medium-maturing	Kufri badshah	Late and early blights
	Kufri pukhraj	Early blight and moderately late blight.
	Kufri jyoti,	Late and early blights & tolerant to viruses. Late blight
	Kufri kundan,	Resistant to frost
	Kufri sheetman	Resistant to frost
	Kufri dewa	Late blight and ideal for intercropping
	Kufri jawahar	-
	Kufri Bahar	Late blight & excellent for chip making
	Kufri chipsona-2	
Late-maturing	Kufri kumar,	Late blight
	Kufri chamatkar,	Early blight
	Kufri sindhuri,	-

Varieties

Potato varieties are primarily distinguished based on their habit, pigmentation on the stem, stucture of leaf, flower and fruit colour and tuber characters like shape, size and colour. Some of the varieties under cultivation at present are:

1. **Kufri Badshah(Kufri jyoti x Kufri Alankar):**

   The variety was released in 1979 for north indian plains and plateau region.it is a medium maturing (90-100 days) variety producing tall, erect, medium compact and vigorous plants with white flowers. The variety possesses resistance to late blight, early blight and potato virus and has an average yield of 32-35 tonnes/ha.

2. **Kufri sindhuri (Kufri Red x Kurfi kundan):**

   The variety was released in 1967 for cultivation in north Indian plains particularly for Eastern Uttar Pradesh and Bihar where red tubers were preferred. It is a late maturing(120-140 days) variety producing tall,erect,open and vigorous plants with flower of light-red-purple colour with white tips.it is moderately resistant to early blight with an average yield of 30-35 tonnes/ha.

3. **Kurfi chandramukhi(sd:4485 x Kurfi kuber):**

   The variety was released in 1968. It is an early maturing (75 days). Tubers are large, white, oval, flattened with shallow eyes. The variety has an average yield of 23-25 tonnes/ha.

4. **Kufri jawahar (Kufri neelamami x Kufri jyoti):**

   The variety was released in 1996 for Punjab, Harayana, plateau region of Madhya Pradesh, Gujarat and Karnataka. It is a medium early maturing (80 days) variety producing short,erect, compact, and vigorous plants with white flowers. The variety is moderately resistant to late blight and has an average yield of 28-30 tonnes/ha.

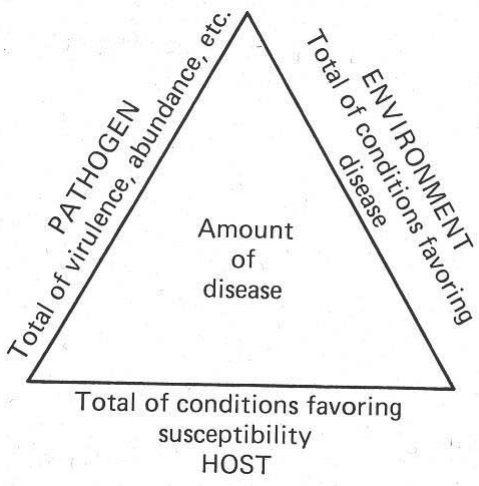
5. **Kurfi pukhraj (craigs defiance x JEX/B-687):**

   The variety was released in 1997 for Punjab, Uttar Pradesh, Madhya Pradesh, Karnataka, Gujarat and Maharashtra. It is a medium maturing (90-100 days) variety producing tall, semi-erect, medium compact and vigorous plants with white flower. The variety is resistant to early blight and moderately resistant to late blight and has an average yield of 30-35 tonnes/ha.

6. An outline of breeding methods for development of disease resistance

Plant disease resistance protects plants from pathogens in two ways: by preformed structures and chemicals, and by infection-induced responses of the immune system. Relative to a susceptible plant, **disease resistance** is the reduction of pathogen growth on or in the plant (and hence a reduction of disease), while the term disease tolerance describes plants that exhibit little disease damage despite

substantial pathogen levels. Disease outcome is determined by the three-way interaction of the pathogen, the plant and the environmental conditions (an interaction known as the disease triangle).



Defense activating compounds can move cell-to-cell and systematically through the plant's vascular system. However, plants do not have circulating immune cells, so most cell types exhibit a broad suite of antimicrobial defenses.

Host plant resistance

A physiological deviation from the normal functioning of the organism (i.e., the crop plant) caused by pathogenic organisms is a disease and may be caused by fungi, bacteria or viruses. The inherent ability of an organism (i.e., the crop plant) to resist or withstand the pathogen is called resistance.

Resistant varieties can be the simplest, practical, effective and economical method of plant disease control. The use of resistant varieties cannot only ensure protection against diseases but also save the time, energy and money spent on other measures of control. Virulence is due to the action of one or a few genes. An aggressive strain of a pathogen causes severe disease on all the host genotypes which they are able to attack and aggressiveness is polygenically inherited. A disease is the result of an interaction of genes governing resistance in the host with those governing pathogenicity in the pathogen. The resistance of a crop to a physiological race of the pathogen depends not only on the genotype of the host for resistance, but also upon the genotype of the pathogen for virulence or aggressiveness. Flor (1942) proposed the gene-for-gene hypothesis, according to which, for every gene for resistance in the host, there is a corresponding gene for pathogenicity in the pathogen.

It means that there are atleast two alleles at a locus controlling resistance/susceptibility in the host (R-r) and two alleles at a corresponding locus in the pathogen (V-v) controlling virulence / aggressiveness. Out of the four possible interactions between these alleles, only one combination leads to the expression of resistance. The demonstration of gene-for-gene relationship requires genetic studies of both the host and the pathogen.

Vertical resistance (VR) and horizontal resistance (HR)

Van der Plank (1960) has discussed the whole issue of disease resistance in a different perspective. He calls the unstable and often complete type of resistance as vertical resistance and the more stable but somewhat incomplete resistance as horizontal resistance. If resistance to some races of a pathogen is

more than to other races, it is called Vertical resistance. It is also called Perpendicular resistance, Physiological resistance, seedling resistance, hypersensitivity, race specific resistance or qualitative resistance. As it is conditioned by one or a few genes, it is called major gene or monogenic of oligogenic resistance.

Resistance to more than one race of the pathogen or to many or all races of the pathogen is called Horizontal Resistance. It is non-specific resistance governed by polygenes. It is severally termed as non-specific, general, polygenic, minor gene, mature plant, adult, quantitative resistance, partial or field resistance or tolerance. HR causes reduction in the number and rate of sporulation of the pathogen on the host and slows down the infection rate. HR includes tolerance slow development of disease, escape and exclusion mechanisms besides hypersensitive reaction.

Differences between vertical and horizontal diseae resistance * Detectable by analysis of variance of a suitable experiment

Feature	**Vertical resistance**	**Horizonal resistance**
Pathotype-specificity	Race specific	Race nonspecific
Nature of gene action	Oligogenic	Polygenic; rarely oligogenic
Response to pathogen	Usually, hypersensitive	Resistant response
Phenotypic expression	Qualititative	Quantitative
Stage of expression	Seedling to maturity	Expression increases as plant matures (Adult plant)
Selection and evaluation	Relatively easy	Relatively difficult
Risk of 'boom and burst'	Present (rarely durable)	Absent (durable)
Suitable for: a. Host b.Pathogen	Annuals but not perennials Immobile pathogen, e.g., Soil pathogens, but for mobile air-borne, pathogens	Both annuals and perennials All pathogens
Need for specific deployment of resistant varieties	Critical for success with mobile pathogens	None
Need for other control measures	Likely	Much less likely
Host-pathogen interaction *	Present	Absent
Efficiency	Highly efficient against specific races	Variable, but operates against all races

Vertical resistance to specific races is generally governed by a single (monogenic) dominant gene or by a few dominant genes. Some of these genes may be multiple alleles as in leaf rust gene, Lr2 that accords resistance to Puccinia recondite tritici. In that locus, four genes designated as Lr2a, Lr2b, Lr2c and Lr2d are present and are tightly linked. Each of these genes accord resistance to a different spectrum of races and hence can be differentiaited from one another. Such multiple alleles exist on Sr9 locus of wheat for P.graminis tritici and gene Pi-k in rice for resistance to *Pyriculariva grisea*. The tight linkage between the multiple alleles permits an efficient transfer of all these genes in one attempt.

'Horizontal resistance' (HR) reduces the rate of disease spread and is evenly spread against all races of the pathogen. The low terminal disease severity in HR is assumed to result from polygenic resistance. Morphological features such as size of stomata, stomatal density per unit area, hairiness, waxiness and several others influence the degree of resistance expressed. Partial resistance, dilatory resistance, lasting resistance are some other terms coined for denoting horizontal resistnce.

The phenomenon of slow rusting manifested as lesser number of pustules per unit leaf area, smaller size of uredosori and increased latent period in some wheat cultivars is a typical example of this type of resistance. Although it is preferable to use varieties that have both vertical and horizontal resistance, most of the resistant varieties carry only one or few (2 or 3) major genes of vertical resistance. If varieties are resisitant only to some of the races of pathogen and if the pathogen is airborne, then new races evolve easily, as happens with cereal rusts, the powdery mildlew and Phytophthora infestans. Appearance of new races lead to breakdown of resistance of the popular, ruling genotype. As a result, varieties with vertical resistance need to be replaced at frequent intervals.

Disease resistance is governed by several in built mechanisms of the host, plants against infection by the pathogen. They are disease escape, disease endurance or tolerance and true resistance.

a. Disease escape

It is a prevention mechanism that causes the host to escape pathogenic infection. Early or late maturity of the crop may prevent physical contact of the pathogen with the host. Mechanical and anatomical barriers such as thick cuticle, waxy bloom on leaves and stem, stomatal regulation prevent penetration of spores. Ergot, a fungal disease of inflorescence in cereals caused by *Claviceps purpurea* does not affect varieties of wheat and barley in which the flowers remain closed until pollination occurs. Erect leaves of barley avoid deposition of spores of *Erysiphe graminis* tritici in contrast to prostrate leaves. Early maturing varieties of groundnut escape early leaf spot infection (*Cercospora arachidicola*) and early varieties of wheat escape rust and loose smut infection.

Gene for gene hypothesis:

For each gene conditioning rust reaction in the host there is a specific gene conditioning pathogenicity in the parasite.

For each resistance gene in the host there is a corresponding gene for avirulence in the pathogen conferring resistance and viceversa.

Flor (1946, 47) showed correlation between inheritance of pathogenicity and resistance to linseed rust caused by *Melampsora lini* which is now commonly known as gene -for -gene hypothesis. that "for each gene conditioning rust reaction in the host there is a specific gene conditioning pathogenicity in the parasite.

The concept has been applied with varying degree of proof to other host pathogen combinations including viruses, bacteria, fungi, nematodes, insects and a flowering plant (Orobanche).

RR = homozygous resistant ; Rr = heterozygous resistant; rr = homozygous susceptible
AVR AVR = homozygous avirulent; AVR avr = heterozygous avirulent; avr avr = homozygous virulent

		HOST GENOTYPE	
		RR or Rr	rr
PATHOGEN GENOTYPE	AVR avr or AVR AVR	Disease-resistant	Susceptible to disease
	avr avr	Susceptible to disease	Susceptible to disease

Gene-for-gene hypothesis
Multifactor Interactions

Avirulence/virulence	Resistance/susceptibility			
	R1 R2	r1 R2	R1r2	r1r2
A1A2	-	-	-	+
a1A2	-	-	+	+
A1a2	-	+	-	+
a1a2	+	+	+	+

where, – = Resistance
+ = Susceptible

(Agrios 2007)

Resistance genes:

Classes of resistance gene

There are several different classes of R Genes. The major classes are the NBS-LRR genes and the cell surface pattern recognition receptors (PRR). The protein products of the NBS-LRR R genes contain a

nucleotide binding site (NBS) and a leucine rich repeat (LRR). The protein products of the PRRs contain extracellular, juxtamembrane, transmembrane and intracellular non-RD kinase domains.

Within the NBS-LRR class of R genes are two subclasses:

1. One subclass has an amino-terminal Toll/Interleukin 1 receptor homology region (TIR). This includes the N resistance gene of tobacco against tobacco mosaic virus (TMV).
2. The other subclass does not contain a TIR and instead has a leucine zipper region at its amino terminal.

The protein products encoded by this class of resistance gene are located within the plant cell cytoplasm.

The PRR class of R genes includes the rice XA21 resistance gene that recognizes the ax21 peptide and the Arabidopsis FLS2 peptide that recognizes the flg22 peptide from flagellin

here are other classes of R genes, such as the extracellular LRR class of R genes; examples include rice Xa21D [11] for resistance against *Xanthomonas* and the cf genes of tomato that confer resistance against *Cladosporium fulvum*.

The Pseudomonas tomato resistance gene (Pto) belongs to a class of its own. It encodes a Ser/Thr kinase but has no LRR. It requires the presence of a linked NBS-LRR gene, prf, for activity.

Specificity of resistance genes

R gene specificity (recognising certain Avr gene products) is believed to be conferred by the leucine rich repeats. LRRs are multiple, serial repeats of a motif of roughly 24 amino acids in length, with leucines or other hydrophobic residues at regular intervals. Some may also contain regularly spaced prolines and arginines.

LRRs are involved in protein-protein interactions, and the greatest variation amongst resistance genes occurs in the LRR domain. LRR swapping experiments between resistance genes in flax rust resulted in the specificity of the resistance gene for the avirulence gene changing.

Recessive resistance genes:

Most resistance genes are autosomal dominant but there are some, most notably the mlo gene in barley, in which monogenic resistance is conferred by recessive alleles. mloprotects barley against nearly all pathovars of powdery mildew.

Avirulence genes:

The term “avirulence gene” remains useful as a broad term that indicates a gene that encodes any determinant of the specificity of the interaction with the host. Thus, this term can encompass some conserved microbial signatures (also called pathogen or microbe associated molecular patterns (PAMPs or MAMPs)) and pathogen effectors (e.g. bacterial type III effectors and oomycete effectors) as well as any genes that control variation in the activity of those molecules.

Biotrophy and gene for gene systems:

All the parasites in which gene for gene relationship has been proved are essentially biotrophic or biotrophs at least for some time after start of infection.

- (*Xanthomonas campestris* pv. *malvacearum, Phytophthora infestans, Venturia inaequalis* (Vander Plank, 1978).
- The genes-for-gene systems thus involve biotrophy.

- But the converse is not necessarily true. For example, *Plamodiophora brassicae* , the cause of club root of crucifers, is biotrophic but no evidence has yet been presented in the literature to suggest that host-pathogen interaction in them is based on a gene for gene systems.

Table. The diagonal check for specificity in a gene-for gene relationship

Pathogen	Plant				
	R_1R_1[b]	R_2R_2	R_3R_3	R_4R_4	R_5R_5
v_1v_1	S	R	R	R	R
v_2v_2	R	S	R	R	R
v_3v_3	R	R	S	R	R
v_4v_4	R	R	R	S	R
v_5v_5	R	R	R	R	S

a. Plant reaction when resistance gene R_1,R_2,R_3,R_4,R_5 at interact with virulence genes v_1,v_2,v_3,v_4,v_5 at five loci pathogen

b. Resistance is assumed to be dominant and RR can be r by Rr. Virulence is assumed to be recessive. However, re resistance and dominant virulence are also known.

R= resistant S= susceptible

Vander Plank (1978) elaborated protein for proteins hypothesis as a biochemical explanation of gene for gene interaction.

The protein for protein hypothesis states that in **gene for gene diseases the mutual recognition of host and pathogen is not by the genes themselves but by their coded proteins**.

Vander Plank (1978) hypothesized that in susceptibility the pathogen excretes a protein (virulence for product) into the host cell which copolymenizes with a complementary host protein (resistance gene product). This co-polymerization interferes with one auto regulation of the host gene that codes for the protein and by so doing turns the gene on to produce more protein.

In resistance, the protein specified by the gene for avirulence in the pathogen and excreted into the host does not polymerize with the protein coded for by the gene for resistance. It is not recognized by the host at all.

From a practical point of view, gene for gene relationship can be used to study the following:

1. The source of pathogenic variability in pathogens
2. The mutability of resistance and virulence genes
3. Why host resistance is expressed under one set of conditions and not others
4. Prediction of putative genotypes
5. Race nomenclature
6. Genetic dissection of complex loci

7. Cataloguing and storing of R genes in the form of plant seeds or cuttings and V genes in the form of pathogen strains
8. Management and deployment of resistance genes in space and tome
9. Detection of linkage and allelic relationship
10. Geographic distribution of R and V genes
11. Synthesis of multilines and multigene cultivars.

Vertifolia effect:

The vertifolia effect was discovered by Van der Plank (1963) who named it after a potato cultivar of this name, in which the effect was very pronounced. The vertifolia effect is a loss of horizontal resistance which occurs during breeding for vertical resistance. Its meaning was later extended to include the loss of horizontal resistance that occurs during breeding under the protection of pesticides.

The level of horizontal resistance can only be assessed by the level of parasitism. Clearly, if there is no parasitism because of a functioning vertical resistance, or a pesticide, the level of horizontal resistance cannot be assessed. Because individual plants with a high level of horizontal resistance are rather rare in a mixed screening population, the chances are that individuals with a relatively low level of horizontal resistance will then be selected on the basis of their other attributes. The loss is usually quite small in a single breeding cycle but, after many cycles, it can become very serious indeed.

The prime example of the vertifolia effect is the loss of horizontal resistance to potato blight (*Phytophthora infestans*) that has continued ever since both the discovery of Bordeaux mixture in the late nineteenth century, and the discovery of vertical resistance in the twentieth century. A loss of horizontal resistance to cotton pests has continued ever since the discovery of DDT in the 1940s.

The vertifolia effect is a very modern phenomenon. Its overall consequences are seen in the high levels of horizontal resistance in heritage cultivars, when they are compared to modern cultivars. This is the main reason why heritage cultivars are so valued by organic farmers.

One of the main objectives of most amateur plant breeders will be to restore the horizontal resistances that were lost to the vertifolia effect.

Methods of breeding for disease resistance

The methods of breeding varieties resistant to diseases do not differ greatly from those

adopted for other characters. The following methods are used:

1. Introduction,

2. Selection,

3. Hybridization followed by selection,

4. Back cross method,

5. Induced mutagenesis,

6. Development of multilines and

7. Tissue culture techniques

1. Introduction

It is a very simple and inexpensive method. Varieties resistant to a particular disease elsewhere may be thoroughly tested in the regions in which they are proposed to be introduced. Their yield performance and disease resistance should be confirmed by large scale cultivation.

Introductions have served as a useful method of disease control. For example, Ridley wheat introduced from Australia has been useful as a rust resistant variety. Manila, a rice variety introduced in Karnataka from the Philippines, has tolerance to blast, bacterial leaf blight and sheath blight. Intan, a Javanica type rice variety introduced in Karnataka from Indonesia is highly resistant to blast. Munal, a rice variety introduced in West Bengal from the U.S.A. is tolerant to blast, bacterial leaf blight and leaf folder (pest). Some of IRRI rice varieties such as IR 20, IR.24, IR.28, IR.34, IR.36 and IR .50 possess resistance to one or more diseases. Early varieties of groundnut introduced from U.S.A. havebeen resistant to laf spot (*Cercosora arachidicola*). Kalyan Sona and Sonalika wheat varieties originated from the segregating materials introduced from CIMMYT, Mexico and were rust resistant.

2. Selection

This is better method than introduction and has more chances of success in obtaining disease-resistant plants. The work of selection is carried out either in the naturally infected fields under field conditions or under artificially inoculated conditions. To ensure the resistant character of a plant, large population of crop plant may be exposed to the attack of pathogen under artificial conditions and the non-infected plants may be chosen. Suvarnamodan rice of Kerala is a pure line of ARC. 11775 and is highly tolerant to blast. Sugandh of Bihar is a selection from Basmati rice of Orissa tolerant to bacterial leaf blight. Rice varieties Sudha (Bihar), Sabita, Nalini (West Bengal), Patel 85 (Madhya Pradesh), Janaki(Bihar) are some of rice selections resistant to one or more diseases. MCU 1 cotton, a selection from Co 4, is resistant to Kufri Red a potato selection from Darjeeling Red Round is a disease resistant variety.

3. Hybridization

When selection of resistant varieties is not feasible, resistant varieties may be evolved by crossing the susceptible popular variety with resistant wild variety where in the resistant gene or genes transferred into the genetic make up of susceptible variety. Very often the F1 from crosses may be resistant but carry the other undesirable qualities of the resistant parent. The bad qualities are removed by several back crossing of F1 with the susceptible parent may ultimately yield a resistant progeny with good agronomic characteristics.

Under certain circumstances pedigree or bulk method of selection is followed to obtain a resistant variety. In this method, the crosses are made till F2 population is got. Selections are made in F2 generation for superior genetic traits including disease resistance. By continued selfing, selections are made through F3 to F5 or F6 generations and the best variety is selected. This method is suited for small grains and beans but unsuited to fruits and vegetables.

4. Back cross method

Back cross method is widely used to transfer disease resistance from wild species. Wild species are rice sources of disease resistance. Interspecific hybridization is made to transfer the gene or genes for

resistance to the cultivated species. Resistance to grassy stunt virus from *Oryza nivara* to *O.sativa*, late blight resistance from *Solanum demissum* to cultivated potato, rust resistance from durum to *aestivum* wheat are some of the examples involving interspecific hybridization.

5. Induced mutagenesis

While following mutation breeding for disease resistance, a large number of mutation progeny should be produced and screened under artificial epiphytotic condition to select resistant plants. MCU10 cotton, a resistant variety to bacterial blight was evolved in Tamil Nadu by subjecting seeds of a susceptible variety CO4 to gamma rays followed by rigorous screening and selection 6. Development of multilines The concept of multilines was first suggested by Jensen(1952) and developed by Borlaug (1959) for evolving multiline varieties to resist stem rust in wheat. A multiline variety is a composite of genetically similar lines, except that each line possesses a different gene for resistance to the pathogen.

Lines that are genetically similar, except for one gene, are called isoline. It is assumed that gene for resistance in each isoline contributes resistance to a separate physiological race or group of races. Genes for disease resistance are transferred by backcrossing from donor varieties to a common disease susceptible, but agronomically superior, recurrent parent. Isolines are generated differing only in the gene for disease resistance. The isolines are composited to synthesize a multiline variety.

Care should be taken to see that there is uniformity for height, maturity and other features in the multiline. Multiline varieties have been developed for resistance to stem rust and stripe rust of wheat and crown rust of oats. The first multiline variety in wheat, 'Miramar 60' was developed and released in Columbia to combat the attack of yellow rust. 'Miramar 63' and 'Miramar 65' were resistant to stem rust and stripe rust. 'Yoqui 50', 'Crew' and 'Tumult' are a few other wheat multilines. Kalyan sona and Sonalika-based multilines of wheat resistant to different races of rust have been developed in India.

7. Tissue culture technique

Tissue culture techniques to produce somaclonal variation for disease are developed in different crops. Somaclonal variations for disease resistance are reported in *Zea mays* for *Drechslera maydis* race T-toxin resistance, in *Brassica napus* for resistance/tolerance to Phoma lingam, early and late blight resistance in potato, *Pseudomonas* and *Alternaria* resistance in tobacco, besides smut and rust disease resistance in sugarcane.

5. Molecular marker assisted breeding – an outline

Marker Assisted Breeding (MAS):

Marker assisted breeding is defined as the application of molecular markers in combination with linkage maps and genomics to alter and improve plant traits on the basis of genotypic assays. This term is used to describe several modern breeding strategies including marker-assisted selection (MAS). MAS is based on the concept that it is possible to infer the presence of a gene from the presence of a marker which is tightly linked to the gene of interest.

Features of Marker Assisted Selection (MAS):

1. Pre-Requisites:

There are two pre-requisites for marker assisted selection. These are: (i) a tight linkage between molecular marker and gene of interest, and (ii) high heritability of the gene of interest.

2. Markers Used:

MAS makes use of various types of molecular markers. The most commonly used molecular markers include amplified fragment length polymorphisms (AFLP), restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) or micro satellites, single nucleotide polymorphisms (SNP), etc. The use of molecular markers differs from species to species also.

3. Efficiency:

The relative efficiency of MAS is greatest for characters with low heritability, if a large fraction of the additive genetic variance is associated with the marker loci. In other words, MAS is useful when the heritability of the trait is low. Moreover, MAS is more efficient than purely phenotypic selection in quite large populations.

It has been found by some workers that MAS may become less efficient than phenotypic selection in the long term. This is because the rate of fixation of unfavourable alleles at QTLs with small effects is higher under MAS than under phenotypic selection. It may be a consequence of the strong selection applied to QTLs with large effects under MAS in early generation. However, such problem comes after a long period.

4. Accuracy:

Molecular markers have very high accuracy. They are not affected by environmental conditions. MAS is a new breeding tool which is available to make more accurate and useful selections in breeding populations. MAS allows heritable traits to be linked to the DNA which is responsible for controlling that trait.

5. Speed of Progress:

MAS is a rapid method of crop improvement. For example, in conventional breeding when we transfer a recessive character through backcross, one selfing is required after every backcross for identification of recessive character. MAS permits identification of recessive alleles even in heterozygous condition and thus speeds up the progress of crop improvement work.

6. Traits Improved:

MAS can be used for improvement of both oligogenic and polygenic traits. In the past, MAS has been mostly used for the genetic improvement of oligogenic traits and little progress has been made with polygenic traits.

7. Material Developed:

MAS leads to development of non-transgenic genotypes or cultivars. In other words, MAS is used for development of non-transgenic cultivars. The transgenic cultivars face public resistance. On the other hand, cultivars developed by MAS are acceptable by consumers.

8. Cost:

MAS is very costly as compared to phenotypic selection. In MAS, the costly items include equipment's, consumables, infrastructure, labour and DNA extraction process. MAS requires sophisticated and well equipped laboratory.

9. Application:

MAS is applicable for genetic improvement of plants as well as animals. In plants, it is equally applicable in both self-pollinated and cross pollinated species.

Steps in Marker Assisted Selection (MAS):

In the marker aided selection, RFLP markers are widely used for genetic improvement of crop plants for various economic characters.

The marker aided selection consists of five important steps, viz:

(i) Selection of parents

(ii) Development of breeding population

(iii) Isolation of DNA from each plant

(iv) Scoring RFLPs

(v) Correlation with morphological traits

i) Selection of Parents:

Selection of suitable parents is an important step in marker aided selection. The parents should be such so that we can get usable level of polymorphism (variation) in the RFLP markers. In other words, parents with contrasting characters or divergent origin should be chosen. This will help in identification of DNA of both the parents and also their segments in F2 generation in various recombinations.

For selection of parents, we have to screen germplasm and select parents with distinct DNA. The parents that are used for MAS should be pure (homozygous). In self- pollinated species, plants are usually homozygous. In cross-pollinated species, inbred lines are used as parents.

ii) Development of Breeding Populations:

This is the second important step for application of marker aided selection. The selected parents are crossed to obtain F1 plants. F1plants between two pure-lines or inbred lines are homogeneous (alike phenotypically) but are heterozygous for all the RFLPs of two parents involved in the F1. The F2 progeny is required for the study of segregation pattern of RFLPs. Generally 50-100 F2 plants are sufficient for the study of segregation of RFLP markers.

iii) Isolation of DNA:

The third important step is isolation of DNA from breeding population. The main advantage of MAS is that DNA can be isolated even from the seedlings and we need not to wait for flowering or seed development stage. The DNA is isolated from each plant of F2population. Standard procedures are available for DNA isolation.

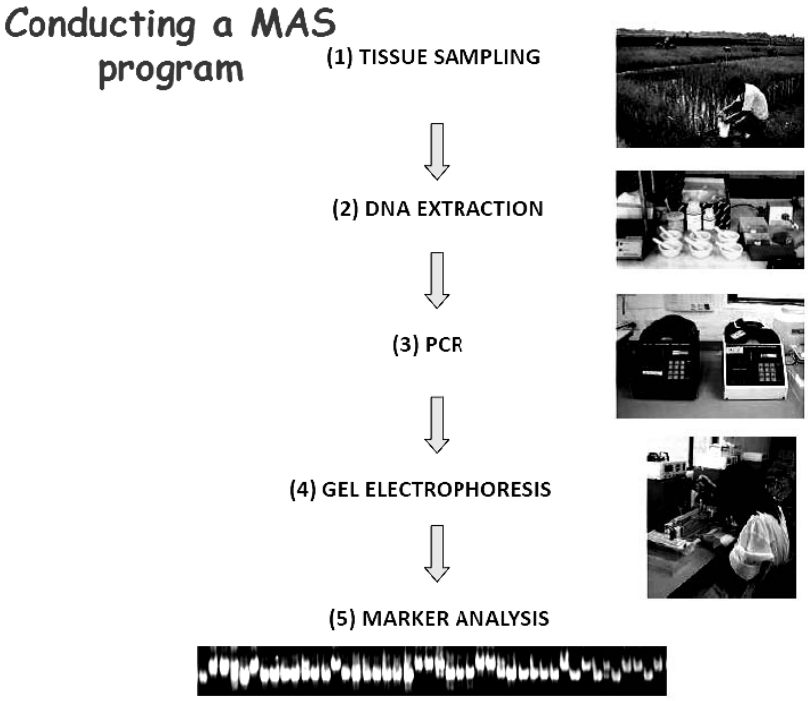
The isolated DNA is digested with specific restriction enzyme to obtain fragments of DNA. The DNA fragments of different sizes are separated by subjecting the digested DNA to agarose gel electrophoresis. The gel is stained with ethidium bromide and the variation in DNA fragments can be viewed in the ultraviolet light.

The DNA of chloroplasts, when digested with specific enzyme, produces about 40 fragments of different sizes. The nuclear DNA of higher plants, when digested with specific restriction enzymes, produces millions of fragments in a continuous range of sizes. It is a tedious job to identify individual DNA fragment in such cases.

iv) Scoring RFLPs:

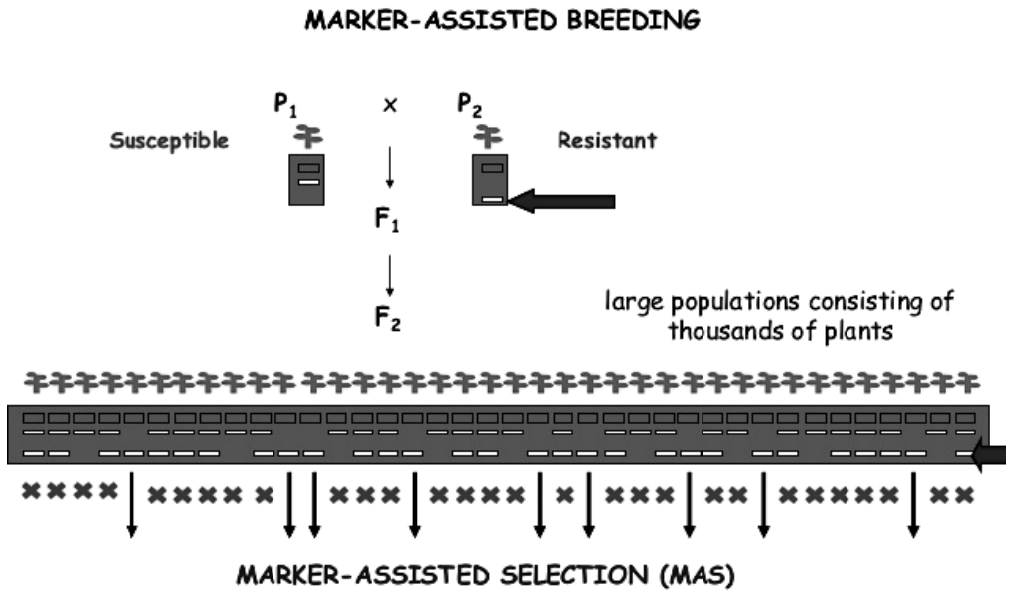
The polymorphism in RFLPs between the parents and their involvement in the recombinants in F2 population is determined by using DNA probes. The labelled probes are used to find out the fragments having similarity.

The probe will hybridize only with those segments which are complementary in nature. Generally 32P is used for radioactive labelling of DNA probe. Now non-radioactive probe labelling techniques are also available. In this way RFLPs are determined.



v) Correlation with Morphological Traits:

The DNA marker (say RFLPs) are correlated with morphological markers and the indirect selection through molecular markers is confirmed. Once the correlation of molecular markers is established with morphological markers, MAS can be effectively used for genetic improvement of various economic traits.



Method whereby phenotypic selection is based on DNA markers

Applications of Marker Assisted Selection (MAS):

In crop improvement programmes MAS can be used in various ways. In other words, MAS has several useful applications in plant breeding.

Important applications of MAS in plant breeding are briefly presented below:

i. MAS is very effective, efficient and rapid method of transferring resistance to biotic and abiotic stresses in crop plants.

ii. It is useful in gene pyramiding for disease and insect resistance.

iii. It is being used for transfer of male sterility and photo period insensitivity into cultivated genotypes from different sources.

iv. MAS is being used for improvement of quality characters in different crops such as for protein quality in maize, fatty acid (linolenic acid) content in soybean and storage quality in vegetables and fruit crops.

v. MAS can be successfully used for transferring desirable transgene (such as Bt gene) from one cultivar to another.

vi. MAS is very effective in introgression of desirable genes from wild into cultivated genotypes.

vii. MAS is equally effective in genetic improvement of plants and animals.

viii. MAS is useful in genetic improvement of tree species where fruiting takes very long time (say 20 years) because for application of phenotypic selection we have to wait for such a long time.

ix. MAS has wide application for genetic improvement of oligogenic traits as compared to polygenic traits.

Achievements of Marker Assisted Selection (MAS):

MAS has been used for genetic improvement of different field crops such as maize, barley, rice, wheat, sorghum, soybean, chickpea, pea, sunflower, tomato, potato and some fruit crops for various economic characters. Some notable examples of the use of MAS:

i) Rice:

In rice MAS has been successfully used for developing cultivars resistant to bacterial blight and blast. For bacterial blight resistance four genes (Xa4, Xa5, Xa13 and Xa21) have been pyramided using STS (sequence tagged site) markers.

The pyramided lines showed higher level of resistance to bacterial blight pathogen. In Indonesia, two bacterial blight resistant varieties of rice viz Angke and Conde have been released through MAS. For blast resistance, three genes (Pil, Piz5 and Pita) have been pyramided in a susceptible rice variety Co 39 using RFLP and PCR based markers.

ii) Maize:

In maize, normal lines have been converted into quality protein maize (QPM) lines through MAS using opaque 2 recessive allele. This work has been done at CIMMYT (International centre for wheat and maize improvement, Mexico).

Three SSR markers (Umc 1066, Phi 057 and Phi 112) present within opaque 2 gene have been used for this purpose. The MAS used for conversion of normal maize lines into QPM is simple, rapid and accurate.

iii) Soybean:

In soybean cyst nematodes pose serious problem and most of the varieties are susceptible to this parasite. The resistant gene (rhg 1) is available. In soybean, nematode resistant lines have been developed through MAS using SSR marker (Sat 309).

Advantages of Marker Assisted Selection (MAS):

MAS has several advantages over phenotypic selection and other breeding techniques.

i) Accuracy:

The accuracy of MAS, is very high because molecular markers are not affected by environmental conditions. It is very effective even with the characters having low heritability.

ii) Rapid Method:

MAS is a rapid method of crop improvement. It takes 3-5 years for developing a new cultivar against 10-15 years taken by the conventional method of breeding.

iii) Non-transgenic Product:

MAS leads to development of non-transgenic cultivars which are acceptable to everybody. In other words, it does not involve transgene. Hence there is no question of gene silencing.

iv) Identification of Recessive Alleles:

MAS permits identification of recessive alleles even in heterozygous condition and thus speeds up the progress of crop improvement programmes. In other words, it is equally effective for the genetic improvement of recessive characters.

v) Early Detection of Traits:

MAS permits early detection of traits that are expressed late in the life of plant. For example characters such as grain or fruit quality, flower colour, male sterility, photoperiod sensitivity that express late in the life of a plant can be screened in the seedling stage. In other words, DNA tested at seedling stage can through light about the trait which are expressed later on.

vi) Screening of Difficult Traits:

MAS permits screening traits that are extremely difficult expressive and time consuming to score phenotypically. For example, screening for traits such as root morphology and resistance to biotic (insects and diseases) and abiotic stresses (drought, salinity, heat, frost etc.) is very easy through MAS.

vii) Gene Pyramiding:

MAS is very effective method in accumulating multiple genes for resistance to specific pathogens and pests within the same cultivar. This process is called gene pyramiding. Maker assisted backcrossing is routinely applied in breeding programmes for gene introgression. MAS can provide an effective and efficient breeding tool for detecting, tracking, retaining, combining and pyramiding genes for disease resistance.

viii) Small Sample for Testing:

MAS requires only a small amount of plant tissue for DNA testing. In other words, MAS can be carried out with small breeding populations. Moreover, MAS can be applied at any stage of plant growth.

ix) Permits QTL Mapping:

MAS permits mapping or tagging of quantitative trait loci (QTL) which is not possible by conventional method.

x) Highly Reproducible:

The MAS is based on DNA fingerprinting technique and the results of DNA fingerprinting pattern are highly reliable and reproducible.

Limitations of Marker Assisted Selection (MAS):

i. MAS is a costly method. It requires well equipped laboratory viz. expensive equipment's, glassware and chemicals.

ii. MAS requires well trained manpower for handling of sophisticated equipments, isolation of DNA molecule and study of DNA markers.

iii. The detection of various linked DNA markers (AFLP, RFLP, RAPD, SSR, SNP etc.) is a difficult, laborious and time consuming task.

iv. MAS sometimes involves use of radioactive isotopes in labelling of DNA, which may lead to serious health hazards. This is a major disadvantage of RFLP based markers. The PCR, markers are safe in this regard.

v. It has been reported that MAS may become less efficient than phenotypic selection in the long term.

vi. The use of MAS is more difficult for QTL because they have minor cumulative effects and are greatly influenced by environmental conditions and genetic background.

8. Population samples, sampling methods

Meaning of Sampling:

In biological experiment, it is not possible to collect complete information about a population. If the number of pods/plant is to be collected from a field then it is really time consuming and also rarely possible to do it.

Then few plants are taken into account for studying the whole population of plant in that field. The method by which only few items are selected from the population in such a way so that they will represent the population in unbiased way is called sampling.

The size of sample is an important factor in statistical analysis which depends on the number of sampling units selected from a population for investigation. The size should not be too big or too small. Taking only 10 values from a plot of 1000 plants will give erroneous result and also it is difficult to handle with 1000 number of values.

Population sampling is the process of taking a subset of subjects that is representative of the entire population. The sample must have sufficient size to warrant statistical analysis.

Why Sampling is Essential?

A. Sampling saves time, the data can be collected and summarised more quickly with a sample than a complete count of the whole population.

B. In case of infinite population, sampling is the only method for statistical analy-sis.

C. Sampling reduces the cost of experiment because only a few selected items are studied in sampling.

Types of Sampling:

1. **Non-Probability Sampling**

In this type of population sampling, members of the population do not have equal chance of being selected. Due to this, it is not safe to assume that the sample fully represents the target population. It is also possible that the researcher deliberately chose the individuals that will participate in the study.

Non-probability population sampling method is useful for pilot studies, case studies, qualitative research, and for hypothesis development.

This sampling method is usually employed in studies that are not interested in the parameters of the entire population. Some researchers prefer this sampling technique because it is cheap, quick and easy.

2. Probability Sampling

In probability sampling, every individual in the population have equal chance of being selected as a subject for the research.

This method guarantees that the selection process is completely randomized and without bias.

The most basic example of probability sampling is listing all the names of the individuals in the population in separate pieces of paper, and then drawing a number of papers one by one from the complete collection of names.

The advantage of using probability sampling is the accuracy of the statistical methods after the experiment. It can also be used to estimate the population parameters since it is representative of the entire population. It is also a reliable method to eliminate sampling bias.

Limitations of Sampling:

A. If the sampling is not done properly, i.e., if it is biased then it misleads which results in false, inaccurate interpretation.

B. There may be personal biasness during sampling or choice of method of sam-pling which may also lead to erroneous interpretation.

Criteria for Good Sampling:

A. Selected samples from the population should be homogenous and should not have any differences when compared with the population.

B. Reasonable number of items is to be included in the sample to make the result more reliable.

C. The selected sample should have the similar characteristics as the original population from which it has been selected.

D. The individual items composing the sample should be independent from each other.

E. The number of observations included in a sample should be more to make the results more reliable.

Methods of Sampling:

The proper method of selection of samples and the relation between the sample and population is the matter which determines the method of sampling. There are various methods of sampling; it totally depends upon a statistician which method will be appli-cable for proper selection of method of sampling

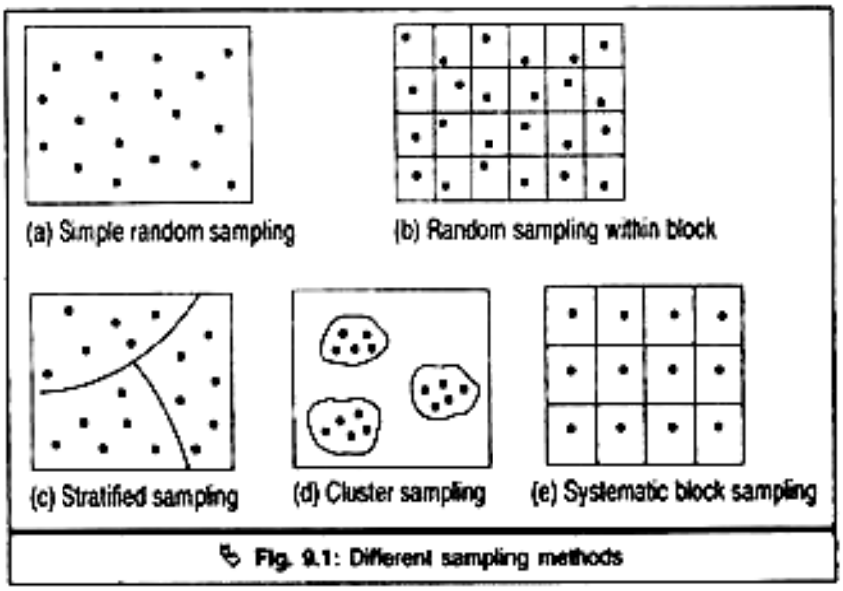


Fig. 9.1: Different sampling methods

Different Sampling Methods

A. Simple Random Sampling:

This method is followed where each item of the population has an equal chance of being included in the sample. Random sampling suggests that selection should be done without any biasness.

To ensure the randomness of selection one can adopt either lottery method or refer to table of random numbers. Lottery method is the simplest and most popular method, all the items are numbered and slips of identical size and shape are made. All are shuffled together and selection is done blindly.

Table of random numbers can be used in place of blind selection. For this purpose, Random Number Table (5 digit) prepared by Snedecor and Cochran (1988) can be used either horizontally or vertically for selection of sample without biasness. This method of sampling is more scientific because there are less chances of personal biasness in sampling from the population and chances of selection of every item are equal.

B. Systematic Sampling:

This method is applied when the population is large, scattered and not homogeneous.

Systematic procedure follows to choose a sample by taking every K the individual, where K refers the sample interval calculated by the formula:

K = Total population/Sample size desired

Example 1:

20% sample to be taken from 1000 individual of a population,

K= 1000/20% of 1,000 = 5

So the, First sample will be the 5th individual,

Second sample will be the 10th individual,

Third sample will be the 15th individual, and like this way.

C. Stratified Sampling:

This method of sampling is followed when the population is not homogeneous, hence the population is first divided into several homogeneous groups or strata and the sample is drawn from each stratum at random. This method is useful as it represents the proportion-ate representative sample from each group and it gives greater accuracy.

D. Cluster Sampling:

A cluster is randomly selected group. This method is used when the units of popula-tion are natural groups such as school, hospitals, etc. The technique of cluster sampling allows small number of target population and the data provided statistically valid at 95% confidence limits.

E. Non-Random Sampling:

This method is called as judgement sampling. In this method the selection process of sample is somewhat subjective. The choice of sample items depends exclusively on the judgement of the investigator.

The investigator exercises his judgement in the choice and includes those items in the sample which he considers most typical for his investigation. For example, from a rice field one investigator may select only the healthy plants for artificial inoculation of a pathogen.

9. Frequency distribution, histogram, normal curve, mean, median, mode, variance, standard deviation, standard error

Biometry:

Statistics is a branch of science which deals with methods of collection, classification and analysis, i.e., drawing inferences from collected data; then testing of hypothesis and making comment on these.

The statistics can be applied in all scientific branches like social, physical and bio-logical. The use of statistics in Biology is known as Biostatistics or Biometry. It deals with the application of different statistical methods and analysis of data collected from biological system.

Application of Biometry:

In biological system, it is very difficult to draw any concrete conclusion about any happening, as there is much difference among the individuals. Such as in the same plant, the leaves are all different; again in a field, all the same kind of plants are not identical; in the same place, the same crop does not respond equally in different plots. So it is very difficult to reach at a definite and reliable conclusion unless we take the help of statistical analysis of the observed data.

In case of plant breeding, the application of biometry has immense importance, as the statistical analysis only helps to conclude about a population of plant; such as:

(a) How the character, which is acquired by the population, is distributed, how the character is being inherited, etc.

(b) Whether two characters in a population are correlated or not, if correlated, how much they are correlated.

(c) In case of plant breeding whether Mendelian Genetics is being followed or not, can be tested by chi-square test.

(d) The application of fertilizer or irrigation or any kind of cultural practice can be effectively justified by application of test of significance.

(e) The laws of probability is of importance in genetics for forecasting the chance of obtaining certain result from a cross and elucidating the operation of ge-netic principle.

(f) The effects of two or more different fertilizer application on the same field can be analysed by analysis of variance.

(g) Study of alleles of genes in populations and the factors which maintain or change the frequencies of particular genotype in populations can be studied.

Limitation of Biometry:

(a) All the conclusions about statistical analysis depend on the availability of sample data. If the sampling is biased, the analysis will be eronotic.

(b) Statistics can be used and applied only on collective data, not on individual data.

(c) Statistical laws are always true in case of large population on the average, not on the small sample.Frequency distribution

For any statistical analysis, the handling with raw data requires some treatment, i.e., the classification of data to organise the available values in a more compact way. The frequency distribution presents the data very concisely indicating how frequently a vari-able occurs in a group of study.

Construction of Frequency Distribution Table:

If there are repetitions in individual values or items of any investigation, suitable frequency table can be formed. These frequency tables may be discrete or may be continu-ous in nature. The available raw data at first should be converted into arrayed data. For biostatistics the raw data are arranged in ascending order to make it arrayed data (Example-2).

Example 1:

Following raw data is obtained in an investigation. 100 pea plants bore pods ranging from 15 to 41 in a garden of pea plants (see Raw Data Table A):

Raw Data Table A:

33	31	28	15	17	17	16	18	16	18	20	22	24
25	31	27	30	29	33	28	20	22	23	25	41	39
30	36	37	27	33	28	31	29	32	31	29	34	19
22	25	40	19	21	24	30	26	37	27	28	32	32
31	29	34	21	23	25	40	26	38	27	26	33	28
34	29	30	30	35	29	23	29	26	38	27	32	28
34	35	29	30	33	32	35	29	24	26	38	27	36
28	34	29	35	30	33	32	36	37				

Arrayed Data Table B:

15	16	16	17	17	18	18	19	19	20	20	21	21
22	22	22	23	23	23	24	24	24	25	25	25	25
26	26	26	26	26	27	27	27	27	27	27	28	28
28	28	28	28	28	29	29	29	29	29	29	29	29
29	30	30	30	30	30	30	30	31	31	31	31	31
32	32	32	32	32	32	33	33	33	33	33	33	34
34	34	34	34	35	35	35	35	36	36	36	37	37
37	38	38	38	39	39	40	40	41				

Then for discrete frequency distribution table, the values of variables are written in one column and the repetition of that value is written against it which is the frequency (Table 9.1, 9.2).

Table 9.1 : Determination of frequency of each variable

No. of Pods Variable	Tally mark	Repetition number of Plants or Frequency
15	I	1
16	II	2
17	II	2
18	II	2
19	II	2
20	II	2
21	II	2
22	III	3
23	III	3
24	III	3
25	IIII	4
26	卌	5
27	卌 I	6
28	卌 II	7
29	卌 IIII	9
30	卌 II	7
31	卌	5
32	卌 I	6
33	卌 I	6
34	卌	5
35	IIII	4
36	III	3
37	III	3
38	III	3
39	II	2
40	II	2
41	I	1

Table 9.2 : Variables and respective frequency			
No. of Pods (Variables)	No. of Plants (Frequency)	No. of Pods (Variables)	No. of Plants (Frequency)
15	1	29	9
16	2	30	7
17	2	31	5
18	2	32	6
19	2	33	6
20	2	34	5
21	2	35	4
22	3	36	3
23	3	37	3
24	3	38	3
25	4	39	2
26	5	40	2
27	6	41	1
28	7		Σf = 100

But for continuous frequency distribution table, the values are grouped in fixed interval and then the frequency within that interval is observed and noted.

The number of classes or the range of class interval is an important factor for making this kind of frequency distribution table. There is no fixed rule for how many classes can be formed, generally it depends on observation of available data, minimum 3 classes and maximum 20 classes can be formed.

The size of class interval also depends on the range of data and the number of classes, it is equal to the difference between highest and lowest value divided by the number of classes,

i – H-L/K

where i = class interval K.

H = Highest value

L = Lowest value

K = number of classes.

Overlapping Frequency Distribution Table:

Values of variables are grouped in such a fashion that the upper limit of one class interval is represented in next class interval. In an example (Example 2), number of pods ranges from 15 to 41, the classes may be 15-17, 17-19, 19-21, etc. (Table 9.3).

Table 9.3 : Overlapping frequency table	
No. of Pods in class interval	No. of plants in frequency
15–17	3
17–19	4
19–21	4
21–23	5
23–25	6
25–27	9
27–29	13
29–31	16
31–33	11
33–35	11
35–37	7
37–39	6
39–41	4
	Σf = 100

Non-Overlapping Frequency Distribution Table:

Values of variables are grouped in such a fashion that the upper level of one class interval does not overlap the preceding class interval. In the above example, number of pods ranges from 15 to 41, the classes can be prepared like 15-17, 18-20, 21-23, etc. (Table 9.4).

Table 9.4 : Non-overlapping frequency table

No. of Pods in class interval	No. of plants in frequency
15–17	5
18–20	6
21–23	8
24–26	12
27–29	22
30–32	18
33–35	15
36–38	9
39–41	5
	$\Sigma f = 100$

Cumulative Frequency Distribution Table:

Cumulative frequency is determined by adding the frequency of a class interval with the frequency of the preceding class interval. The cumulative frequency table can be prepared from both the overlapping and non-overlapping frequency distribution table (Table 9.5, 9.6).

Table 9.5 : Overlapping cumulative frequency distribution table

Class interval	Frequency	Cumulative frequency
15–17	3	3
17–19	4	3 + 4 = 7
19–21	4	7 + 4 = 11
21–23	5	11 + 5 = 16
23–25	6	16 + 6 = 22
25–27	9	22 + 9 = 31
27–29	13	31 + 13 = 44
29–31	16	44 + 16 = 60
31–33	11	60 + 11 = 71
33–35	11	71 + 11 = 82
35–37	7	82 + 7 = 89
37–39	6	89 + 6 = 95
39–41	5	95 + 5 = 100

Table 9.6 : Non-overlapping cumulative frequency distribution table

Class interval	Frequency	Cumulative frequency
15–17	5	5
18–20	6	5 + 6 = 11
21–23	8	11 + 8 = 19
24–26	12	19 + 12 = 31
27–29	22	31 + 22 = 53
30–32	18	53 + 18 = 71
33–35	15	71 + 15 = 86
36–38	9	86 + 9 = 95
39–41	5	95 + 5 = 100

Relative frequency of a class = Cumulative frequency of that class/Total no. of sample.

Class Limit:

It is defined as two boundaries of a class, i.e., the highest and lowest values of a class, which can be represented by L1 and L2.

Mid-Value of Class-Interval:

The central point of a class interval is called its mid-value or mid-point of that class, which is obtained by using the following formula.

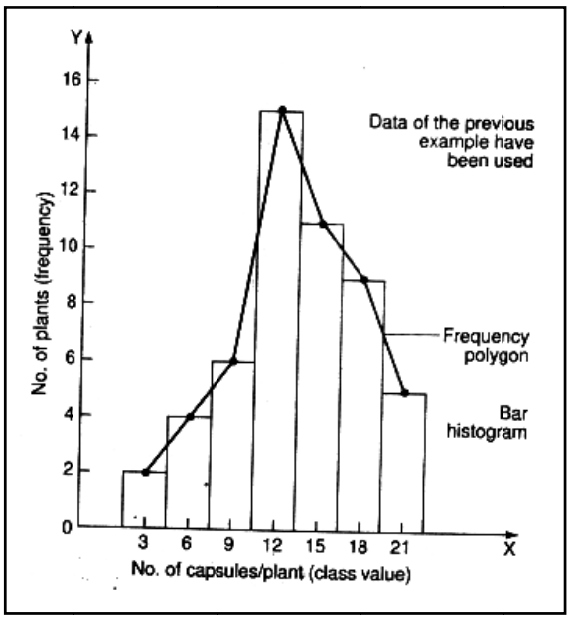
Mid – value of a class = L1 + L2/2

Frequency distribution

The diagrams commonly used to depict statistical data, given in the form of frequency distribution are: 1. Histogram, 2. Frequency polygon, 3. Ogive.

1. **Histogram**: It is the most common form of diagrammatic representation of a grouped frequency distribution. It consists of a set of adjoining rectangles drawn on a horizontal base line with area proportional to the class frequencies.

Uses: (a) give a visual representation of the relative size of the various groups; (b) the surface of the tops of rectangles gives an idea of nature of frequency curve for the population; (c) may be used to find out the mode graphically.



2. **Frequency polygon**: It is alternative to histogram and derived by joining the mid-points of the tops of consecutive rectangles.

3. **Ogive**: It is the graphical representation of cumulative frequency distribution and hence called cumulative frequency polygon.

Normal Distribution:

If we observe in any population any attribute is distributed mostly near the mean value and equally distributed to the higher and lesser value gradually in decreasing order then the distribution pattern is called normal distribution.

When this kind of normally distributed attribute is plotted graphically with the help of available data, the normal distribution pattern gives a bell shaped symmetrical curve which is called 'normal distribution curve'. In this curve the mean value lies in the peak of the curve.

Properties of Normal Distribution Curve:

1. It is a continuous bell shaped curve which is associated with continuous variable.

There is only one maximum peak (unimodal). The normal curve is symmetrical and asymptotic (touches at infinity).

3. The height of normal curve is maximum at its Mean. Mean, Median and Mode coincides in normal curve.

4. The peak divides the distribution in two equal halves.

5. Most of the observations are clustered around the Mean and there are rela-tively a few observations at the extremes.

6. The normal distribution curve has a fixed mathematical characteristic feature independent of the scale. (unit of measurement) of magnitude.

Skewness and Kurtosis:

In normal distribution, most of the cases fall in the middle but there are cases in which central tendency do not exhibit normal behaviour.

There are two types of divergence from normal distribution:

(i) Skewness and
(ii) Kurtosis

Skewness means that the curve is not symmetrical. In a skewed distribution, the Mean, Median and Mode do not coincide it pulls the Median and Mean away from Mode either left or right. In a skewed distribution, the frequency curve is not bell shaped and values do not lie on both sides of measure of central tendency equally. Here Mean Median and Mode fall at different points.

In symmetrical distribution curve, Mode coincides with Mean and Median. In positively skewed curve, the value of Mean and Median lie away from Mode values (right hand), the values are greater than Mode. In negatively skewed curve, the value of Mean and Median lie left hand to Mode value, the values are lesser than the Mode value.

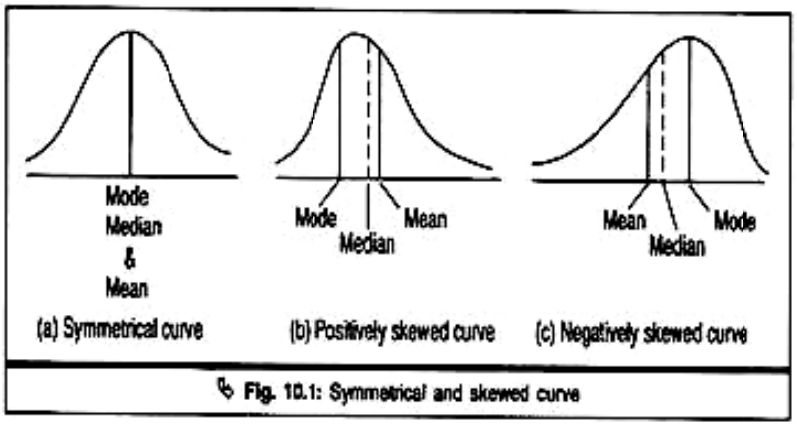


Fig. 10.1: Symmetrical and skewed curve

Measures of Central Tendency

Mean:

Arithmetic mean

It is most commonly used of all the averages. It is the value which we get by dividing the aggregate of various items of the same series by the total number of observations.

When observations are denoted by x values showing x1, x2, x3… xn; the total number of observations is calculated by summing up the observations and dividing the sum by the total number of observations (n)

Find out the average pod length of the plant.

$$\bar{x} = \frac{x_1 + x_2 + x_3 + \cdots + x_n}{n}$$

Example 1: The pod length of ten pods of a plant shows following data:

5.2 cm,	5.3 cm,	5.6 cm,	5.7 cm,	5.4 cm,
5.2 cm,	5.3 cm,	5.3 cm,	5.4 cm,	5.2 cm.

Find out the average pod length of the plant.

$$\bar{x} = \frac{\sum x}{n} = \frac{5.2 + 5.3 + 5.6 + 5.7 + 5.4 + 5.2 + 5.3 + 5.3 + 5.4 + 5.2}{10} \text{cm}$$

$$= \frac{53.6}{10} \text{cm} = 5.36 \text{cm}$$

When the series is discrete, each value of the variable is multiplied by their respec-tive frequencies, sum of all values is divided by total number of frequencies. Variable x has the values like x1, x2, x3, …, xn and their frequencies are f1 , f2, f3, …, f respectively.

Then Arithmetic Mean:

$$\bar{x} = \frac{f_1 x_1 + f_2 x_2 + f_3 x_3 + \cdots + f_n x_n}{f_1 + f_2 + f_3 + \cdots + f_n} = \frac{\sum fx}{\sum f}$$

When the series is continuous, the arithmetic mean is calculated after taking the midpoint value of class intervals.

$$\bar{x} = \frac{\sum fm}{\sum f}$$

where, $\bar{x}$ = Arithmetic mean

$\sum fm$ = Sum values of midpoint value multiplied by their frequencies

If = Sum of frequencies

m = Mid points of various class intervals.

Example 2:

An observation on 32 Balsam plants shows the following data. Calculate the arithmetic mean.

No. of flowers/plant (x)	4	5	6	7	8	9
No. of plants (f)	3	5	6	9	5	4

No. of flowers / plant (x)	No. of plants (f)	f x x
4	3	12
5	5	25
6	6	36
7	9	63
8	5	40
9	4	6
	$\Sigma f = 32$	$\Sigma fx = 212$

$$\bar{x} = \frac{\Sigma fx}{\Sigma f} = \frac{212}{32} = 6.62 \text{ (approx.)}$$

The average number of flowers/plant is 6.62.

No. of pods/plant	Mid points of class (m)	No. of plants frequency (f)	m.f.
15-17	16	5	80
18-20	19	6	114
21-23	22	8	176
24-26	25	12	300
27-29	28	22	616
30-32	31	18	558
33-35	34	15	510
36-38	37	9	333
39-41	40	5	200
		$\Sigma f = 100$	$\Sigma mf = 2,887$

$$\text{Arithmetic Mean} = \bar{x} = \frac{\Sigma mf}{\Sigma f} = \frac{2.887}{100} = 28.87.$$

Merits, Demerits and Uses of Arithmetic Mean:

Merits:

1. It has the simplest formula to calculate and it is easily understood.
2. It is rigidly defined mathematical formula the same result will come on repeated calculations.
3. The calculation is based on all the observations.
4. It is least affected by sampling fluctuation.
5. The arithmetic mean balances the value on either side.

6. It is the best measure to compare two or more series.

7. Arithmetic mean is totally dependent on values not on the position.

Demerits:

1. It cannot be calculated if all the values are not known.

2. The extreme values have greater effect on mean.

3. The qualitative data cannot be measured in this way.

Uses:

1. The arithmetic mean is mostly used in practical statistics.

2. Mean helps to calculate many other estimates in statistics.

3. The arithmetic mean is most popular method of any measurement used by common people to get the average of any data.

(b) Geometric Mean:

The geometric mean is defined as the n-th root of the product of n observations.

$$\textbf{Geometric Mean (GM)} = \sqrt[n]{x_1 \cdot x_2 \cdot x_3 \cdots x_n}$$

Where n = number of observations; x1, x2, x3… xn = variable values.

When n is small then the above formula can be applied but in case of large 'n' number the logarithms are used to find out the GM

$$GM = \text{Anti log}\frac{\log x_1 + \log x_2 + \log x_3 + \cdots + \log x_n}{n} = \text{Anti log}\frac{\log x}{n}$$

Example 3:

Find out the geometric mean of the following seeds, x denotes the weight of each seed in mg.

5 mg, 7 mg, 8 mg, 6 mg and 4 mg.

x	log x
5 mg	0.70
7 mg	0.85
8 mg	0.90
6 mg	0.78
4 mg	0.60
	Σ log x = 3.83

$$\frac{\Sigma \log x}{5} = \frac{3.83}{5} = 0.77$$

Antilog of $\frac{\Sigma \log x}{5}$, i.e., antilog of 0.77 = 5.89 mg

So the geometric mean of seed weight = 5.89 mg.

This mean is based on all observations, rigidly defined, less affected by extreme values. This mean is difficult to understand, compute and interpret.

This mean is mostly helpful in averaging ratios, percentage and determining ratio of change. This mean is important in construction of index number.

(c) Harmonic Mean:

When the variables are expressed in ratios or rates, the proper average to be calcu-lated through harmonic mean. The harmonic mean is defined as the reciprocal of arith-metic mean of the reciprocal of the given values.

The harmonic mean is applicable only in restricted field such as oxygen consump-tion/hour, calorie requirement/hour, CO_2 evolution/hour, flow of sap/min, etc.

$$\text{Harmonic mean (HM)} = \frac{n}{\frac{1}{x_1}+\frac{1}{x_2}+\frac{1}{x_3}+\cdots+\frac{1}{x_n}} = \frac{n}{\Sigma\left(\frac{1}{x}\right)}$$

Where n = Total number of observation; x1, x2, x3 are the values of variables.

Example 4:

In a particular experiment, 5 different sets of *Hydrilla* plants showed O_2, evolution/hour, was recorded.

2.5 c.c./hour, 1.8 c.c./hour, 2.0 c.c./hour

2.2 c.c./hour, 2.4 c.c./hour.

$$HM = \frac{5}{\frac{1}{2.5}+\frac{1}{1.8}+\frac{1}{2.0}+\frac{1}{2.2}+\frac{1}{2.4}} = \frac{5}{.4+.55+.5+.45+.41} = \frac{5}{2.3} = 2.17 \text{ c.c./hour.}$$

So, harmonic mean of the observation is 2.17 c.c./hour. This HM determination is based on all the observations of a series. It gives more weightage to the smaller items and also not much affected by sample fluctuation.

It is not very easy to calculate and also the positive and negative, both values, cannot be computed.

Mode:

Most frequent value in a series. Mode cannot be determined from a series of individual observations unless it is converted into either a discrete or continuous series. In a discrete series the value of the variable against which the frequency is the largest would be the modal value.

For example, 2, 4, 4, 4, 6, 9, 3, 2, 4, 6, 11, 13 mode is 4 as it is occurring maximum.

For example, 5, 3, 6, 3, 5, 10, 7, 2 mode is 3 and 5 such series is known as bi-modal series.

Similarly in a continuous frequency distribution the class interval having the maximum frequency would be the modal class.

Mode can be determined from grouped data using the following formula:

$$\text{Mode} = l_1 + \frac{\Delta_1}{\Delta_1 + \Delta_2} \times i,$$

where l_1 = lower limit of modal class, Δ_1 = difference of frequencies be-tween modal class and the preceding class, Δ_2 = difference of frequencies between modal class and post modal class and i = class interval.

For example:

Class interval (Marks obtained)	*Class value*	*Frequency*
1–9	5	3
10–20	15	5
21–29	25	12
30–40	35	15
41–49	45	25
50–60	55	40
61–69	65	18
70–80	75	11
81–89	85	3
90–100	95	2

Modal class (*maximum frequency*) is 50–60.

$$\text{Mode} = l_1 + \frac{\Delta_1}{\Delta_1 + \Delta_2} \times i$$

$$l_1 = 50, \Delta_1 = 40 - 25 = 15, \Delta_2 = 40 - 18 = 22, i = 10.$$

$$\text{Mode} = 50 + \frac{15}{15 + 22} \times 10 = 50 + 4.054 = \mathbf{54.05.}$$

Merits of Mode:

i. It is simple and easily understood.

ii. Mode is not affected by the values of extreme items provided they follow to the natural law relating to extremes.

iii. For determination of mode all values in the series are not considered.

Demerits of Mode:

i. As mode is not based on all observations of a series, therefore, it is not rigidly defined.

ii. Mode is not capable of further mathematical treatment.

iii. Mode may be unrepresentative in many cases and it may be impossible to set a definite value of mode as in a set of observations 2 or 3 or more modal values may occur.

Median:

Median is the value of the middle item of a series when arranged in order of magnitude (ascending or descending order).

M = size of (n+ 1)/2 th item, where M stands for median and n for the number of items.

For example,

from ungrouped data: 3, 15, 6, 9, 21, 7, 12, 10
arrange the series is magnitude: 3, 6, 7, 9, 10, 12, 15, 21

$n = 8$ (*the series is even*)

$$M = \text{size} = \frac{(8+1)}{2} = 4.5.$$

In such case 4th and 5th items would be added and divided by 2.

$$\therefore \text{Median} = \frac{9+10}{2} = 9.5.$$

For example,

series: 2, 12, 9, 6, 17, 5, 8, 14, 23, 27, 11
arrange in magnitude: 2, 5, 6, 8, 9, 11, 12, 14, 17, 23, 27

$n = 11$ (*odd series*),

$$\text{Median} = \text{size of } \left(\frac{11+1}{2}\right) \text{ th item} = \text{size of 6th item} = 11.$$

Median from grouped data can be calculated from the following formula:

$$\text{Median} = l_1 + \frac{N/2 - F}{fm} \times i,$$

Where, l_1 = lower limit of median class,

N = total frequency,

F = cumulative frequency,

f_m = frequency of the median class and

i = class interval of median class.

For example, find out median value from the following distribution:

Age group of children	*No. of children (frequency)*	*Cumulative frequency*
1–3	5	5
4–6	10	15
7–9	12	27
10–12	35	62
13–15	20	82
16–18	17	99
19–21	9	108
	N = 108	

$$\text{Median} = \text{the value of } \left(\frac{n+1}{2}\right) \text{th item} = \frac{108+1}{2} = 54.5,$$

which lies in 7–9 class. Therefore, 7–9 is the median class.

Applying the formula

$$M = 7 + \frac{54.5 - 27}{12} \times 2 = 11.58.$$

Another formula can be used

$$M = l_1 + \frac{l_2 - l_1}{f_1}(m - c),$$

l_1 = lower limit of the median class, l_2 = upper limit, f_1 = frequency of median class, m = middle item; c = cumulative frequency of the group preceding the median group.

$$M = 7 + \frac{9-7}{12}(54.5 - 27) = 7 + \frac{2}{12}(27.5) = 11.58.$$

Merits of Median:

i. Easily calculated and better understood and not affected by the values of the extreme items.

ii. It can be located merely by inspection in many cases.

iii. It gives best result in a study of those phenomena which are incapable of direct quantitative measurement, for example, assessment of madness among mentally retarded patients. In such case it is possible to arrange a group of patients in ascending or descending order in relation to the degree of madness and thus to locate a person whose madness can be said to be average.

Demerits of Median:

i. Median may not be representative of a series in many cases.

ii. It is not suitable for further algebraic treatment.

iii. When median has to be calculated in continuous series it requires inter-polation.

iv. Median ignores the values of extremes in the series.

v. Median is likely to be affected by the fluctuations of sampling.

Example 1:

Find out mean, mode and median from the following data and draw the frequency distribution curve:

Solution:

No. of capsules/plant [Class interval]	*Class value (x)*	*No. of plants [frequency]*	*Cumulative frequency*	fx
1–5	3	3	3	9
6–10	8	7	10	56
11–15	13	12	22	156
16–20	18	20	42	360
21–15	23	27	69	621
26–30	28	32	101	896
31–35	33	21	122	693
36–40	38	16	138	608
41–45	43	10	148	430
46–50	48	6	154	288
51–55	53	2	156	106
		N = 156		Σfx = 4223

$$\text{Mean} = \bar{x} = \frac{\Sigma fx}{n} = \frac{4223}{156} = 27.07.$$

Mode = Modal class 26–30.

$$\text{Mode} = l_1 + \frac{\Delta_1}{\Delta_1 + \Delta_2} \times i = 26 + \frac{5}{5+11} \times 4 = 27.25.$$

$$\text{Median} = \text{the value of } \left(\frac{n+1}{2}\right)\text{th item} = \left(\frac{156+1}{2}\right) = 78.5,$$ thus median class is 26–30.

$$\text{Median} = l_1 + \frac{l_2 - l_1}{f_1}(m - c) = 26 + \frac{30-26}{21}(78.5 - 69) = 26 + \frac{4}{21}(9.5) = 27.81.$$

Variance:

Variance is a measure of variation and is the sum of square of deviation (d) divided by the number of degree of freedom (n — 1).

Variance of the sample $S^2 = \sum fd^2/(n-1)$

It is also denoted by σ^2.

Standard Deviation:

A statistical measuring the spread or variability of the sample around the mean or in other words it may be defined as the measure of dispersion of different variables around the central value.

It is square root of variance:

$$\sigma = \sqrt{\frac{\Sigma(x_i - \bar{x})^2}{n-1}} = \sqrt{\frac{\Sigma d^2}{n-1}}, \text{ or, } \sqrt{\frac{\Sigma fd^2}{n-1}}.$$

Merits:

1. The calculation is based on all observations.
2. It is more rigidly defined.
3. Less affected by fluctuations of sampling compared to other measures of dispersion.
4. It summarizes the deviation of large number of observations from mean and is expressed as one unit of variation.

Demerits:

1. It requires a lengthy calculation, i.e., squaring of deviations and then again square root of summed up values.
2. Not very simple to understand.
3. The calculation gives more weightage to extreme values.

Uses of Standard Deviation:

1. It helps in correlating and comparing of different samples.
2. It helps in finding the suitable size of sample for valid conclusion.
3. It helps in finding the standard error which determines whether the difference between means of two similar samples by chance or real.
4. The value of mean and standard deviation help to comment on the population on the basis of observation of sample (Fig. 10.3).

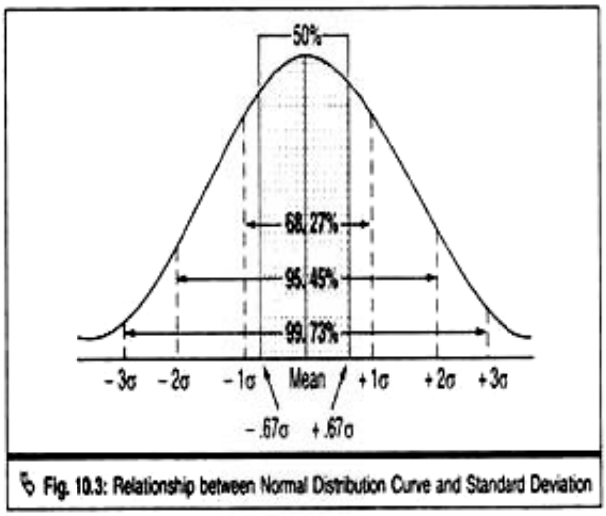


Fig. 10.3: Relationship between Normal Distribution Curve and Standard Deviation

(a) 50% of total observations lie in an area bounded by a distance of 0.6745 σ on both side of the mean.

(b) Mean ± 1σ covers the 68.27% area of the curve.

(c) Mean ± 2σ covers the 95.45% area of the curve.

(d) Mean ± 3a covers the 99.73% area of the curve.

Coefficient of Variation:

In measurement of dispersion, we use the units which are used for observation. But if we want to compare the dispersion of two different characters in the same population, then the calculation of coefficient of variation is needed.

This measurement is expressed in percentage ignoring the units. For example, from the same population if we like to study the length of pod and number of seeds/pod and to compare the dispersion of both the two characters, we must calculate the coefficient of variation.

Standard deviation expressed as percentage of the mean and is denoted by the formula:

Coefficient of variation = Standard deviation/Mean x 100

$$\text{or, c.v.} = \frac{\sigma}{\bar{x}} \times 100 \quad \text{or} \quad \frac{s}{\bar{x}} \times 100 \text{ in case of samples or population respectively.}$$

This coefficient of variation is also helpful to get an idea or compare two different populations about the dispersion of a character. More the c.v., more is the inconsistency about the dispersion of character.

Example 7:

In two different populations (Batch I and Batch II) the seed number/fruit is calculated:

Batch I 7, 9, 6, 8, 6, 5, 7, 8, 6, 8

Batch II 10, 8, 9, 10, 11, 10, 5, 6, 4, 7

Batch I

Seed number/fruit (x)	Frequency (f)	f × x	Deviation (|d|)	Deviation² (d²)	f × d²
5	1	5	2	4	4
6	3	18	1	1	3
7	2	14	0	0	0
8	3	24	1	1	3
9	1	9	2	4	4
	Σf = 10	Σfx = 70			Σfd² = 14

$$\bar{x} = \frac{\sum fx}{n} = \frac{70}{10} = 7$$

$$\sigma = \sqrt{\frac{\sum fd^2}{n-1}} = \sqrt{\frac{14}{9}} = 1.25$$

$$\text{c.v.} = \frac{\sigma}{\bar{x}} = \frac{1.25}{7} \times 100 = 17.8\%$$

Batch II

Seed number/fruit (x)	Frequency (f)	f × x	Deviation (|d|)	Deviation² (d²)	f × d²
4	1	4	4	16	16
5	1	5	3	9	9
6	1	6	2	4	4
7	1	7	1	1	1
8	1	8	0	0	0
9	1	9	1	1	1
10	3	30	2	4	12
11	1	11	3	9	9
	Σf = n = 10	Σfx = 80			Σfd² = 52

$$\bar{x} = \frac{\sum fx}{n} = \frac{80}{10} = 8$$

Standard error:

Standard error is the approximate standard deviation of a statistical sample population. Standard error is a statistical term that measures the accuracy with which a sample represents a population. In statistics, a sample mean deviates from the actual mean of a population; this deviation is the standard error.

The formula for the standard error of the mean is:

$$\sigma_M = \frac{\sigma}{\sqrt{N}}$$

where σ is the standard deviation of the original distribution and N is thesample size (the number of scores each mean is based upon).

Solved Problem:

Find out mean, standard deviation, mean deviation, coefficient of variation and standard error from the given sample:

Class value (x)	*Frequency* (f)	fx	$(x - \bar{x}) = d$	fd	d^2	fd^2
48	8	384	−4.75	+38.00	22.56	180.50
50	32	1600	−2.75	+88.00	7.56	242.00
52	75	3900	−0.75	+56.25	0.56	42.18
54	52	2808	+1.25	+65.00	1.56	81.25
56	28	1568	+3.25	+91.00	10.56	295.75
58	5	290	+5.25	+26.25	27.56	137.81
	n = 200	Σfx = 10,550		Σfd = 364.50		979.49

$$\text{Mean } \bar{x} = \frac{\Sigma fx}{n} = \frac{10,550}{200} = \mathbf{52.75.}$$

$$\text{Mean deviation} = \frac{\Sigma fd}{n}$$

[Note: The positive signs exactly cancel negative sign of deviations and the summation of deviations gives a value of zero and, therefore, the spread of distribution cannot be shown. In calculation of mean deviation, the algebraic signs may be ignored.]

$$= \frac{364.50}{200} = 1.8225 = \mathbf{1.82.}$$

$$\text{Standard deviation } \sigma = \sqrt{\frac{\Sigma fd^2}{n-1}} = \sqrt{\frac{979.49}{200-1}}$$

$$= \sqrt{4.9221} = 2.2185 = \mathbf{2.22.}$$

$$\text{Coefficient of variation (CV)} = \frac{\sigma}{\bar{x}} \times 100 = \frac{2.22}{52.75} \times 100$$

$$= 4.2085 = \mathbf{4.21\%.}$$

$$\text{Standard error} = \frac{\sigma}{\sqrt{n}} = \frac{2.2}{\sqrt{200}} = 0.1556 = 0.16.$$

Therefore, mean is represented as $52.75 \pm \mathbf{0.16.}$

10. Probability & test of significance (Student & paired t) $\chi 2$ test (detection of segregation ratio & linkage, test of independence & heterogeneity), analysis of variance (F test) CD, genetic advance, heritability estimation.

Probability is a number expressed in a quantitative scale. When one event will not occur at all then the probability of that event is 0, and if there is any event which will happen positively without fail then the probability of that event is 1. But in biological science, mostly we find the probability of any event lies between impossibility to certainty i.e., the value ranges from 0 to 1.

Mathematically probability can be explained in the following way:

If an event can happen in 'a' number of ways, and fails to happen in 'b' number of ways, then the probability of its happening 'p' is written as.

$$p = \frac{a}{a+b} \quad \text{or} \quad p = \frac{\text{Number of events occurring}}{\text{Total number of events}}$$

Similarly **probability of failure** of any event is denoted as 'q', where,

$$q = \frac{b}{a+b} \quad \text{or} \quad q = \frac{\text{Number of events not happening}}{\text{Total number of events}}$$

Therefore, $p + q = \frac{a}{a+b} + \frac{b}{a+b} = 1$

So, if the probability of happening any event is 0.7, then the probability of not happening of that event is 0.3.

Events:

The results of any experiment in all possible forms are said to be events of that experiment. Such as, throwing of a dice has 6 possible outcomes, either 1 or 2 or 3 or 4 or 5 or 6. All these six outcomes are called events of that single experiment.

Null Event:

When there is no chance of getting an event is called null or impossible event. It is symbolically denoted by ϕ. Such as, survival of any human being forever is an impossible event or null event.

Sure Event:

If the likelihood of occurrence of any event is sure then the event is called sure event. Such as, the death of a human being is a sure event.

Equally likely events:

If the likelihood of the occurrence of every event in an experiment is same then those are called as equally likely events. Such as, when a dice is thrown, there is no biasness, there are the possibilities of coming any number 1 to 6 in equal probability, so the events are equally likely events.

Mutually Exclusive Events:

If in an experiment the occurrence of one event prevents or rules out the happening of all other events, then these are called as mutually exclusive events. Such as, when a coin is tossed either 'head' or 'tail' will come.

The occurrence of one event affects the occurrence of another event, both events cannot occur together, i.e., occurrence of 'head' rules out getting 'tail' in the same trial. Here the events are connected by the words 'either' or 'or'.

Simple Event and Compound Event:

Any event having only one sample point of a sample space is called simple event and if any event is decomposable into a number of simple events then it is called as compound event.

Such as, if a bag contains 4 white and 6 red balls, and if one ball is drawn then it is simple event, but if two balls are drawn together then the events will be — 'both the balls are white', 'both the balls are red', 'one ball is white and another ball is red' — these are compound events.

The compound events may be of two types:

Independent Event:

Two or more events are said to be independent events when the outcome of one event does not affect or is not affected by the other events. For example, if a coin is tossed twice, the result of second tossing would in no way be affected by the result of first tossing, so these are independent events.

Dependent Event:

The occurrence or non-occurrence of one event in one trail affects the probabilities of other events in other trails are called dependent events. For example, the probability of drawing a queen from a pack of 52 cards is 4/52, but if the card drawn for the first time (queen) is not replaced then the probability of second drawing of a queen is 3/51, as the pack now contains 3 queens and 51 cards.

Addition and Multiplication Rules:

Probability is estimated usually on the basis of following two rules of chances:

1. Addition rule

2. Multiplication rule

Addition Rule:

This rule is applied when two events are mutually exclusive, i.e., both events cannot occur simultaneously. The birth of a male child excludes the birth of a female child in the same trial. The probability that one of several mutually exclusive events will occur is the sum of the probabilities of the individual events.

Mathematically, when two events A and B are mutually exclusive, the chance of occurrence or probability of occurrence of either A or B can be calculated from the following formula:

$p (A \text{ or } B) = p(A) + p(B)$

This rule is applicable to any number of mutually exclusive events as follows:

$p(E_1 \text{ or } E_2 \text{ or } E_3 \ldots E_n) = p(E_1) + p(E_2) + p(E_3) + \ldots + p(E_n)$

Example 1:

If a dice is rolled, what is the probability of getting either 3 or 5?

Probability of getting 3 is p(3) = 1/6

Probability of getting 5 is p(5) = 1/6

∴ Probability of getting either 3 or 5 is p(3) + p(5) = 1/6 + 1/6 = 1/3

Example 2:

What is the probability of getting a king or a joker from a pack of 54 cards?

Probability of getting a king is p(K) = 4/54 = 2/27

Probability of getting a joker is P(J) = 2/54 = 1/27

So, the probability of either a king or a joker is

p(K or J) = p(K) + p(J) = 2/27 +1/27 = 3/27 = 1/9

Addition rule changes when the events are not mutually exclusive, i.e., if two events A and B can occur simultaneously in few cases, then the rule becomes modified in the following way:

p(A or B) = p(A) + p(B) – p(A and B)

Example 3:

What is the probability of getting a king or club from a pack of 52 cards?

In this example, getting a king and a club are not mutually exclusive events as there will be one king which is king of club. So the chance or probability of getting that event should be subtracted.

p(King or Club) = p(King) + p(Club) – p(King and Club)

Probability of King = p(King) = 4/52 = 1/13

Probability of Club = p(Club) = 13/52 = 1/4

Probability of King and Club = p (King and Club) = 1/52

So, p(King or Club) = (1/13 + 1/4) – 1/52 = 4/13

Multiplication Rule:

(a) When the Events are Independent:

Probability of two or more independent events occurring together is the product of the probabilities of individual events.

Symbolically, if p(A) and p(B) are the probabilities of two respective events A and B, and the happening of these two events are independent then the probability of happening both the events together can be calculated with the following formula:

p(A and B) = p(A) x p(B)

Thus the rule may be extended to any number of independent events like E_1, E_2, E_3… E_n, and the formula will be as follows:

$p(E_1$ and E_2 and E_3 … and $E_n) = p(E_1)$ x $p(E_2)$ x $p(E_3)$ x … x $p(E_n)$

Example 4:

If two dice are thrown simultaneously what is the probability of getting 3 in both the dice?

The probability of getting 3 in first dice is p(A) = 1/6

The probability of getting 3 in 2nd dice is p(B) = 1/6

So, the probability of getting 3 in both the dice is

p(A and B) = p(A) × p (B)= 1/6 × 1/6 = 1/36

(b) When the Events are Dependent:

When the probability of happening one event is affected by the occurrence of another event then it is called conditional probability. Such as, conditional probability of happening A, when B has already happened, is denoted as p(A/B); conditional probability of B, and A has already happened, is denoted as p(B/A).

When the two events A and B are occurring simultaneously but any one event has conditional probability then the multiplication rule will be written as:

p(ab) = p(A)p(B/A) or p(B)-p(A/B)

where p(A/B) = Conditional probability of A given that B has happened

p(B/A) = Conditional probability of B given that A has happened

Example 5:

Four cards are drawn consecutively four times from a pack of 52 cards. Find the chances of drawing an ace, a king, a queen and a jack. The cards are not replaced after each withdrawal.

Probability of drawing an ace = p(A) = 4/52

Probability of drawing a king = p(K) = 4/51

Probability of drawing a queen = p(Q) = 4/50

Probability of drawing a jack = p(J) = 4/49

So, the combined probability

p(A and K and Q and J) = p(A) x p(K) x p(Q) x p(J)

= 4/52 x 4/51 x 4/40 x 4/49 = 0.317

Example 6:

Four cards are drawn in four consecutive drawals from a pack of 52 cards without replacing the cards after each drawal. What is the probability of drawing a king in each drawal?

The probability of getting a king in 1st drawal = 4/52

The probability of getting a king in 2nd drawal = 3/51

The probability of getting a king in 3rd drawal = 2/50

The probability of getting a king in 4th drawal = 1/49

So, the combined probability of getting a king in 4 consecutive drawals is

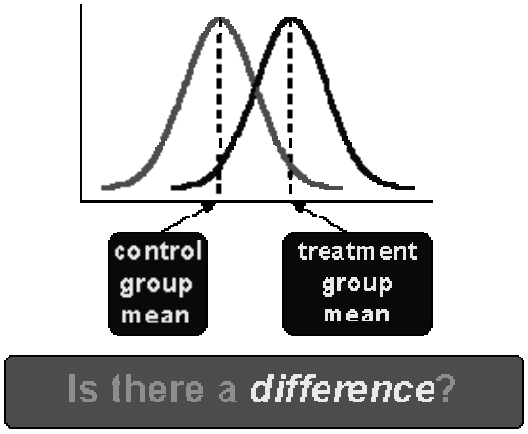
4/52 x 3/51 x 2/50 x 1/49 = 1/270725

Test of Significance:

In biological research when we compare any character of two samples, we calculate the significance of difference in the mean and variance to draw a meaningful conclusion.

Student's 't'-Test:

To test the significance of difference of means of two samples, W.S. Gosset applied a statistical tool called 't'-test. According to nick name of Gosset, the test has been named as Student's 't'-test.



In this test we make a choice between two alternatives:
1. To accept the null hypothesis (no difference between the two means);
2. To reject the null hypothesis (the difference between the means of two samples significant).
The test is applied to small sample (n<30), and samples must be drawn randomly from normal population. The 't' is defined as quantity representing the difference between the sample mean or true means or population mean expressed in terms of the standard error.
T = Difference between sample means/Standard error of the difference between means

$$t = \frac{\bar{x}_1 - \bar{x}_2}{SE_D}$$

$\bar{x}_1$ and $\bar{x}_2$ = Mean of two samples

SE_D = Standard error of the difference between means

Degree of Freedom:

To calculate the standard error of the difference, the degree of freedom can be obtained by one less than the number of observations of sample. In case of unpaired 't' test, i.e., when the comparison between two samples is done, degree of freedom is calculated by the formula
$(n_1 + n_2) - 2$, where n_1 = no. of observations in sample I
n_2 = no. of observations in sample II.
Whereas, in case of paired 't' test, the degree of freedom is (n – 1), as there is the same number of observations.

Determination of Significance:

Probability of occurrence of any calculated value of 't' is determined by comparing it with the value given in the 't'-table corresponding to the calculated degree of freedom, derived from the number of observations in the samples under study. If the calculated value of 't' exceeds the value given at p = 0.05 (5% level) in the table, it is said to be significant. If the calculated value of 't' is less than the value given in 't'-table, it is not significant.

The 't'-test procedure is done to locate the observed value of 't' in the student's 't'- distribution curve. The 't' distribution curve is a symmetrical curve with mean zero, it extends to infinity on either sides. When degree of freedom is less in number, 't'- distribution resembles to normal distribution curve. If the calculated value of 't' is near the centre then the data is regarded as compatible with H_0, which concludes that the observed deviation is due to chance factor or only due to sampling. If the 't' value is situated at tail region then $H_0$is not accepted.

Unpaired t-Test:

This test is applied to unpaired data of independent observations made on individuals of two different or separate groups or samples drawn from two different populations.

Steps to be followed for calculation of 't':

1. The means of two samples are calculated and the differences between the means of two samples are calculated.

$$(\overline{x}_1 - \overline{x}_2)$$

2. The standard error of the difference between two means is calculated.

$$\sigma_d = \sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}$$

$$\sum fx_1^2 - \frac{(\sum fx_1)^2}{n_1} \qquad \sum fx_2^2 - \frac{(\sum fx_2)^2}{n_2}$$

3. 't' value is calculated by the ratio between the observed difference of means and its standard error.

For Large Sample,

$$\sigma_d = \sqrt{\frac{\Sigma(x_1 - \overline{x}_1)^2 + \Sigma(x_2 - \overline{x}_2)^2}{n_1 + n_2 - 2}} \quad \text{or} \quad \sqrt{\frac{\Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n_1} + \Sigma x_2^2 - \frac{(\Sigma x_2)^2}{n_2}}{n_1 + n_2 - 2}}$$

$$\sigma_d = \sqrt{\frac{\Sigma(x_1 - \overline{x}_1)^2 + \Sigma(x_2 - \overline{x}_2)^2}{n_1 + n_2 - 2}} \quad \text{or} \quad \sqrt{\frac{\Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n_1} + \Sigma x_2^2 - \frac{(\Sigma x_2)^2}{n_2}}{n_1 + n_2 - 2}}$$

4. The calculated 't' value is compared with 't' table to find out the significance at that particular degree of freedom.

Practical sheet for 't' analysis:

No. of Replicates	Sample 1 x_1	Sample 1 x_1^2	Sample 2 x_2	Sample 2 x_2^2
1				
2				
3				
4				
5				
6				
—				
—				
—				
n				
	$\Sigma x_1 =$	$\Sigma x_1^2 =$	$\Sigma x_2 =$	$\Sigma x_2^2 =$
	$\overline{x}_1 =$		$\overline{x}_2 =$	
	$\frac{(\Sigma x_1)^2}{n_1} =$		$\frac{(\Sigma x_2)^2}{n_2} =$	

$$\sigma_d = \sqrt{\frac{\Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n_1} + \Sigma x_2^2 - \frac{(\Sigma x_2)^2}{n_2}}{n_1 + n_2 - 2}}$$

Small Sample

$$t = \frac{\overline{x}_1 - \overline{x}_2}{\sigma_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

Large Sample

$$t = \frac{\overline{x}_1 - \overline{x}_2}{\sigma_d}$$

Example 1:

In an experiment to find out the effect of a hormone spray on the seed yield of dwarf French bean, the following results were obtained. Analyse the data using the 't' test for inference on the effect of hormonal spray on the seed yield.

No. of seeds/plant

Control :	30	35	31	36	38	32	25	39	26	27	40	41	35	38	32
	31	38	30	35	36	25	28	29	37	36	36	30	31	34	33
	35	40	30	32	28	26	29	30	30	35	36	37	38	39	30
	31	39	40	35	36										
Treated :	35	38	40	45	40	42	46	48	49	50	35	38	45	47	45
	46	50	51	46	41	42	48	49	51	52	50	53	54	55	43
	45	50	44	51	52	53	52	48	50	49	47	49	50	49	51
	52	50	51	52	40										

Control (x_1) ← No. of seeds/plant → Treated (x_2)									
x_1	f	fx_1	x_1^2	fx_1^2	x_2	f	fx_2	x_2^2	fx_2^2
25	2	50	625	1250	35	2	70	1225	2450
26	2	52	676	1352	38	2	76	1444	2888
27	1	27	729	729	40	3	120	1600	4800
28	2	56	784	1568	41	1	41	1681	1681
29	2	58	841	1682	42	2	84	1764	3528
30	7	210	900	6300	43	1	43	1869	1849
31	4	124	961	3844	44	1	44	1936	1936
32	3	96	1024	3072	45	4	180	2025	8100
33	1	33	1089	1089	46	3	138	2116	6348
34	1	34	1156	1156	47	2	94	2209	4418
35	6	210	1225	7350	48	3	144	2304	6912
36	6	216	1296	7776	49	5	245	2401	12005
37	2	74	1369	2738	50	7	350	2500	17500
38	4	152	1444	5776	51	5	255	2601	13005
39	3	117	1521	4563	52	5	260	2704	13520
40	3	120	1600	4880	53	2	106	2809	5618
41	1	41	1681	1681	54	1	54	2916	2916
					55	1	55	3025	3025
	$\Sigma f=50$	$\Sigma fx_1=1670$		$\Sigma fx_1^2=56726$		$\Sigma f=50$	$\Sigma fx_2=2359$		$\Sigma fx_2^2=112499$

$$\bar{x}_1 = \frac{\Sigma fx_1}{\Sigma f} = \frac{1670}{50} = 33.4$$

$$\bar{x}_2 = \frac{\Sigma fx_2}{\Sigma f} = \frac{2359}{50} = 47.18$$

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sigma_d} = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}} = \frac{|33.4 - 47.281|}{\sqrt{\frac{19.347}{50} + \frac{24.518}{50}}} = \frac{13.88}{\sqrt{0.387 + 0.49}} = \frac{13.88}{\sqrt{0.877}} = \frac{13.88}{0.936} = 14.829$$

$$\sigma_1^2 = \frac{\Sigma fx_1^2 - \frac{(\Sigma fx_1)^2}{\Sigma f}}{\Sigma f - 1} = \frac{56726 - \frac{(1670)^2}{50}}{49} = \frac{56726 - 55778}{49} = \frac{948}{49} = 19.347$$

$$\sigma_2^2 = \frac{\Sigma fx_2^2 - \frac{(\Sigma fx_2)^2}{\Sigma f}}{\Sigma f - 1} = \frac{112499 - \frac{(2359)^2}{50}}{49} = \frac{112499 - 111297.62}{49} = \frac{1201.38}{49} = 24.518$$

Degrees of freedom = n1 + n2 – 2 = 50 + 50 – 2 = 98

Calculated t = 14.829

Tabulated t = 3.37 (p = 0.001)

Since the calculated t (14.829) far exceeds the tabulated t (3.37) with 98 d.f. at p = 0.001, the null hypothesis stating that there is no difference in the two sample means, is rejected. Alternatively there is a highly significant difference between the two sample means, i.e., the hormonal spray has a very significant effect on the seed yield.

Example 2:

Two varieties of potato plants (A and B) yielded tubers as shown in the following table. Does the mean weight of tubers of the variety A significantly differ from that of variety B.

Variety A : 2.2 2.5 1.9 2.6 2.3 1.8 2.0 2.1 2.4 2.3 kg/plant
Variety B : 2.8 2.5 2.7 3.0 3.1 2.3 2.4 3.2 2.5 2.9 kg/plant

Variety A		Variety B	
x_A	x_A^2	x_B	x_B^2
1.8	3.24	2.3	5.29
1.9	3.61	2.4	5.76
2.0	4.00	2.5	6.25
2.1	4.41	2.5	6.25
2.2	4.84	2.7	7.29
2.3	5.29	2.8	7.84
2.3	5.29	2.9	8.41
2.4	5.76	3.0	9.00
2.5	6.25	3.1	9.61
2.6	6.76	3.2	10.24
$\Sigma x_A = 22.10$	$\Sigma x_A^2 = 49.45$	$\Sigma x_B = 27.40$	$\Sigma x_B^2 = 75.94$

$$\bar{x}_A = \frac{22.10}{10} = 2.21 \qquad \bar{x}_B = \frac{27.40}{10} = 2.74$$

$$\Sigma x_A^2 - \frac{(\Sigma x_A)^2}{n_A} = 49.45 - \frac{(22.1)^2}{10} = 49.45 - 48.84 = 0.61$$

$$\Sigma x_B^2 - \frac{(\Sigma x_B)^2}{n_B} = 75.94 - \frac{(27.4)^2}{10} = 75.94 - 75.08 = 0.86$$

$$n_A + n_B - 2 = 10 + 10 - 2 = 20 - 2 = 18$$

Pooled variance,

$$\sigma_d^2 = \frac{0.61 + 0.86}{18} = \frac{1.47}{18} = 0.082$$

Pooled standard deviation,

$$\sigma_d = \sqrt{\sigma_d^2} = \sqrt{0.082} = 0.286$$

$$\therefore\ t = \frac{|\bar{x}_A - \bar{x}_B|}{\sigma_d\sqrt{\frac{1}{10} + \frac{1}{10}}} = \frac{2.74 - 2.21}{0.286\sqrt{\frac{1}{5}}} = \frac{0.53}{0.286 \times 0.45} = \frac{0.53}{0.129} = 4.108$$

Calculated t = 4.108

Tabulated t = 3.92 (0.001 p) for 18 d.f.

As the calculated t (4.108) for the difference of the two sample means is greater than the tabulated t (3.92) for 18 d.f. at $p \leq 0.001$, the difference between the two means is therefore highly significant. The null hypothesis stating that the two sample means are not different is rejected at $p \leq 0.001$.

Example 3:

Application of fertilizer (NPK) was tested on the yield of rice grown in 10 plots. Another set of 10 plots of similar size and conditions was taken as the control. Determine whether the fertilizer has any significant effect on the yield of rice.

	NPK applied			Control	
Plot Nos.	Rice yield (x_1) (Quintal/hectare)	x_1^2	Plot Nos.	Rice yield (x_2) (Quintal/hectare)	x_2^2
1	16	256	1	10	100
2	14	196	2	12	144
3	18	324	3	11	121
4	15	225	4	9	81
5	13	169	5	13	169
6	17	289	6	13	169
7	16	256	7	12	144
8	15	225	8	14	196
9	14	196	9	13	169
10	13	169	10	11	121
Total	$\Sigma x_1 = 151$	$\Sigma x_1^2 = 2305$	Total	$\Sigma x_2 = 118$	$\Sigma x_2^2 = 1414$

$$\therefore \bar{x}_1 = \frac{\Sigma x_1}{n_1} = \frac{151}{10} = 15.1 \qquad \therefore \bar{x}_2 = \frac{\Sigma x_2}{n_2} = \frac{118}{10} = 11.8$$

$$\Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n_1} = 2305 - \frac{(151)^2}{10} \qquad \Sigma x_2^2 - \frac{(\Sigma x_2)^2}{n_2} = 1414 - \frac{(118)^2}{10}$$

$$= 2305 - 2280.1 = 24.9 \qquad = 1414 - 1392.4 = 21.6$$

Pooled variance,

$$\sigma_d^2 = \frac{\Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n_1} + \Sigma x_2^2 - \frac{(\Sigma x_2)^2}{n_2}}{n_1 + n_2 - 2} = \frac{24.9 + 21.6}{10 + 10 - 2} = \frac{46.5}{18} = 2.58$$

$$\sigma_d = \sqrt{2.58} = 1.61; \quad |\bar{x}_1 - \bar{x}_2| = |15.1 - 11.8| = 3.30$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sigma_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} = \frac{3.30}{1.61\sqrt{\frac{1}{10} + \frac{1}{10}}} = \frac{3.30}{1.61\sqrt{0.2}} = \frac{3.30}{1.61 \times 0.45} = \frac{3.3}{0.72} = 4.58$$

Tabulated t = 3.92 (p = 0.001) for 18 df. is found to be less than the calculated t (4.58). Thus the difference between the means of two samples is highly significant. The null hypothesis stating that the two means have no difference is rejected at p = 0.001.

Paired 't'-Test:

Paired 't'-test is applied when each individual gives a pair of observations. Here the paired data of independent observation from one sample only to be compared.

This kind of observations is made available in biological sciences, such as:

(a) To study the effect of fertilizer, pesticide, drug on plants.

(b) To compare the effect of two different fertilizers or drugs.

(c) To compare the result of two techniques or the accuracy of two different instruments.

Steps to be followed for calculation of 't':

1. The difference in each set of paired observations are made.

$$\bar{x}_1 - \bar{x}_2 = D$$

2. The mean of difference $\bar{D}$ is calculated,

$$\bar{D} = \frac{\sum D}{n}.$$

3. Standard deviation of the difference (S_D) and then standard error of mean from the same is calculated,

$$SE_D = \frac{S_D}{\sqrt{n}}, \quad \text{where} \quad S_D = \sqrt{\frac{\sum D^2 - \frac{(\sum D)^2}{n}}{n-1}}$$

4. The 't' value is determined by substituting the above values in the formula,

$$t = \frac{\bar{D}}{\text{Standard error of difference}(SE_D)} \quad \text{or} \quad t = \frac{\bar{D}}{\frac{S_D}{\sqrt{n}}}$$

5. The calculated value of 't' is compared with 't'-table to find out the significance at that particular degree of freedom.

Example:

Determine the effect of a new growth regulator (x) on the callus growth of Nicotiana in laboratory condition. The observations are made on particular 10 calli.

Callus no.	1	2	3	4	5	6	7	8	9	10
Initial wt. of callus (g)	2.9	2.7	2.8	2.8	2.9	2.9	2.8	3.0	2.6	2.8
Final wt. of that callus (g)	3.0	2.8	2.9	2.8	3.2	3.1	3.0	3.2	2.7	2.9
Difference (D)	0.1	0.1	0.1	0	0.3	0.2	0.2	0.2	0.1	0.1
D^2	0.01	0.01	0.01	0	0.09	0.04	0.04	0.04	0.01	0.01

$$\Sigma D = 1.4 \qquad \Sigma D^2 = 0.26 \qquad n = 10$$

$$\bar{D} = \frac{1.4}{10} = 0.14$$

$$\text{Standard deviation of the difference} = \sqrt{\frac{\Sigma D^2 - \frac{(\Sigma D)^2}{n}}{n-1}}$$

$$= \sqrt{\frac{0.26 - \frac{1.96}{10}}{9}} = \sqrt{\frac{.06}{9}} = \sqrt{.0066} = .08$$

$$SE_{\bar{D}} = \frac{.08}{\sqrt{10}} = \frac{.08}{3.16} = .025$$

$$t = \frac{\bar{D}}{SE_{\bar{D}}} = \frac{0.14}{.025} = 5.6.$$

Tabulated t = 4.78 at p .001 with 9 degrees of freedom.

The calculated 't' far exceeds the tabulated 't' at p = 0.001 with 9 degrees of freedom. So the result is highly significant, i.e., the hormone has the positive effect on callus growth.

$\chi 2$ test:

It is used for testing the agreement of observed frequencies with those expected upon a given hypothesis or in other words it can be said that it is test of deviation between theoretical and observed frequencies and to see whether the deviation is significant or not. If the deviation is significant then the assumed hypothesis on which the test is performed is rejected.

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

O = Observed frequency;
E = Expected frequency;
Σ = Summation over all classes.

2. Salient Features of χ2 test:

1. Complete agreement of observed and expected frequencies will give χ2 -value as zero; but due to chance deviation (from sample fluctuations) positive val-ues will be scored.

2. The use of χ2 test requires that the frequency in any class is not 5 or less.

3. The test is applicable only to comparisons of observed and expected values of absolute frequencies.

3. Applications of χ2 test:

1. Testing goodness of fit.

2. To determine genetic ratios [gene(s) involved for a particular trait] from F2/BC1 segregating data.

3. Estimation of linkage.

4. Use in testing independence of classifications.

5. Use in testing heterogeneity of data.

4. Testing Goodness of Fit of χ2 test:

Example 1:

A chlorophyll mutant (viridis) was spotted in M_2 generation of black cumin (*Nigella sativa* L.) following treatments of dry seeds with 5kR gamma-rays. The mutant was crossed with pollens from normal plants and F_1 progenies were raised. All F1's were normal and on selfing 72 normal and 8 viridis were obtained in F2 segregating population.

Question:

State the nature of inheritance of the mutant trait.

Solution:

(a) First see how many phenotypic classes are there. In the present case there are 2 classes—normal and mutant. Therefore, the hypothesis to be assumed will be from the following genetic ratios: 1 : 1, 3 : 1, 9 : 7, 13 : 3,15 : 1.

(b) Add the total observations and select ratio which will be closest to observe values (as goodness of fit of χ2 test is performed).

(c) As the trait concerned is qualitative, χ2 test of goodness of fit has to be ap-plied to ascertain inheritance of the mutant trait.

(d) Hypothesis considered in the present case is 15 : 1. Therefore, it has to be seen whether 15 : 1 is good-fit or not.

	Normal	*Mutant*	*Total*
Observed	72	08	80
Expected	75	05	80
$O - E = d$	−3	3	
$(O - E)^2 = d^2$	9	9	
$\frac{(O-E)^2}{E} = \frac{d^2}{E}$	$\frac{9}{75}$	$\frac{9}{5}$	
	0.12	1.80	

$$\chi^2 = \sum \frac{(O - E)^2}{E} = 0.12 + 1.80 = 1.92.$$

As there are two phenotypic classes, degree of freedom will be n – 1 =1 [degree of freedom is the choice of the experiment; n = number of classes in the present case).

Comment: The $\chi 2$ value for 1 DF has been found to be 1.92, which is much less than the $\chi 2$-value given at table (3.84) at 5% (standard check) for 1 DF. There-fore, the deviation that occurred between observed and expected values is not significant and considered to be good-fit at the probability level of 0.10 to 0.20 (from $\chi 2$-table). Thus, the assumed hypothesis is accepted (15 : 1).

Inference:

1. 15 : 1 genetic ratio indicated that the nature of inheritance of chlorophyll trait (present case) is digenic (15 : 1 is a modification of Mendel's di-hybrid ratio), that is, 2 genes (two pairs of alleles) are involved for the trait.

2. As the mutant crossed with normal gave normal at F_1 and in F_2 frequency of mutants was much lower than normal it may be concluded that mutation was recessive in nature.

Thus, the mutant trait was disgenic recessive to normal.

Note: Why it is $(O - E)^2/E$?

1. To eliminate negative sign, the deviation is squared.

2. As the deviation from expected is tested, therefore, deviation is divided by expected.

Example 2:

A brown seed-coat (bb) mutant of Nigella sativa was crossed with plants having black seed-coat (BB). The F_1 plants were black seeded and were crossed with brown seed-coat mutants and in BC_1 generation 32 black seed-coat and 28 brown seed-coat plants were observed.

Question:

Test the hypothesis at 5% level and comment on the nature of inheri-tance of the mutant trait (brown seed-coat trait).

Solution:

1. As there are only two phenotypic classes and the F_1's were crossed with re-cessive parent so the assumed hypothesis can only be 1 : 1.

2. Instead of F_2 generation the data has been obtained from BC_1 segregating population.

	Phenotypic classes		
	Brown seed-coat	*Black seed-coat*	*Total*
Observed	28	32	60
Expected	30	30	60
$(O - E) = d$	−2	+2	
$(O - E)^2 = d^2$	4	4	
$\frac{(O-E)^2}{E} = \frac{d^2}{E}$	$\frac{4}{30}$	$\frac{4}{30}$	
$\chi^2 = \sum \frac{(O-E)^2}{E}$	0.13	0.13	
$\chi^2 = 0.26$ at 1 DF			

The calculated $\chi 2$-value 0.26 at 1 DF is much less than the $\chi 2$-table value (3.86) at 5% for 1 DF. Thus, the observed and expected values are good-fit (p-value 0.50 to 0.70) and, therefore, the deviation between them is not sig-nificant and consequently the assumed hypothesis is accepted.

Inference:

As the hypothesis 1 : 1 is accepted, it can be concluded that the mutant trait is monogenic (one pair of alleles) recessive to normal.

In practical classes seeds are given in petri-plates to test goodness of fit of a particular data. For some data careful test should be conducted.

Example 3:

Black seeds	Brown seeds	Total
18	22	40

Solution:

Let the assumed hypothesis be 1 : 1. Therefore, expected frequencies will be 20 for black seeds and 20 for brown seeds.

$$\frac{(O-E)^2}{E} = \frac{4}{20} \text{ and } \frac{4}{20} = 0.2 \text{ and } \dot{0}.2;\ \chi^2 = 0.2 + 0.2 = 0.4.$$

As χ2-value 0.4 at 1 DF is much less than table value 3.87 at 1 DF, the de-viation between expected and observed is not significant rather good-fit at the probability level of 0.50 to 0.55. Therefore, the hypothesis 1:1 is accepted and seed-coat colour is controlled by one pair of alleles.

But for the same set of data—hypothesis 9 : 7 may be considered.

	Black seeds	*Brown seeds*
Observed	18	22
Expected	17.5	22.5
$\frac{(O-E)^2}{E} = \frac{(0.5)^2}{17.5} = 0.014$		$\frac{(0.5)^2}{22.5} = 0.011$
$\chi^2 = 0.025$		

In this case also the hypothesis 9:7 is accepted as the deviation between observed and expected values is not significant and is good-fit at 0.80 to 0.90 probability level. Acceptance of this hypothesis is indicated that the seed-coat colour had a digenic mode of inheritance.

Inference:

9:7 is the correct hypothesis as it shows lesser deviation between observed and expected values than 1:1. Thus, assumption of hypothesis in χ2– test of goodness of fit should be based on close fit of expected with observed values.

5. Test of Linkage following the use of χ2-Test:

Example 4:

A tall homozygous pea plant (TT) bearing yellow pods (YY) was crossed with a dwarf plant (tt) having green pods (yy).

The F_1 plants raised were all tall and yellow poded and on selfing of these plants F_2 plants were developed in the following frequencies:

Tall Yellow–120 *Tall Green–10*
Dwarf Yellow–10 *Dwarf Green–20*

Question:

Comment on the assortment of T and Y genes.

Solution:

χ2-test of goodness of fit will be applied to test the hypothesis 9:3:3:1 is the assumed hypothesis as it is a data from di-hybrid cross.

	Phenotypic classes				
	Tall Yellow	*Tall Green*	*Dwarf Yellow*	*Dwarf Green*	*Total*
Observed	120	10	10	20	160
Expected	90	30	30	10	160
(O – E)	30	–20	–20	10	
$(O - E)^2$	900	400	400	100	
$\frac{(O-E)^2}{E}$	$\frac{900}{90}$	$\frac{400}{30}$	$\frac{400}{30}$	$\frac{100}{10}$	
$\chi^2 = \sum \frac{(O-E)^2}{E}$	10.0	13.33	13.33	10.0	
$\chi^2 = 46.66$					

Therefore, χ2 = 46.66 at 3 DF (4 phenotypic classes; n-1 =3).

As computed χ22-value 46.66 at 3 DF is much higher than table value 7.8 at 3 DF, the deviations between observed and expected values are not good-fit and, therefore, the assumed hypothesis 9 : 3 : 3 : 1 is not accepted.

This significant deviation may be due to failure of independent assortment of T and Y genes as the consequence of linkage. High frequency of parental combinations than recombinant also indicated the possibility of linkage.

Linkage can be estimated from F^2 data by the following formula:

$P^2 = E – M/N$,

where P = linkage value, E = sum of end classes (parental classes), M = sum of middle classes (recombinant classes) and N = number of progenies.

$p^2 = 140 – 20/160 = 0.75$; $P = \sqrt{0.75} = 0.8660$.

Therefore, percentage of linkage in the given data is 86.6. Recombination value is thus 13.4%. As 1% recombination is considered as 1 map unit, therefore, T and Y genes are 13.4 map units distance apart.

χ2 Test of Independence:

Another common use of the χ2 Test is in testing Independence of classifications in what are known as contingency table. Data are set out in a table with rows and columns, i.e., each observation is assigned to one of the cells in the table.

For example, if there are r rows and c columns, the table is generally called r x c contingency table, where r and c may represent any number and the simplest table of this kind is 2 x 2 contingency table.

			Total
	a	b	a + b
	c	d	c + d
Total	a + c	b + d	a + b + c + d

$$DF = (r - 1)(c - 1).$$

χ^2-value for 2 × 2 table is given by the following formula:

$$\chi^2 = \frac{(ad - bc)^2(a + b + c + d)}{(a + b)(b + d)(d + c)(c + a)}.$$

For a 2 x 2 table, there is only one degree of freedom, i.e., only one of the four cell frequencies can be arbitrarily given if the row and column total remain fixed. It is, therefore, necessary to make a correction of formula, so that its approximation to the continuous chi-square distribution can be improved. This is known as Yates correction for continuity.

$$\chi^2 = \frac{N\{(ad - bc) - N/2\}^2}{(a + b)(b + d)(d + c)(a + c)}.$$

Example 8:

In a survey of 200 boys of which 75 were intelligent, 40 had skilled fathers; while 85 of the unintelligent boys had unskilled fathers. Do these figures, support the hypothesis that skilled father have intelligent boys?

Solution:

The data are shown in the following 2 x 2 table:

Intelligence of sons	*Skill of fathers*		*Total*
	Skilled	*Unskilled*	
Intelligent	40	35	75
	(*a*)	(*b*)	
Unintelligent	40	85	125
	(*c*)	(*d*)	
Total	80	120	200

Null hypothesis—The two attributes skill of father and intelligence of son are inde-pendent (As it is a test of independence—the assumed hypothesis is considered to be independent between attributes).

$$\chi^2 = \frac{N\{(ab - bc) - N/2\}^2}{(a+b)(b+d)(d+c)(a+c)}$$

$$= \frac{200\left[(40 \times 85) - (35 \times 40) - \frac{200}{2}\right]^2}{80 \times 120 \times 75 \times 125}$$

$$= 8.02 \text{ at } 1 \text{ DF } [(r-1)(c-1) = (2-1)(2-1)] = 1.$$

As the observed value 8.02 at 1 DF is greater than the table value (3.84 at 1 DF) at 5% level of significance, there exist significant deviation between ob-served and expected cell frequencies and, therefore, the assumed hypothesis is rejected.

Inference:

By the use of $\chi 2$-test of independence it can be concluded that skilled fathers have intelligent sons (the attributes are dependent).

Example 9:

Anaphase I chromosomal segregation in *Allium cepa* (2n = 16) showed seasonal variation and the data obtained has been represented in 2 x 2 contingency table. Test whether the attributes are independent or not.

	Anaphase I separations		
Seasons	8 : 8	9 : 7	*Total*
Summer	65(a)	35(a)	
Winter	80(c)	20(d)	100
Total	145	55	200

Solution

Null hypothesis—Anaphase I separation of chromosomes is independent of seasons:

$$\chi^2 = \frac{N\{(ad - bc) - N/2\}^2}{(a+b)(b+d)(d+c)(a+c)}$$

$$= \frac{200\{(65 \times 20) - (35 \times 80) - 200/2\}^2}{100 \times 55 \times 100 \times 145}$$

$$= 6.42 \text{ at } 1 \text{ DF.}$$

The calculated $\chi 2$-value 6.42 at 1 DF is greater than the table value 3.87 (1 DF at 5% level of significance) and, therefore, the hypothesis is rejected as there exist significant deviation between observed and expected cell frequencies.

Inference:

Anaphase I separation of *Allium cepa* chromosomes varied with seasons (the traits are dependent to one another).

Example 10:

500 PMCs of *Nigella sativa* (black cumin) were assessed in 2 different seasons (summer and winter) for studying anaphase I segregation of chromo-somes (2n = 12) and the data obtained have been tabulated in r x c contingency table. Test the independence of the characters (anaphase I chromosome segrega-tion and seasons,).

Seasons	*Anaphase I chromosome separation*			*Total*
	4 : 8	6 : 6	5 : 7	
Summer	42	137	61	240
	a	b	c	
Winter	58	113	89	260
	d	e	f	
Total	100	250	150	500

Solution:

Null hypothesis—Anaphase I chromosome segregation is independent to seasons.

The expected value for each class can be calculated from the following formula:

E = r x c / G, where r = row, c = column and G = grand total.

Expected values:

$$a = \frac{100 \times 240}{500} = 48; \quad b = \frac{250 \times 240}{500} = 120;$$

$$c = \frac{150 \times 240}{500} = 72; \quad d = \frac{100 \times 260}{500} = 52;$$

$$e = \frac{250 \times 260}{500} = 130; \quad f = \frac{150 \times 260}{500} = 78.$$

$$\chi^2 = \frac{(42-48)^2}{48} + \frac{(137-120)^2}{120} + \frac{(60-72)^2}{72} + \frac{(58-52)^2}{52} + \frac{(113-130)^2}{130} + \frac{(89-78)^2}{78}$$

$$= 0.75 + 2.41 + 2.0 + 0.69 + 2.22 + 1.55$$

$$= 9.62 \; DF = (r-1)(c-1) = (2-1)(3-1) = 2.$$

The calculated χ2-value 9.62 at 2 DF is higher than table value (5.9 at 2 DF) at 5% level of significance, thereby indicating that the expected and observed frequencies are not good-fit rather showed significant deviations. Thus, the hy-pothesis is rejected.

Inference:

Anaphase I chromosome segregation is dependent to seasons.

χ2-Test of Heterogeneity:

χ2-test of heterogeneity is very similar to that of testing independence and widely used in genetic experiments, where the consistency (randomness) or otherwise (inconsistency = non-randomness = significant variation) of several groups of data can be tested. The advantage of this method is that it shows actual proportions observed in each class.

Example 12:

Metaphase I chromosome associations in tetraploid *Nigella damascena* (2n = 12) have been documented over 4 generations. Test whether chromo-somal configurations (I, II and IV) per cell were consistent over the generations or not.

For bivalent/cell:

$$78 \times 7.78 - C_0 - 606.84 = O_1$$
$$84 \times 8.12 - C_1 - 682.08 = O_2$$
$$116 \times 8.26 - C_2 - 958.16 = O_3$$
$$127 \times 9.19 - C_3 - 1167.13 = O_4$$

Pooled: 3414.21/405 = 8.43 – Pooled expected frequency.

$$E_1 = 657.54$$
$$E_2 = 708.12$$
$$E_3 = 977.88$$
$$E_4 = 1070.61$$

$$\chi^2 = \frac{(606.84 - 657.54)^2}{657.54} + \frac{(682.08 - 708.12)^2}{708.12} + \frac{(958.16 - 977.88)^2}{977.88} + \frac{(1167.13 - 1070.61)^2}{1070.61}$$
$$\chi^2 = 3.91 + 0.96 + 0.40 + 8.70$$
$$= \boxed{13.97}.$$

For quadrivalent/cell:

$$78 \times 1.54 - C_0 - 120.12 = O_1$$
$$84 \times 1.45 - C_1 - 121.80 = O_2$$
$$116 \times 1.33 - C_2 - 154.28 = O_3$$
$$127 \times 0.91 - C_3 - 115.57 = O_4$$

Pooled: 511.77.

Expected: 511.77/405 = 1.26.

$$E_1 = 1.26 \times 78 = 98.28$$
$$E_2 = 1.26 \times 84 = 105.84$$
$$E_3 = 1.26 \times 116 = 146.16$$
$$E_4 = 1.26 \times 127 = 160.02$$

$$\chi^2 = \frac{(120.12 - 98.28)^2}{98.28} + \frac{(121.80 - 105.84)^2}{105.84} + \frac{(154.28 - 146.16)^2}{146.16} + \frac{(115.57 - 160.02)^2}{160.02}$$
$$= 4.85 + 2.41 + 0.45 + 12.35$$
$$= \boxed{20.06} \text{ at 3 DF.}$$

Thus, analysis of χ2-test of heterogeneity of chromosome configurations over the generations at 3 DF revealed that frequency of quadrivalents ($p > 0.001$) and bivalents ($p > 0.01$) per cell at metaphase I among tetraploids were inconsis-tent (non-random/significant) over the generations; while, the univalents were random ($p = 0.30$) in distribution [uniformly distributed over the generations].

Example 13:

Univalent and bivalent frequency per cell have been presented in Table from 10 plants of *Ocimum basilicum* (basil 2n = 72).

Question:

Find out from the given data whether univalent and bivalent frequen-cies per cell were consistent among the plants.

Plant number	*No. of MI cells scored*	*Frequency/cell*	
		II	*I*
1	33	34.76	2.48
2	35	35.14	1.71
3	30	35.33	1.33
4	25	35.32	1.36
5	29	35.10	1.79
6	32	34.94	2.13
7	28	35.11	1.79
8	33	35.21	1.58
9	35	35.29	1.43
10	24	35.00	2.00

Solution:

X2-test of heterogeneity has been performed to assess distribution of univalent and bivalent per cell over the plants. Result has been tabulated (process of calculation have been shown in the previous example).

Configuration	χ^2*-value*	DF	*Probability level*
II	0.26	9	> 0.95
I	21.27	9	< 0.05

Result indicated that bivalents/cell was random (consistent) among the plants; while, frequency of univalent/cell was non-random (significant variants occurred among plants).

Analysis of variance (F test):

The analysis of variance is based on F-statistics which is a ratio of variances. The ratio of variances due to treatments and variances due to random differences within the treatment is calculated and the estimate, known as the ‘F’, is then used for comparison.

It is a collection of statistical models used to analyze the differences among group means and their associated procedures (such as "variation" among and between groups), developed by statistician and evolutionary biologist Ronald Fisher.

ANOVA statistical significance result is independent of constant bias and scaling errors as well as the units used in expressing observations.

Example:

Four rice varieties were grown in 4 replications in randomized block design and their yield/plot was assessed. From yield data do you consider that the mean yield of varoieties differ among themselves?

Solution:

A_5	C_{10}	B_2	D_{10}
B_4	A_6	C_{12}	D_9
D_9	A_5	B_2	C_{11}
C_{12}	B_3	A_7	D_8

Significance:

No biasness over the experiment—Wide range of treatments with no restriction on number of replications.

Demerits:

Sectoral representation of some variety/treatments (that is, the treatments were not distributed uniformly) may occur.

Yield of the varieties—kg/plot.

Replication	*Varieties*				
	A	B	C	D	
1	5	2	10	10	27
2	6	4	12	9	31
3	5	2	11	9	27
4	7	3	12	8	30
	23	11	45	36	115 (Grand Total)

In the present case null hypothesis is assumed as:

(a) Varietal means are same, i.e., the varieties do not differ among themselves in yield.

(b) Varieties do not differ in replication.

First Step:

Add row and column totals to find out grand total = 115(T).

CF (Correction factor) = $T^2/n = (115)^2/16 = 826.56$.

Second Step:

Grand total sum of squares = $5^2 + 6^2 + 5^2 + 7^2 + \ldots + 9^2 + 9^2 + 9^2 + 8^2 = 1003$.

Third Step:

(Total SS = Grand total SS – CF) = 1003 – 826.56 = 176.44.

Fourth Step:

SS due to variety (column) = $23^2 + 11^2 + 45^2 + 36^2/4 - CF = 166.19$.

It is divided by 4 as each value is sum of four items.

Fifth Step:

SS due to replication (row) = $27^2 + 31^2 + 27^2 + 30^2/4 - CF = 3.19$.

Sixth Step:

SS due to error

= Total SS – (SS of variety + SS of replication)

= 176.44 — (166.19 + 3.19) = 7.06.

Anova Table

Sources	df	SS	MSS	F-*value*	*Table Value*		
					5%	1%	0.1%
Variety	$3(n_1)$	166.19	55.40	71.03***	3.9	7.0	13.9
Replication	$3(n_1)$	3.19	1.06	1.36			
Error	$9(n_2)$	7.06	0.78				
Total	15						

$$\text{MSS} = \text{mean sum of squares} = \frac{\text{SS}}{\text{df}}$$

$$\text{Calculated F-value} = \frac{\text{MSS of source}}{\text{MSS of error}}.$$

Results indicated that the varieties varied significantly among themselves at 0.1% level of significance; however, the varieties did not varied in replication.

Critical Difference (CD):

The square root of the error mean square measures the standard error per plot due to uncontrolled environmental effects. The varietal means were obtained from four plots (replication) and, therefore, standard error of varietal mean in the present case will be

$\sqrt{0.78/4} = 0.44$.

The standard error of difference of means of two varieties will be:

$0.44 \times \sqrt{2} = 0.44 \times 1.41 = 0.6204$.

From the value of standard error of difference we can calculate the value of the difference which will be just significant at a chosen level of significance. The difference is known as the critical difference for the particular level of significance (generally 5% level of significance is considered adequate).

Hence, CD is t-value x standard error of difference (5%) for 9 DF

= 2.26 (t-value at 5% for 9 DF in the present case) x 0.6204

= 1.4.

That is, if the difference between 2 varieties is 1.4, then it is significant.

	A	B	C	D
Total	23	11	45	36
Mean	5.75	2.75	11.25	9.0
CD at 5%	1.4			

From CD-value it is apparent that yield varied significantly between the varieties.

Estimation of Heritability from ANOVA:

$$\text{Genotypic variance} = \text{MSS due to genotypes (}\textit{variety}\text{)} - \frac{MS_e \text{ (error)}}{\text{No. of Replications}}$$

$$= 55.40 - \frac{0.78}{4} = 13.66.$$

$$\text{Phenotypic variance} = \text{Genotypic variance } (\sigma_g^2) + \sigma_e^2 \text{ (variance due to error} = MS_e)$$

$$= 13.66 + 0.78 = 14.44.$$

$$\text{Heritability (\%) (}\textit{broad sense}\text{)} = \frac{\sigma_g^2}{\sigma_p^2} \times 100 = \frac{13.66}{14.44} \times 100 = 94.6\%.$$

Genetic Advance = Genetic Gain:

The genetic gain is the difference between the mean of the progeny of selected individuals ($\bar{X}_p$) and the base population ($\bar{X}_0$)

R (genetic gain) = $\bar{X}_p$ — $\bar{X}_0$.

Genetic gain can also be predicted using the following formula R = ih.$^2\sigma_p$; where, i is the standardized selection differential (5% level has been used mostly 2.06), h^2 is heritability, i.e., σ^2_g/σ^2_p, op is the phenotypic standard deviation.

11. Correlation and Regression

Correlation:

Association between variables or attributes or characteristics at a given time is known as correlation.

Example:

(i) The amount of rainfall and yield of a certain crop;

(ii) Age of husband and wife;

(iii) Height and weight of students and

(iv) Different concentrations of mutagen and their effect on seed germination frequency.

In plant breeding the breeders targets improvement of yield. Relationship between yield and yield related traits (plant height, number of primary branches/ plant; total branches/plant, number of capsules/plant, capsule length, seeds/capsule, 100-seed weight, etc.) and between the yield related components can be worked out through correlation studies.

Significant correlation obtained will be helpful for selection and ascertaining the model plant type for the concerned species.

Precisely correlation may be defined as movement of one variable tend to be accompanied by corresponding movements in the other. Such simultaneous movement of two variables can be graphically plotted using value of one variable on x-axis and the other variable along y-axis.

Such representation of variables in-dicates the nature of association between the attributes and is called as scattered diagram or correlation chart.

Correlation may:

(a) Positive Correlation:

Increase in plant height is related to increase in number of branches per plant. On the scattered diagram the dots (each pair of obser-vation) representing the variables are in a linear path diagonally across the graph paper from bottom left-hand corner to the top right.

(b) Negative Correlation:

For example, increase in plant height of a species is re-lated to decrease in branch number per plant. The pattern of dots be such as to indicate a straight line path from the upper left-hand corner to the bottom right.

(c) Zero Correlation:

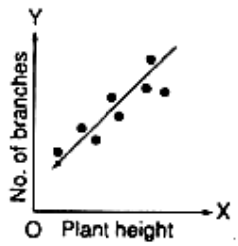
The dots are scattered and do not indicate any straight line.

(d) Perfect Correlation:

When the dots lie exactly on a straight line.



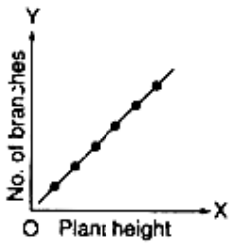
Positive correlation



Negative correlation



Zero correlation



Perfect correlation

In the present example height of plants represented independent variable and on the other hand the variable which changes with the change in the independent variable is called dependent variable (branches/plant).

It is customary to use the horizontal axis (x-axis) for the independent variable and the vertical axis (y-axis) for dependent variable.

The degree of relationship between 2 attributes can be determined by calcu-lating a coefficient called as correlation coefficient. The correlation coefficient is expressed by the letter 'r'. r varies from 0 to 1 and can be + (positive correlation) or — (negative correlation). Practically, r is never zero or 1 (complete/absolute).

Whenever correlation coefficient analysis is made, r-value ranges from 0 to 1 but it is necessary to compare the calculated r-value with table value at specific de-gree of freedom. If the value is significant, i.e., if the calculated r-value is greater than table value, then only we can say that the two attributes are statistically associated to one another. Degree of significance level has also to be assessed (5%, 1% and 0.1% levels).The correlation coefficient is expressed by 'r' which varies from 0 to 1.

$$r = \frac{\Sigma(x-\bar{x})(y-\bar{y})}{\sqrt{\Sigma(x-\bar{x})^2\Sigma(y-\bar{y})^2}} = \frac{\Sigma d_x d_y}{\sqrt{\Sigma d_x^2 \Sigma d_y^2}},$$

where x and y are the variables.

$$\Sigma d_x d_y = \Sigma xy - \frac{\Sigma x \Sigma y}{n};$$

$$\Sigma d_x^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n};$$

$$\Sigma d_y^2 = \Sigma y^2 - \frac{(\Sigma y)}{n}.$$

In correlation degree of freedom is n — 1, where n represents pairs of observations.

Example 1:

Ten plants have been assessed in sesame (Til) for plant height (cm) and number of branches per plant. From the given data do you consider that there exist correlation (significant) between the variables?

Solution:

No. of plants	*Plant height (cm) x variable*	*Branches per plant y variable*	x^2	y^2	xy
1	10	12	100	144	120
2	15	16	225	256	240
3	20	20	400	400	400
4	22	25	484	625	550
5	30	35	900	1225	1050
6	35	40	1225	1600	1400
7	40	45	1600	2025	1800
8	45	50	2025	2500	2250
9	50	52	2500	2704	2600
10	55	60	3025	3600	3300
$n=10$	$\Sigma x = 322$	$\Sigma y = 355$	$\Sigma x^2 = 12484$	$\Sigma y^2 = 15079$	$\Sigma xy = 13710$

$$r = \frac{\Sigma d_x \Sigma d_y}{\sqrt{\Sigma d_x^2 \Sigma d_y^2}}$$

$$\Sigma d_x d_y = \Sigma xy - \frac{\Sigma x \Sigma y}{n} = 13710 - \frac{322 \times 355}{10} = 2279.$$

$$\Sigma d_x^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n} = 12484 - \frac{(322)^2}{n} = 2115.6.$$

Inference: The calculated value 0.93 for 9 DF is higher than the tabulated value at 5%, 1% and 0.1% levels and it can be suggested that the two variables are positively and significantly correlated between them.

The calculated value 0.996 for 9 DF is higher than the tabulated value at 5%, 1% and 0.1% levels and hence it can be suggested that the two vari-ables are positively and significantly correlated between them at 0.001 probabil-ity level.

The r-value can be represented as 0.996*** to show the level of significance.

Thus, selection of plants with enhance no. of primary branches will facilitate selection of plants with increase no. of umbels/plant.

How to prepare Correlation Table from Experimental Data:

Following data has been given

a. Plant height and number of primary branches/plant = 0.65

b. Plant height and total branches per plant = 0.57.

c. Height and number of capsules per plant = 0.81**.

d. Height and yield = 0.62.

e. Primary branches and total branches = 0.35.

f. Primary branches and capsules per plant = 0.80**.

g. Primary branches and yield = 0.87***.

h. Total branches and number of capsules = 0.52.

i. Total branches and yield = 0.43.

j. Capsules per plant and yield = 0.82**.

Attributes	*Plant height*	*Primary branches/ plant*	*Total branches per plant*	*Capsules per plant*	*Yield*
Plant height		0.65	0.57	0.81**	0.62
Primary branches per plant			0.35	0.80**	0.87***
Total branches per plant				0.52	0.43
Capsules/plant					0.82**

Inference:

Interrelationship between four yield related traits and their association with yield have been documented in tabular form. Result indicated positive and significant correlation between height and capsules/plant (1% level), primary branches/plant and capsules/plant (1% level), primary branches and yield (0.1% level) and capsules/plant and yield (1% level).

Thus, plants having higher number of primary branches with enhanced cap-sule number should be the selection indices for higher yield in the plant species.

Partial Correlation:

X_1 and X_2 estimated by taking into account the effect of a 3rd variable X_3 and is denoted as $r_{12.3}$.

Partial correlation provides better relationship between the two variables X_1 and X_2 and is given by the formula:

$$r_{12.3} = \frac{(r_{12} - r_{13}r_{23})}{\sqrt{(1 - r_{13}^2)(1 - r_{23}^2)}}$$

r_{12}, r_{13} and r_{23} are the estimates of simple correlation coefficients between the variables X_1 and X_2, X_1 and X_3 and X_2 and X_3, respectively.

Multiple Correlation:

Estimate of joint influence of two or more variables on a dependent variable is called multiple correlation. Such an estimate helps in understanding the dependence of one variable, say x_1 on a set of independent variable say X_2, X_3.

$$R^2_{1.23} = \frac{[(r^2_{12} + r^2_{13}) - 2(r_{12} \cdot r_{13} \cdot r_{23})]}{(1 - r^2_{23})}.$$

The square root of $R^2_{1.23}$ is the estimate of multiple correlation coefficient. $R^2_{1.23}$ is coefficient of determination.

Linear Regression Analysis:

The statistical analysis employed to find out the exact position of the straight line is known as Linear regression analysis. From simple correlation analysis if there exist relationship between independent variable x and dependent variable y then the relationship can be expressed in a mathematical form known as Re-gression equation.

From regression equation we can work out the actual value of y variable (dependent) based on X variable (independent) and such values plot-ted graphically will give precise nature of the straight line (point of interception to y-axis can be noted).

Simple regression equation Yx = a + bx, where a and b are constant which minimize the residual error of Y. Y is the dependent variable.

The constants a and b can be obtained from the formula:

$$b = \frac{\Sigma dxdy}{\Sigma dx^2}, \quad \text{where } \Sigma dxdy = \Sigma xy - \frac{\Sigma x \Sigma y}{n};$$

$$\Sigma dx^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n}.$$

$$a = \bar{y} - b\bar{x}.$$

Example:

From the data find out the regression equation and draw a regression line on the graph paper.

No. of Branches (x)	*No. of Capsules* (y)	x^2	xy
2	4	4	8
5	10	25	50
8	15	64	120
10	20	100	200
15	25	225	375
20	30	400	600
25	40	625	1000
$\Sigma x = 85$	$\Sigma y = 144$	$\Sigma x^2 = 1443$	$\Sigma xy = 2353$

Solution:

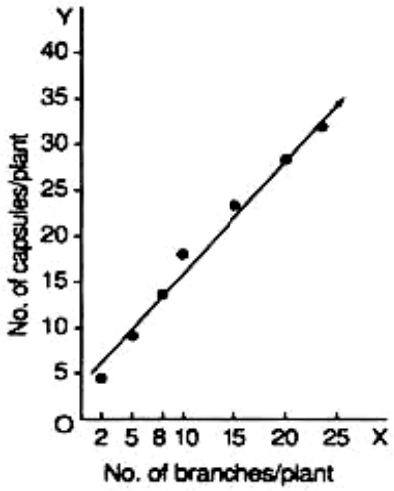
$$\bar{x} = \frac{85}{7} = 12.14; \quad \bar{y} = \frac{144}{7} = 20.57.$$

$$b = \frac{\Sigma xy - \frac{\Sigma x \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}} = \frac{2353 - \frac{85 \times 144}{7}}{1443 - \frac{(85)^2}{7}} = 1.48.$$

$$a = \bar{y} - b\bar{x} = 20.57 - (1.48)(12.14) = 2.6.$$

Therefore, the regression equation $y_x = a + bx$, $y_x = 2.6 + 1.48x$.

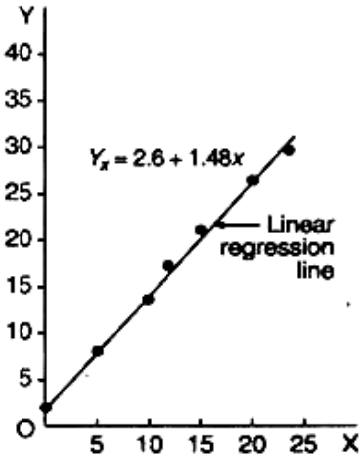
Using the regression equation $y_x = 2.6+1.48x$ the actual values of dependent variable can be worked out.



If $x = 0$, $y = 2.60$
$x = 2$, $y = 5.56$
$x = 5$, $y = 10.00$
$x = 8$, $y = 14.44$
$x = 10$, $y = 17.40$
$x = 15$, $y = 24.80$
$x = 20$, $y = 32.20$
$x = 25$, $y = 39.60$

Using data of the given example the straight line is drawn but the point of interception to y-axis is lacking and, therefore, precise nature of the straight line is not understood. However, from the straight line it is evident that the variables were significantly and positively correlated between themselves.

These set of values plotted graphically will give a straight and the precise nature of the straight line can be obtained from x = 0, y = 2.6 (point of interception to y- axis can be found out).



12. Design of experiments: general principles of field trails, randomized blocks, latin square, split plot designs, layout of breeding experiment.

Design of experiments: general principles of field trails

Meaning of Experimental Designs*:*

Experimental designs are various types of plot arrangements which are used to test a set of treatments to draw valid conclusions about a particular problem. Before dealing with experimental designs, it is necessary to define experiment, treatment and experimental unit.

A scientifically planned method is called an experiment and various objects of comparison are known as treatments. The group of material to which a treatment is applied in a single trial of experiment is known as experimental unit. It may a plot of land, a patient in a hospital, a group of cattle in a dairy, etc.

Experimental designs are useful to a researcher in several ways as given below:

1. In reducing the soil heterogeneity and thereby experimental error to a considerable extent.
2. In testing the significance of difference among various objects of comparison.
3. In screening of various treatments in a scientific manner.
4. In partitioning of variation into different components.
5. In the assessment of variances and co-variances.
6. In proper interpretation of scientific results and drawing valid conclusions.

Principles of Experimental Designs:

There are three basic principles of experimental designs, viz., replication, randomization and local control or error control. All these principles help in reducing the experimental error and thus make the experiment more efficient.

These principles are briefly-described below:

1. Replication:

Repetition of treatments under investigation is known as replication.

There are two main advantages of replications:

i. Increase in replication increases the precision by reducing the error to a great extent. The increase in precision results in two ways. In case of field experiments standard error is calculated as VrVE / r where VE = error variance and r = number of replications. Thus, it is clear that the standard error of mean decreases as the number of replication increases.

Increase in replication increases the degree of freedom of error which decreases the value of t due to decrease in confidence interval. The shortening of confidence interval is a good proof of increased precision.

ii. The error of experiment arises from the difference between the plots of the same treatment. Thus, without replications estimate of error is not possible and without the estimate of error comparisons are not possible.

Another way of reducing the experimental error is to increase the plot size. But the plot size cannot be increased beyond 0.1 acre, because further increase in plot will increase the heterogeneity within the plot and affect the advantage obtained by increasing the plot size.

As the smaller plots are homogeneous in fertility, it is better to keep the experimental area the same and increase the number of replications by shortening the plot size.

2. Randomization:

Allocation of treatments of different plots by a random process is known as randomization of treatments. Randomization gives equal chance to all the treatments for being allotted to a more fertile plot as well as to a less fertile plot.

3. Local Control or Error Control:

The principle of making use of greater homogeneity in groups of experimental units for reducing the experimental error is known as local control.

The fertility of the field may be of two types:

i. A major fertility variation which is usually marked by a fertility gradient.

ii. Sporadic or scattered fertility variations which are not systematic but are distributed in patches.

The first type of fertility effects are reduced by dividing the field into homogeneous blocks or replications and the effects of second type of fertility are minimized by randomization of treatments within the block. The experimental error should be reduced as far as possible because lower error helps in determining the small real differences between the treatments.

Factors of Experimental Designs:

The choice of experimental designs depends on three main factors, viz:

(1) Number and nature of treatments under study,

(2) Objective of the experiment, and

(3) Available resources.

RBD (Anova test) from Unequal Replications:

Example:

Performance of mean height of 4 lines of rice are to be tested. They are sown in the field as single row. Plant number in them were unequal. Plant heights are in cm.

Line 1 12, 12, 13, 16, 14, 10, 10, 14, 15, 13, 17, 11, 11, 10, 7, 14.
Line 2 7, 6, 7, 11, 10, 11, 8, 11, 4, 9, 10.
Line 3 5, 14, 9, 9, 8, 10, 10, 9.
Line 4 27, 11, 14, 10, 12, 11, 13, 20, 12, 11, 10.

Solution:

Total of Line 1 = 199		No. of observations in Line 1 = 16	
" " " 2 = 94		" " " " " 2 = 11	
" " " 3 = 74		" " " " " 3 = 8	
" " " 4 = 151		" " " " " 4 = 11	
Grand Total = 518		Total No. of observations = 46	

$$CF = \frac{(518)^2}{46} = \frac{268324}{46} = \mathbf{5833.13.}$$

$$\text{Total SS} = \left[12^2 + 12^2 + 13^2 + \ldots + 12^2 + 11^2 + 10^2\right] - CF$$
$$= 6506 - 5833.13 = \mathbf{672.87.}$$

$$\text{Between line SS} = \left[\frac{(199)^2}{16} + \frac{(94)^2}{11} + \frac{(74)^2}{8} + \frac{(151)^2}{11}\right] - CF$$
$$= [2475.06 + 803.27 + 684.50 + 2072.82] - CF$$
$$= 6035.65 - 5833.13 = \mathbf{202.52.}$$

Anova Table

Sources	df	SS	MSS	F
Between lines	3	202.52	67.507	5.631**
Error (*within lines = between plants*)	42	470.35		
Total	45	672.87		

** Significant at 1% level.

Conclusion:

Since the observed value of F (5.631) is greater than the value of F at 1% at df $n_1 = 3$, $n_2 = 42$, the test is highly significant indicating thereby that there are significant difference in plant height between different lines.

Treatment means, i.e., line means:

Line 1 = 199/16 = 12.44 cm;
Line 2 = 94/11 = 8.55 cm;
Line 3 = 74/8 = 9.25 cm;
Line 4 = 151/11 = 13.73 cm;

From the above mean heights of the four lines it is found that the mean value of line 2 is lowest and that of line 4 is highest.

Significance of Difference of Means of Line 2 and Line 4:

Standard deviation (σ) of Line 2 and 4

$$= \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}} = \sqrt{\frac{[\text{Line } 2^2 + \text{Line } 4^2] - \frac{[\Sigma \text{ Line } 2 + \Sigma \text{ Line } 4]^2}{n}}{n-1}}$$
$$= \sqrt{\frac{(858 + 2345) - \frac{(94 + 151)^2}{22}}{22 - 1}} = \sqrt{\frac{3203 - \frac{60025}{22}}{21}}$$
$$= \sqrt{\frac{474.6}{21}} = \sqrt{22.6} = \mathbf{4.75.}$$

Now, t between Line 2 and Line 4

$$= \frac{\text{Difference of Line 2 and 4}}{\sqrt{\frac{1}{\text{No. of obs. of Line 2}} + \frac{1}{\text{No. of obs. of Line 4}}}}$$

S = pooled SD of Line 2 and 4

$$= \frac{13.75 - 8.55}{4.75\sqrt{\frac{1}{11} + \frac{1}{11}}} = \frac{5.18}{4.75\sqrt{\frac{1}{22}}} = \frac{5.18}{1.012} = \mathbf{5.128.}$$

Conclusion:

The observe value of t, i.e., 5.128 is greater than the table value of t at 20($n_1 + n_2 - 2 = 11 + 11 - 2 = 20$) at 1% level. Therefore, the value of observed t is highly significant which indicates that the difference in plant height between the Line 2 and 4 is highly significant.

Analysis of Variance using Latin Square Design:

Here the treatments are so designed that each treatment is represented once in each row and once in each column. Number of treatments must be same as number of replications.

Four genotypes of wheat (A, B, C and D) in four replications 4 x 4 assignment.

A	D	B	C
B	C	D	A
C	B	A	D
D	A	C	B

As randomization is non-biased representation, it may so happens that varieties/families/treatments, etc., in field may have sectorial representation which maybe a hindrance to minimization of error (soil fertility). However, Latin square design will be only accommodative in square or rectangular field.

Example:

Seeds of 4 wheat genotypes (A,B,C and D) were given mutagenic treatments of ethyl methane sulphonate at 0.25%, 3h, 0.50%, 3h and 1.0%, 3h (treatments: 0-control, 0.25%, 0.5% and 1.0%) and grown in 4 replications in field plots. On harvest yield was assessed per plots (kg/plot).

Solution:

Test:

1. Yield of the varieties differed or not.
2. Variety yield in relation to replication.
3. Variety yield in relation to treatments.

Given data in tabular form:

Replication	*Treatments (%)*			
	0	0.25	0.50	1.0
1	4(A)	5(D)	12(B)	10(C)
2	6(B)	8(C)	16(D)	10(A)
3	2(C)	6(B)	14(A)	10(D)
4	6(D)	10(A)	8(C)	6(B)

Solution:

Null hypothesis—Mean yield of the genotypes is same:

(a) among themselves,

(b) among replication and

(c) among treatments.

(a) Varieties (Genotypes) in relation to replication:

Rep	*Genotypes*				*Total*
	A	B	C	D	
1	4	12	10	5	31
2	10	6	8	16	40
3	14	6	2	10	32
4	10	6	8	6	30
Total	38	30	28	37	133

$$\text{Correction factor} = \frac{(133)^2}{16} = \mathbf{1105.56.}$$

$$\text{Grand total SS} = 4^2 + 10^2 \cdots 10^2 + 6^2 = \mathbf{1313.}$$

$$\text{SS due to genotypes} = \frac{38^2 + 30^2 + 28^2 + 37^2}{4} - \text{CF} = \mathbf{18.69.}$$

$$\text{SS due to replication} = \frac{31^2 + 40^2 + 32^2 + 30^2}{4} - \text{CF} = \mathbf{15.69.}$$

(b) Genotypes in relation to treatments:

Genotypes	*Treatments*			
	0	0.25	0.50	1.0
A	4	10	14	10
B	6	6	12	6
C	2	8	8	10
D	6	5	16	10
Total	18	29	50	36

$$\text{SS due to treatments} = \frac{18^2 + 29^2 + 50^2 + 36^2}{4} - \text{CF} = \mathbf{134.69.}$$

$$\text{Total SS} = \text{Grand total SS} - \text{CF} = 1313 - 1105.56 = \mathbf{207.44.}$$

$$\begin{aligned}\text{SS due to error} &= \text{Total SS} - (\text{SS due to genotypes} + \\ &\qquad \text{replication} + \text{treatments}) \\ &= 207.44 - (18.69 + 15.69 + 134.69) \\ &= \mathbf{38.37.}\end{aligned}$$

Anova Table

Sources	df	SS	MSS	F-*value*	*Table-values* (F-*value*)		
					0.05	0.01	0.001
Genotypes	$3(n_1)$	18.69	6.23	0.97	4.8	9.8	23.7
Replications	$3(n_1)$	15.69	5.23	0.82			
Treatments	$3(n_1)$	34.69	44.90	7.02*			
Error	$6(n_2)$	38.37	6.40				
Total	15						

Inference:

1. Yield (mean) of the genotypes do not differ among themselves significantly.
2. Mean yield of the genotypes did not vary in replication.
3. Mean yield of the genotypes varied significantly in treatments.

Analysis of Variance from Split Plot Design:

In split plot design the field is divided into identical blocks and such blocks are considered similar to replications. Each block is divided into main plots, where the first factor (treatment) is assigned at random and subsequently each main plot is subdivided into sub-plots to be allotted randomly to the levels of the second factor. It is breeder's choice to consider the treatments as first or second factor.

Significance of this layout is that it gives effective control over treatments and minimizes error. Further, interaction between two factors can also be analyzed.

Thus, in the design the field is:

1. Divided into blocks = replications.
2. Each block is divided into main plots, where the main treatment will be assigned.
3. Each main plot is sub-divided in sub-plots = sub-treatment.
4. Size of the main plots and sub-plots should be identical.
5. Random distribution of the treatments into plots.

Layout of the Experiment with a Specific Example:

Treatments:

1. Date of sowing:

(a) S_1—1st November
(b) S_2—1st December
(c) S_3—1st January
(d) S_4—1st February

} Main treatment ↓ Assigned to main plots

2. Methods of sowing:

(a) m_1—Broadcasting

(b) m_2—Drilling

(c) m_3—Line

Blocks = Replications = 3.

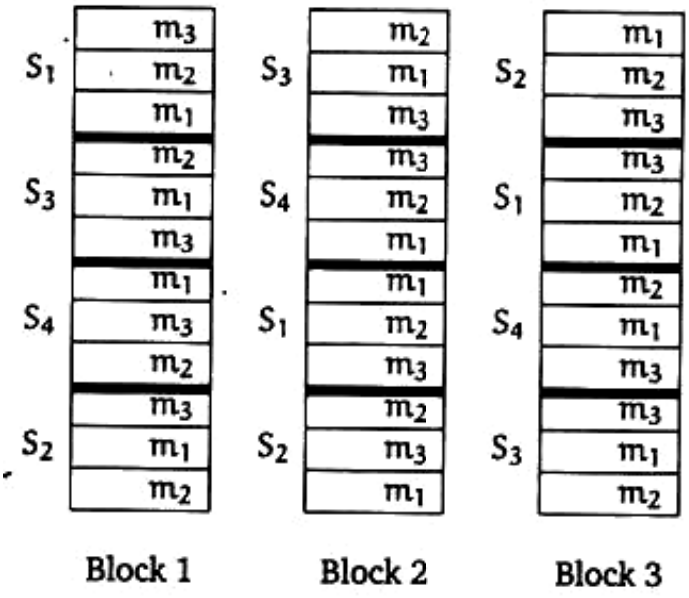


Diagram showing how field is divided in split plot design.

Example:

Split plot design experiment has been conducted to evaluate the differences in yield in relation to sowing dates (A—15th November; B—1st December; C—15th December; D—1st January and E—15th January) in 8 genotypes of Nigella sativa (control and 7 mutant lines) to evaluate the yield differences (seed yield in gm). The experiment was conducted in 3 blocks.

Solution:

Main treatment = Sowing dates = Main plots;

Sub-treatment = Genotypes = Sub-plots.

Results have been tabulated.

Table 7.1: Two-way table of blocks and dates of sowing (*replication and main treatment*)

Blocks	*Dates of sowing*					*Total*
	A	B	C	D	E	
1	12.35	7.46	4.45	1.79	1.78	27.77
2	11.00	11.49	7.78	1.18	1.15	32.60
3	10.91	14.59	4.15	1.44	1.35	32.44
Total	34.26	33.48	16.38	4.41	4.28	92.81

$$CF = \frac{(92.81)^2}{120} \quad [8 \text{ genotypes} \times 5 \text{ sowing dates} \times 3 \text{ blocks} = 120]$$

$$= \mathbf{71.78.}$$

$$\text{Grand total SS} = \frac{(12.35)^2 + (11.0)^2 + \cdots (1.15)^2 + (1.35)^2}{8} - CF$$

[as each x_i-value is sum total of 8 values obtained from genotypes]

$$= \mathbf{41.06.}$$

$$\text{SS due to replication (*blocks*)} = \frac{(27.77)^2 + (32.60)^2 + (32.44)^2}{40} - CF \quad (8 \times 5 = 40)$$

$$= \mathbf{0.37.}$$

SS due to planting dates (*main treatment*)

$$= \frac{(34.26)^2 + (33.48)^2 + (16.38)^2 + (4.41)^2 + (4.28)^2}{24} - CF \quad (8 \times 3 = 24)$$

$$= \mathbf{36.58.}$$

$$\text{Error a} = \text{Total SS} - [\text{SS due to planting} + \text{SS due to replication}]$$
$$= 41.06 - [0.37 + 36.58]$$
$$= 41.06 - 36.95$$
$$= \mathbf{4.11.}$$

Table 7.2: Two-way table of treatment totals for split plot experiment (*main treatments and sub-treatments*)

Genotypes	*Planting dates*					*Total*
	A	B	C	D	E	
1	3.88	6.33	3.44	0.60	0.50	14.75
2	6.25	3.48	1.25	0.71	0.66	12.35
3	3.78	6.85	4.15	0.77	0.60	16.15
4	3.60	2.54	2.58	0.46	0.44	9.62
5	5.94	3.39	1.05	0.63	0.75	11.76
6	6.69	8.17	2.82	0.48	0.60	18.76
7	2.30	1.26	0.75	0.47	0.38	5.16
8	1 82	1.46	0.34	0.29	0.35	4.26
Total	34.26	33.48	16.38	4.41	4.28	92.81

SS due to planting × genotypes

$$= \frac{(3.88)^2 + (6.25)^2 + \cdots + (0.38)^2 + (0.35)^2}{3} - CF$$

[value obtained from 3 replications/blocks]

$$= \mathbf{64.71.}$$

SS due to genotypes

$$= \frac{(14.75)^2 + (12.35)^2 + \cdots + (5.16)^2 + (4.26)^2}{15} - CF$$

(3 replications × 5 dates = 15)

$$= \mathbf{12.12.}$$

SS due to main treatment (*planting dates*) × sub-treatment (*genotypes*)

$$= 64.71 - (12.12 + 36.58)$$

$$= \mathbf{16.01.}$$

Total SS = (Sum of squares of individual values Σx_i's)

$$= 154.42 - 71.78(CF)$$

$$= \mathbf{82.63.}$$

Error b = 82.63 − [SS due to replication + SS due to dates + SS due to genotypes + SS due to interaction + SS due to error a]

$$= 82.63 - [0.37 + 36.58 + 12.12 + 16.01 + 4.11]$$

$$= \mathbf{13.44.}$$

Table 7.3: Complete analysis of variance

Sources of variation	df	SS	MSS	F-*value*
Replication	2	0.37	0.185	0.35
Planting dates	4	36.58	9.145	17.59***
Error a	8	4.11	0.52	
Genotypes	7	12.12	1.73	9.01***
Planting dates × genotypes	28	16.01	0.57	2.96***
Error b	70	13.44	0.192	
Total	119			

*** Significant at 0.001 probability level (0.1%).

Inference:

It has been observed that both treatments and their interactions have been significant at 0.1% level. It has also been noted that the differences between genotypes were of a much smaller order than those between dates of planting.

The estimates of the two error variance were 0.52 [Error a] and 0.192 [Error b], thus confirming the expectation that the main plot error is likely to be higher than sub-plot error. Further, it may also be concluded that sowing period A (1st November) gives maximum yield irrespective of genotypes.

13. Let's sum up

- Plant breeding is the genetic improvement of the crop in order to create desired plant types that are better suited for cultivation, give better yields and are disease resistant.
- Plant introduction is a process of introducing plants from their own environment to a new environment. The central plant introduction agency in India is National Bureau of Plant Genetic Resources (NBPGR), which has its headquarters at New Delhi.
- In mass selection, a large number of plants having similar phenotype are selected and their seeds are mixed together to constitute a new variety whereas in pure line selection, The selected individual plants are grown in individual rows and evaluated and best progeny is selected, yield tested and released as a variety.
- Individual produced as a result of cross between two genetically different parents is known as hybrid. The natural or artificial process that results in the formation of hybrid is known as hybridization.
- Heterosis can be defined as the superiority of F1 hybrid over both the parents in terms of yield and some other character and inbreeding depression is the reduced biological fitness in a given population as a result of inbreeding.
- When mutations are induced for crop improvement, the entire operation of the induction and isolation, etc. of mutants is termed as mutation breeding.
- When the techniques for artificial chromosome doubling became established, investigations on the origin of many of our economic plants were resumed. Many important crop plants like wheat, oat, sugarcane, cotton, tobacco as well as many fruits and vegetables are the Polyploidy of various degrees.

- Worldwide there are more than 40,000 different varieties of *Oryza sativa*, classified into four major categories: *indica, japonica, aromatic and glutinous*. Maize is second leading crop after wheat. United States produces 50% of the world production. Potato is a very popular vegetable grown all over the world and important food crop of grown more than 100 countries in world.
- Plant disease resistance protects plants from pathogens in two ways: by preformed structures and chemicals, and by infection-induced responses of the immune system.
- Marker assisted breeding is defined as the application of molecular markers in combination with linkage maps and genomics to alter and improve plant traits on the basis of genotypic assays. This term is used to describe several modern breeding strategies including marker-assisted selection.
- Then few plants are taken into account for studying the whole population of plant in that field. The method by which only few items are selected from the population in such a way so that they will represent the population in unbiased way is called sampling.
- Mean is the value which we get by dividing the aggregate of various items of the same series by the total number of observations. Mode is the Most frequent value in a series and median is the value of the middle item of a series when arranged in order of magnitude.
- In biological research when we compare any character of two samples, we calculate the significance of difference in the mean and variance to draw a meaningful conclusion.
- $\chi 2$ test is used for testing the agreement of observed frequencies with those expected upon a given hypothesis.
- The analysis of variance is based on F-statistics which is a ratio of variances. The ratio of variances due to treatments and variances due to random differences within the treatment is calculated and the estimate, known as the 'F', is then used for comparison.
- Association between variables or attributes or characteristics at a given time is known as correlation. The statistical analysis employed to find out the exact position of the straight line is known as Linear regression analysis.

13. Suggested Reading

1. Chaudhuri, H.K. Elementary Principles of Plant Breeding, Latest Ed., Oxford & IBH.
2. Singh, B.D. Plant Breeding, Principles & Methods (7th ed.), 2005, Kalyani Publishers.
3. Barley, N.T.J. Statistical Methods in Biology, Latest Ed., Cambridge University Press.
4. Roy, D. Plant Breeding: Analysis & Exploitation of Variation, 2000, Narosa Publishing
5. Kar, D.K. and Halder, S. Plant Breeding & Biometry, 2006, New Central Book Agency.
6. Datta, Animesh K. Basic Biostatistics & its Application 2006, New Central Book Agency.
7. http://www.biologydiscussion.com/
8. https://en.wikipedia.org/wiki/Plant_breeding

14. Assignment

1. What is meant by sample?
2. What is normal curve?
3. How do you calculate $\chi 2$ test of heterogeneity?
4. Short note on a) CV, b) Standard error, c) ANOVA, d) Gene for gene hypothesis.
5. Give the differences between vertical and horizontal disease resistance?
6. What is the importance of allopolyploidy?
7. Write the differences between pureline selection and mass selection.
8. Describe the merits and Demirits of mean, median and mode.
9. Explain Vertifolia effect with suitable examples.
10. What is RAPD, ISSR? States its significance in plant breeding program?
11. What are the possible methods of breeding for disease resistance?
12. Enumerate the broad sense and narrow sense heritability with classical examples.
13. Why CD is important?
14. Write the detail description about breeding practices of rice, potato.
15. How does split plot design differ from randomized block and Latin square designs?
16. How can you differentiate Student's t test from F-test?
17. What does 't-test' signify?
18. Mention the equation for simple regression analysis.
19. What is a basic chromosome number?
20. Name the first two mutants released in India.
21. What is the current unit of gamma irradiations?
22. Mention the basic significance of gene mutation in crop improvement.
23. Mention the chromosome number of cultivated cotton.
24. Mention the cytogenetical status of Rice.
25. Cite two examples to explain disease resistance in plant species
26. What is vertifolia effect?
27. How can you calculate CD from ANOVA table?
28. What are the measures of dispersion?
29. How can you calculate mode from grouped data with regular class interval?
30. What is mean shift?
31. What is the genetical significance of $\chi 2$-test analysis?
32. Mention the names of test of significance.
33. What is 'r' and how it varies?
34. State two applications of student 't' test.

35. What is degree of freedom?
36. Name the first two mutant varieties released in India.
37. What is amphidiploids?
38. Name one released autopolyploid variety.
39. Name the parents of *Nicotiana tabacum*
40. Write notes on the following:
    i. Probability
    ii. Paired t-test
    iii. Phenotypic correlation analysis
    iv. Design of experiments

All the materials are self writing and collected from e-book, journals and web sites

POST GRADUATE DEGREE PROGRAMME (CBCS)

IN

BOTANY

SEMESTER - II

Course: BOHCT 2.4

(Palaeobotany, Palynology and Anatomy)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

KALYANI-741 235

WEST BENGAL

COURSE PREPARATION TEAM

Dr. Bapi Ghosh
Assistant professor
Department of Botany, DODL
Kalyani University

Dr. Pallab Kumar Ghosh
Assistant professor
Department of Botany, DODL
Kalyani University

Dr. Sudipta Roy
HOD & Associate professor
Department of Botany
Kalyani University

Dr. Zahed Hossain
Associate professor
Department of Botany
Kalyani University

Dr. Sudha Gupta
Assistant professor
Department of Botany
Kalyani University

Dr. Malay Kr. Adak
Assistant professor
Department of Botany
Kalyani University

Dr. Neera Sen Sarkar
Assistant professor
Department of Botany
Kalyani University

June, 2019

Directorate of Open and Distance Learning, University of Kalyani

Published by the Directorate of Open and Distance Learning,
University of Kalyani, Kalyani-741235, West Bengal and Printed by
Printtech, 15A, Ambika Mukherjee Road, Kolkata-700056

All right reserved. No part of this work should be reproduced in any form without the permission in writing from the Directorate of Open and Distance Learning, University of Kalyani.

Authors are responsible for the academic contents of the course as far as copyright laws are concerned.

Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2017 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Sankar Kumar Ghosh, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every member of PGBOS (DODL), University of Kalyani. Heartfelt thanks is also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and co-ordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

Self Learning Materials (SLMs) have been published by the Directorate of Open and Distance Learning, University of Kalyani, Kalyani-741235, West Bengal and all the copyright reserved for University of Kalyani. No part of this work should be reproduced in any from without permission in writing from the appropriate authority of the University of Kalyani.

All the Self Learning Materials are-self writing and collected from e-book, journals and websites.

Director
Directorate of Open and Distance Learning
University of Kalyani

SYLLABUS

COURSE–BOHCT 2.4

Palaeobotany, Palynology and Anatomy

(Full Marks–75)

Course	Group	Details Contents Structure		Study hour
BOHCT 2.4	Palaeobotany	**Unit 1. Introduction and Presevation of plants as fossils**	1. Introduction: Definition and application. 2. Preservation of plants as fossils: Definition; taphonomy; environment for fossilization; modes of preservation; types; major rock types, rock cycle and rocks containing Fossils; systematics, reconstruction and nomenclature.	1
		Unit 2. Geologic Time and Early life	3. Geologic Time: Geologic timescale, relative vs. numerical age, physical and biological principles for defining relative and numerical age. 4. Early Life: The origin of earth, earliest environment, theories on origin of life, evidences for the origin of life - prokaryotes, evolution of eukaryotes and fossil records, diversified life - algae and fungi.	1
		Unit 3. Terrestrialization of plants and Early vascular plants to early spore producing trees	5. Terrestrialization of plants: Geologic time, environment, vegetative and reproductive adaptations to land dwelling, fossil evidences - transitional plants with land adaptive features, early non vascular land plants (bryophytes), early vascular land plants (pteridophytes). 6. Early vascular plants to early spore producing trees (arborescent pteridophytes & progymnosperms): Geologic time, environment, advancement in plant adaptive features for land dwelling with fossil evidences.	1
		Unit 4. Early spore producing trees to early seed producing trees andOrigin and evolution of flowering plants	7. Early spore producing trees to early seed producing trees (gymnosperms): From isospores to free sporing heterospores, origin of ovule, hydrasperman reproduction with fossil evidences. 8. Origin and evolution of flowering plants (angiosperms): Geologic time, evolutionary trends -angiosperm derived characteristics, fossil evidences for early flowering plants, place of origin, radiation, phylogeny.	1

Course	Group	Details Contents Structure		Study hour
BOHCT 2.3	Palynology	**Unit 1. Spore-pollen morphology**	1. Spore-pollen morphology: units, polarity, symmetry, shape, size, aperture; NPC system for numerical expression of apertural details; harmomegathic mechanism; evolution of aperture types.	1
		Unit 2. Sporoderm and Pollen Wall:	2. Sporoderm: stratification & sculptures; LO-analysis; pollen wall evolution. 3. Pollen Wall: Development; Ubisch body; sporopollenin; adaptive significance of pollen wall architecture.	1
		Unit 3. Extraexinous wall materials and Natural spore/pollen traps	4. Extraexinous wall materials: pollen connecting threads, perine, pollen-kit. 5. Natural spore/pollen traps: types, their implications in floristic & environment reconstruction	1
		Unit 4. Branches of Palynology & their Application	4. Branches of Palynology & their Application: Branches of palynology; palynology in taxonomic & phylogenetic deductions; palynology in academic & applied aspects including melissopalynology, medical palynology, forensic palynology, entomopalynology & copropalynology..	1
	Anatomy	**Unit 1. Meristems and differentiation**	1. Meristems and differentiation: meristems and growth of the plant, differentiation of primary and secondary plant body: epidermis: stomatal ontogeny, cuticle and epidermal appendages; secretory structure	1
		Unit 2. Phylogeny and evolution of vascular and mechanical tissue elements	2. Phylogeny and evolution of vascular and mechanical tissue elements: origin and development of sclereids and fibres, phylogeny of xylem and phloem elements, wood anatomy: vascular cambium and its seasonal activity	1
		Unit 3. Anatomical variations with ecology	3. Anatomical variations with ecology: leaf and root anatomy in ecological perspectives; hydraulic architecture of plant	1
		Unit 4. Anatomy in applied science	6. Anatomy in applied science: anatomy and pollution, anatomy in forensic science, archaeology and climatology.	1

Content

	COURSE – BOHCT 2.4 Palaeobotany, Palynology and Anatomy	Page No.
Unit 1.	Introduction and Presevation of plants as fossils	2-38
Unit 2.	Geologic Time and Early life	38-52
Unit 3.	Terrestrialization of plants and Early vascular plants to early spore producing trees	52-85
Unit 4.	Early spore producing trees to early seed producing trees and Origin and evolution of flowering plants	86-100
Unit 1.	Spore-pollen morphology	103-133
Unit 2.	Sporoderm and Pollen Wall:	134-141
Unit 3.	Extraexinous wall materials and Natural spore/pollen traps	141-143
Unit 4.	Branches of Palynology & their Application	143-148
Unit 1.	Meristems and differentiation	151-167
Unit 2.	Phylogeny and evolution of vascular and mechanical tissue elements	168-189
Unit 3.	Anatomical variations with ecology	189-200
Unit 4.	Anatomy in applied science	200-211

COURSE – BOHCT 2.4
PALAEOBOTANY, PALYNOLOGY AND ANATOMY

Hard Core Theory Paper **Credit: (Groups A+B+C) = 3**

Group – A (PALAEOBOTANY)

Content Structure

1. Introduction
2. Course Objective
3. Introduction: Definition and application.
4. Preservation of plants as fossils: Definition; taphonomy; environment for fossilization; modes of preservation; types; major rock types, rock cycle and rocks containing Fossils; systematics, reconstruction and nomenclature.
5. Geologic Time: Geologic timescale, relative *vs*. numerical age, physical and biological principles for defining relative and numerical age.
6. Early Life: The origin of earth, earliest environment, theories on origin of life, evidences for the origin of life - prokaryotes, evolution of eukaryotes and fossil records, diversified life - algae and fungi.
7. Terrestrialization of plants: Geologic time, environment, vegetative and reproductive adaptations to land dwelling, fossil evidences - transitional plants with land adaptive features, early non-vascular land plants (bryophytes), early vascular land plants (pteridophytes).
8. Early vascular plants to early spore producing trees (arborescent pteridophytes & progymnosperms): Geologic time, environment, advancement in plant adaptive features for land dwelling with fossil evidences.
9. Early spore producing trees to early seed producing trees (gymnosperms): From isospores to free sporing heterospores, origin of ovule, hydrasperman reproduction with fossil evidences.
10. Origin and evolution of flowering plants (angiosperms): Geologic time, evolutionary trends - angiosperm derived characteristics, fossil evidences for early flowering plants, place of origin, radiation, phylogeny.
11. Let us sum up
12. Suggested Reading
13. Assignment

1. Introduction

The relevance for the unit for attaining a comprehensive knowledge about the sequences of nature's own creations (origin and diversification) and disasters (extinction) occur including interrelations among different spheres geosphere-atmosphere-biosphere through millions of years in the globe. Fossil is one of the authentic evidences in bio-geo-atmospheric evolutionary history of the Earth through geological ages. By introducing this course, students would be acquainted with overall idea about fossils and different techniques for their study. The knowledge would help them to describe primordial life forms and their evolution, to determine the age of the sediments and fossils, to enlighten significance of continental drift hypothesis and plate tectonics, and to explain Indian Gondwana sequence and its importance.

2. Course Objectives

After completion of the course the learners will be able to:

- describe the general idea about fossils focusing on plant fossils and their applications in our life;
- explain the origin of life, early life forms and their evolution through geological ages;
- discuss the Indian Gondwana sequence and its importance;
- explain the concepts and validation of continental drift hypothesis and plate tectonic theory;
- determine the geological age of sediments and fossils

3. Introduction: Definition and application

Definition

The branch of science which deals with the plants of the geological past (plant fossils) is known as 'Palaeobotany'. Palaeobotany deals with the study of Plant Fossils. 'Fossils' are extremely important component of nature being the store house of unique data set that not only throw light on the yet not understood phenomenon of "Origin of life" on earth but also explore sequences on the "Origin of the Biosphere, Geosphere, and the Atmosphere" of the globe. Besides many other applications, Palaeobotany is closely related to exploration of valuable organic deposits.

Brief idea

The word Fossils is derived from the Latin verb fodere, which means "to dig", referred to anything removed from earth (Arnold, 1947). Precisely, any direct or indirect evidences of life and life time activities preserved in the sedimentary layers of the geological past and recent past (Holocene) are considered as Fossils. It may be anything e.g. Plants, Animals, Microbes, Trace fossils, chemical precipitations, organic deposits etc. The data bases are successfully utilized primarily to know about 'Evolution'- in case of plants- from Algae to Angiosperms. Plant Fossil data are excellent guide to understand the dynamics of the moving biosphere of the moving continents. The very important finding of the drifting of the continents was proposed with the support of the Fossil Plants besides other evidences. The fossil data base is closely linked to diverse disciplines such as evolution of the environment (Palaeoenvironment), ecology (Palaeoecoloy and creation, destruction of continents, the mountain ranges, shifting of latitudes, movements of the oceans, coastal evolution, sea level changes,

progradation and regression of delta change of climate, monsoonal activities (palaeoclimate), change of the geography of the globe (palaeogeography), more importantly the complex episode of plant animal interactions in relation to environment and so on.

In the beginning of 21st century we now have enormous amount of data on Fossil plants in chronological succession collected from all over the globe including India. Palaeobotanical study has been enriched with various applications with the knowledge in Fossil Plants since early 19th century. It was assumed initially that no life did exist at the chronologically oldest strata Precambrian. Since long time, Precambrian was considered as Cryptozoic eon or period of Hidden life. The discoveries made from the Precambrian rocks since mid twentieth century by Tyler and Barghoorn (1954) raised the importance of the palaeobotanical data in understanding the origin of life on earth. The discoveries of fossils in the Precambrian rocks thereafter were made from all over the globe; the importance of the Stromatolites, the organo-sedimentary deposits very much common in the Precambrian also gained attention in tracing the microbiota in the oldest strata. Series of discoveries from the Precambrian within about twenty-five years resulted to accumulation of enormous data on fossil microbiota from Precambrian rocks; the authentic evidence about the structurally preserved bio forms started to come from all over the globe including India after the wonderful discovery of microfossils in the rocks as old as more than 2 billion years by Tyler and Barghoorn (1954) from Canadian Shield. Since then many evidences have been accumulated for justifying the fact that life appeared 3500 million years also diversity in the life forms did exist at least since 3500 million years. It is now presumed that life might have emerged much earlier (?300 million years prior) to the earliest recorded life form Schopf (2009, 2010). An elaborate account on the discoveries on Precambrian biosphere was documented in the book “Earth’s Earliest Biosphere” edited by James W. Schopf (1983) which includes records of undoubted and doubtful microfossils. Lately, Schopf (2009, 2010) demonstrated the undoubted documents of early life in the Precambrian with the help of the modern techniques of studying the microfossils by Fluorescence CLSM (Confocal Laser Scanning Microscopy) and molecular structural Imagery (Raman Spectroscopy) by which the three-dimensional morphology and direct analysis of chemical composition of the fossils can be determined.

History of palaeobotanical studies in India

History of palaeobotanical studies in India has been reviewed from time to time by the eminent botanists, palaeobotanists of the country. The first twenty five years of progress in palaeobotanical research in India was published by Professor S. P. Agharkar in 1938 (Proceedings Indian Science Congress Association, Pages 760-765), Fifty years of Science in India by P. Maheshwari and R. N. Kapil (1914-1963) Ind. Sci. congress Association P.147-1963) A. K. Ghosh in A. K. Sharma edited volume (1973) on A decade of science in India (1963-1972; p. 203-220), Venkatachala and Maheshwari, (in B. M. Johri Edited, Botany in India: History and progress, 1995, Oxford and IBH Publishing Co. Pvt . Ltd, P445-463).

Palaeobotanical research centers in India

Pioneer and important Palaeobotanical research centers in India and some palaeopalynological study centers are Department of Geology, Lucknow University; Department of Botany, University of Calcutta; University of Pune; University of Allahabad; Maharashtra Association for the Cultivation of Science, Pune; National Institute of Science, Nagpur; Department of Botany, , Burdwan University; Kalyani University; Department of Geology, Delhi University; Bose Institute, Calcutta; Institute of Petroleum exploration, Oil and Natural Gas Commission, Dehradun; Indian Statistical Institute, Calcutta; Institute of Francaise, Pondichery; Wadia Institute of Himalayan Geology, Dehradun;

National Institute of Oceanography, Goa; Indian Institute of Technology, Powai, Bombay. In addition, in many other Universities, Institutions, colleges of India palaeobotanical, palynogical studies are carried out.

Origin of environment and its successive evolution on the planet earth was possible by the contributions of plants of the geological past. The present world has diverse biosphere surviving in the normal atmospheric condition with 21% O_2 and natural Global warming on the lithosphere of the earth which is made up of at least seven (VII) large crustal blocks regulated by the plate tectonic mechanism. A close interrelationship of these spheres i.e. Atmosphere, Biosphere and litho/Geosphere including hydrosphere creates the Environment on the globe. With the change in these spheres due to various natural conditions the environment on the globe has changed in time and space.

At the time the earth was born, 4600 Million years ago there was no life and no lithosphere and atmosphere. The planet earth became a living planet through successive chemical and biochemical evolution followed by the prolonged efforts of blue green and the green algae in the Precambrian eon. Step by step evolution of biomolecules followed by the evolution of diverse prokaryotes, subsequently the eukaryotes induced liberation of oxygen. The original anoxic atmosphere of the earth thus started to have free O_2; the percentage gradually increased with the flourishing of abundant oxygen producing green algae. The evidences are the Banded Iron Formation and the Stromatolites. The free O_2 partially converted to O_3 through natural process and protection of the planet earth from the lethal rays of the Sun started. This is how the present-day environment originated. The green plants were initially growing in aquatic condition at suitable depth of water for protection from the lethal rays for a long time of about 3000 million years. Gradual increase in the percentage of O_3, and O_2 enabled the plants to adapt in the terrestrial condition about 440 million years ago. The unique evidences of some enigmatic non-vascular plants and the small plants like *Aglaophyton major, Cooksonia, Rhynia* etc have revealed the steps in the terrestrialization of the plants. Evolution of plants and environment was rapid since terrestrialisation and the percentage of oxygen in the atmosphere was almost like present condition (21%) by 245 million years by the end of Permian. By this time the planet was covered by the all time thick forests which later transformed to Coal. The forest started to evolve from Devonian with the *Archaeopteri*s tree of Progymnosperms and then the *Lepidodendron, Lyginopteris, Medullosa, Cordaitales, Glossopteris* trees flourished in the Carboniferous – Permian Period.

The forests of the Carboniferous–Permian Period are known as the Lepidodendron forest of Northern hemisphere and the Glossopteris forest of the southern hemisphere and Indian subcontinent which was at that time a part of southern hemisphere. The Geosphere at that time was different from present condition. The Northern hemisphere is recognized as Laurasia while the Southern hemisphere together with Indian sub continent is well known as Gondwanaland.

Application

Plant fossils have enormous applications in both academic and applied aspects which are enumerated below-

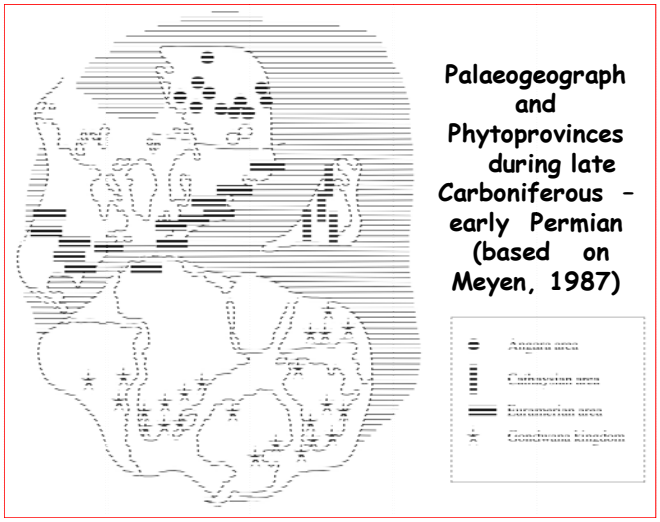
Fossils reveal the mysteries of the living planet and help to resolve the mystery behind the origin of life, when & how evolution of O_2 and O_3 occurred in the primitive earth, primordial life forms and their evolution.

Palaeofloristics Study for determination of palaeovegetation, palaeogeography and their subsequent changes: We know flroistics is the geographical distribution of taxa, the plotting of the plant system against the geographical background and palaeofloristics is the study of the distribution

of the taxa — in time and space. In palaeofloristics both the spatial distribution and the chronological ranges of the taxa are considered.

The concentration of the boundaries in certain areas of the Earth can be observed by plotting the taxa areas on maps. This implies that the taxa form natural associations, each of which is confined to a definite territory. Each association corresponds to a territorial floristic unit — the phytochorion. Phytochorion is a geographic area with a relatively uniform composition of plant species. Adjacent phytochoria do not usually have a sharp boundary, but rather a soft one, a transitional area in which many species from both regions overlap. Phytochoria may be of different ranks. In descending order, they are as follows: kingdom, area, province, district, region. Each is denoted by a specific geographical name. The taxa list of a phytochorion or its part is termed flora.

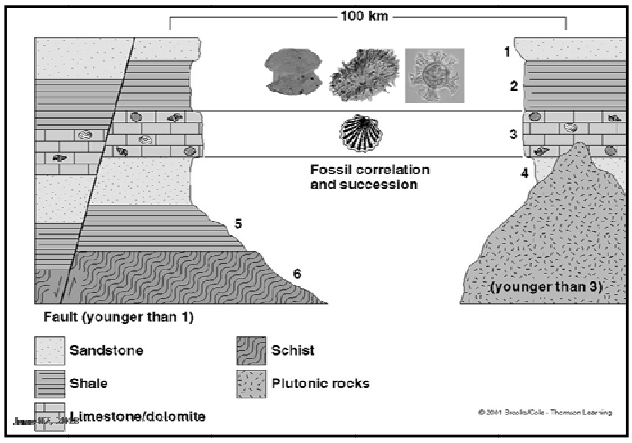
Fig.1: Floral diversification in late Palaeozoic (Late Carboniferous – Early Permian)



Plant Fossil evidences show that apparently uniform pattern of pre-Carboniferous vegetation of the globe was changed by the influence of Glacial cycle of mid Carboniferous and thus the floral diversification in the late Palaeozoic was initiated with changed climatic and geographic conditions of the globe. The diversified late Palaeozoic flora was designated as four geographic regions specific namely, **Angara flora** (in modern Siberia), **Cathaysian Flora** (in modern China & South East Asia), **Euramarian flora** (in Western part of Laurasia- North eastern North America, Western Europe) and **Gondwana flora** (in South America, Africa, India, Australia, Antarctica) (Fig.1).

Biostratigraphic analysis for determination of relative age and correlation of rock: Biostratigraphy is the branch of stratigraphy which focuses on correlating and assigning relative ages of rock strata and correlation between and among the strata by using the fossil assemblages contained within them. The methods by which the age relationship between various strata of Earth's crust is established is called correlation (Fig. 2). Such relationships can be established, in general, in one of two ways: by comparing the physical characteristics of strata with each other (physical correlation through lithostratigraphy); and by comparing the type of fossils found in various strata (fossil correlation through biostratigraphy). It provides information about changes that have taken place at various times in Earth history.

Fig. 2: Correlation of rock strata



Facies analysis for determination of depositional environment: Facies is a body of rock with specified characteristics. Ideally, facies are distinctive rock unit that forms under certain conditions of sedimentation, reflecting a process or environment. Thus, sedimentary facies refer to all the characteristics of a rock unit which come from the depositional environment (Fig. 3). In this regard, **Walther's Law of Facies** is well known which describes the vertical succession of facies reflects lateral changes in environment. Conversely, it states that when a depositional environment "migrates" laterally, sediments of one depositional environment come to lie on top of another. A classic example of this law is the vertical stratigraphic succession that typifies marine transgressions (sea level rise) and regressions (sea level fall).

Fig. 3: Sedimentary Facies showing depositional environment

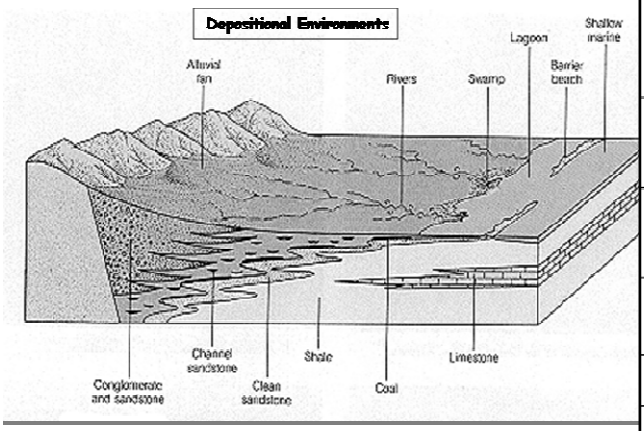
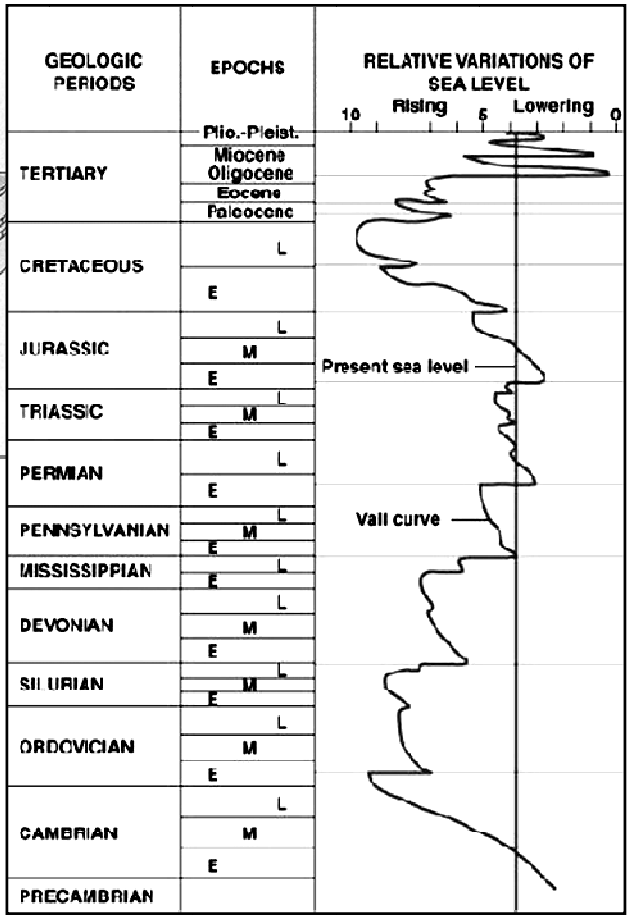


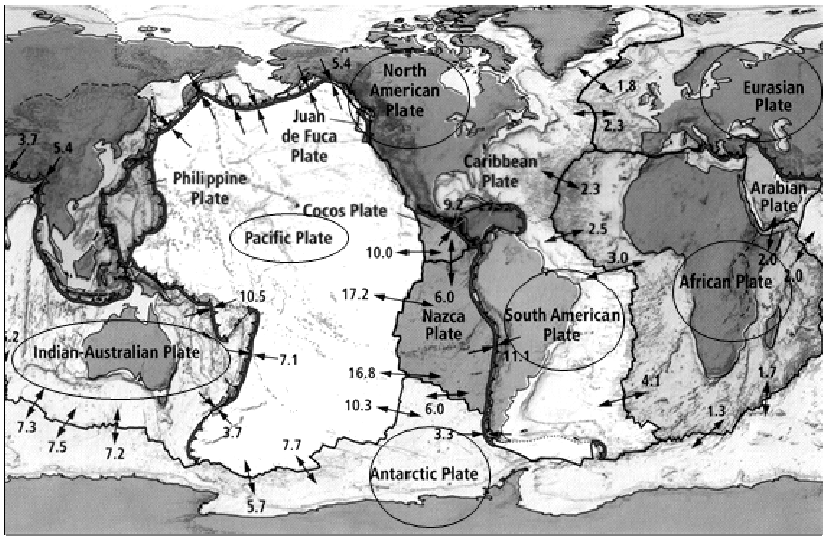
Fig. 4: Vail Curve



Determination of sea level change: The sea level throughout the geological history is changed by transgression and regression which is represented by the sea-level curve (Fig. 4). The first such curve is the **Vail curve or Exxon curve**. The names of the curve refer to the fact that in 1977 a team of Exxon geologists from Esso Production Research headed by Peter Vail published a monograph on global eustatic sea-level changes. Their sea-level curve was based on seismic and biostratigraphic data accumulated during petroleum exploration.

Plant fossil as provenance of Continental Drift & Plate Tectonic Theory: The rigid lithosphere of our planet Earth consists of numerous variable sized pieces called plates. At present, 7 large lithospheric plates and a few dozen smaller plates make up the outer shell of Earth (Fig. 5). These plates move slowly over the hotter and weaker semiplastic asthenosphere (*uppermost mantle underlying the lithosphere)* and change in size because of some type of heat transfer system within the asthenosphere.

Fig. 5: Lithospheric plates of outer shell of modern Earth
(Source: https://geology.com)

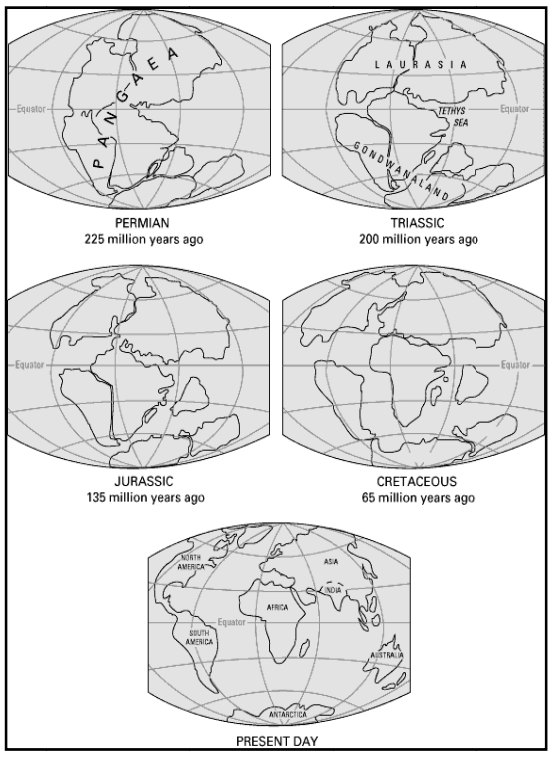


Consequently, plates separate, mostly at oceanic ridges *(underwater mountain system formed by plate tectonics)*, and/or collide, usually at oceanic trenches *(depressions of the sea floor)*, where they are subducted back into the mantle

Concept of Continental drift hypothesis

The continental drift hypothesis was proposed by Alfred Wegener in 1915. He proposed that all the land masses were originally united in a single supercontinent – Pangaea (Greek meaning all land). The supercontinent Pangaea started to break up about 200 million years ago by the movement of crustal plates over the mantle and uultimately continents "drifted" to their present positions (Fig. 6).

Fig. 6: Changing position of crustal plates of Earth over millions of years
(Source: https://futuremaps.com/blog/plate-tectonics)



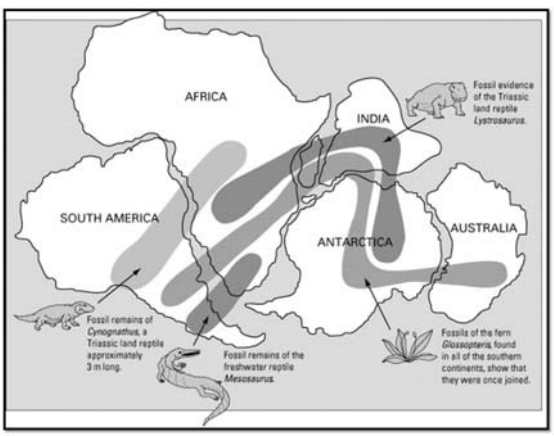
Evidences in support of Continental drift hypothesis

This hypothesis was supported by fossils, rock types and by matching the coastline shapes. Importance of the Gondwanaland concept and the present distribution of the Glossopteris flora was realized by Wegener (1915), Du Toit (1932) and other geoscientists while they were advocating the famous Continental drift hypothesis.

A. Fossil correlations: Identical members of Glossopteris flora including fertile organs and some reptiles distributed in all the Southern hemisphere continents and Indian subcontinent (Fig. 7). Important examples are *Senotheca murulidihensis* fertile organ in organic connection with *Glossopteris clarkei* (Fig. 7) are recorded from Lower Gondwana sediments of both India

and Australia. Restricted occurrence of the small fresh water aquatic reptile *Mesosaurus* (Fig. 7) in the eastern part of Brazil, South America and western part of Africa (Fig. 7).

Fig. 7: Fossil evidences in support of continental drift hypothesis



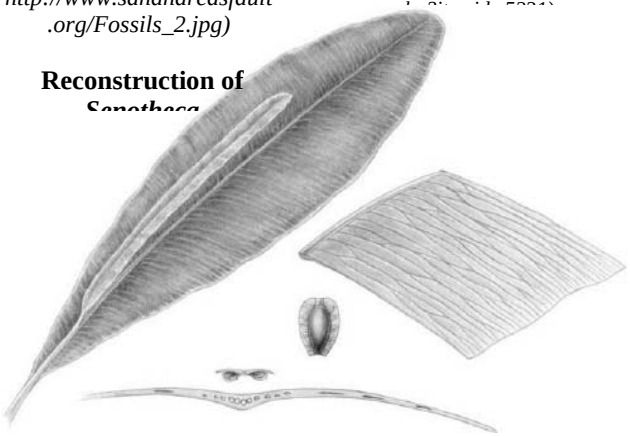
Fossil Correlations
(Source: http://www.sanandreasfault.org/Fossils_2.jpg)



Mesosaurus
(Source: https://www.busaccagallery.com/catal



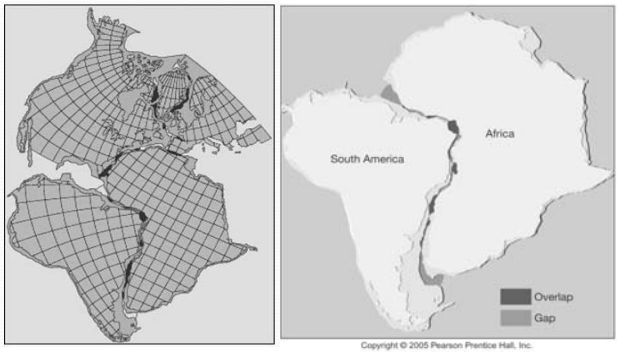
Reconstruction of *Senotheca*



***Senotheca*-fertile organ of *Glossopteris clarkei* leaf**
(Source:https://www.sciencedirect.com/science/article/pii/

B. Tight fit of the Continents: Shape of the present-day continents look like jigsaw puzzle pieces especially the coastal contours between eastern South America and western Africa suggesting that once they fitted together geographically (Fig. 8) and drifted apart later.

Fig. 8: Geographical fit of the Continents



C. Correlation of mountains: The mountain range with nearly identical rocks and structures are found in the eastern USA and north-west Europe (Fig. 9).

Fig. 9: Correlation of mountains with nearly identical rocks and structures

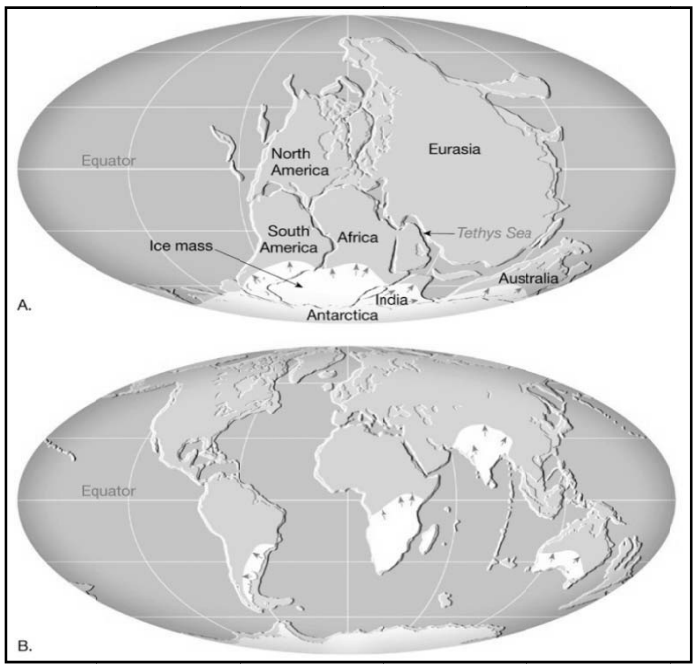


Copyright © 2005 Pearson Prentice Hall, Inc.

D. Glacial features: Similar glacier deposits are found in Antarctica, South America and India which are now thousands of km apart (Fig. 10A). The identical glacial features of the same age establish a tight polar distribution of the Gondwana continents (Fig. 10B).

Fig. 10: Identical glacial features in Gondwana continents

A: Tight polar distribution during past B: Present-day distribution



E. Similar climate change event: Similar sequence of climate change from glacial climate to warm humid climate occurred in the entire Gondwanaland as revealed from the lithological, and fossil evidences.

Validation of Continental drift hypothesis

The Continental drift hypothesis was received well in Europe and southern hemisphere continents but rejected in U.S.A, as because of lack of a suitable mechanism to explain how continental crusts, composed of granitic rocks, could seemingly move through the denser basaltic oceanic crust. But, this conflict remained unresolved because seafloors were almost completely unexplored until 1960s, when Military Spending U.S. Navy mapped seafloor with echo sounding to find and hide submarines during the World War II and the Cold War. This detailed oceanographic research provided convincing evidence that the continents had once been joined together and subsequently separated, and finally the hypothesis of continental drift became widely accepted.

Concept of Plate Tectonics

Seafloor mapping is the basis for rising of the Plate Tectonic theory. Plate Tectonic is a unifying theory of Geology which combines the earlier hypotheses of 'continental drift' (moving continent) and 'seafloor spreading' (moving sea floor). The generalized map of seafloor explores different unique structures like-

- **Oceanic ridges**—submerged mountain ranges
- **Fracture zones**—cracks perpendicular to ridges
- **Trenches**—narrow, deep gashes (cuts)
- **Abyssal plains**—vast flat areas
- **Seamounts**—drowned undersea islands

Furthermore, marine geologists found that seafloor magnetism has a striped pattern which is completely unlike patterns on land and they hypothesized that these stripes are indicatives of periodic reversal of the direction of Earth's magnetic field. Scientists validated the hypothesis by determining the eruptive ages and the polarity of young basalts using the K-Ar (Potassium-Argon) radiometric dating. Afterwards (1962-1963) geologists realized that the patterns are symmetrical across oceanic ridges and the K-Ar dating determines the age of rocks at the ridges as youngest. They also resolved that bands of seismicity chiefly exist at trenches and oceanic ridges which are the origins of different plate boundaries where earthquakes and volcanic eruptions happen. Three types of relative motions like divergent, convergent, and transform (Fig. 11) exist between plates.

Fig. 11: Different boundaries between the tectonic plates

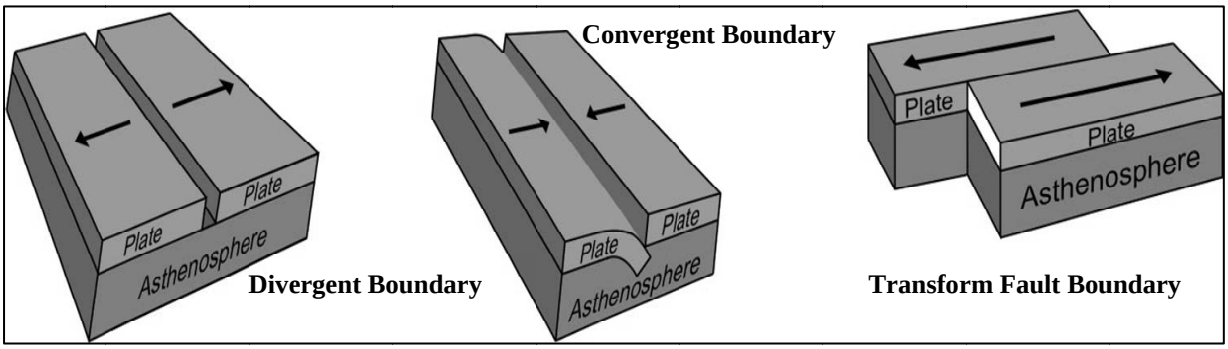
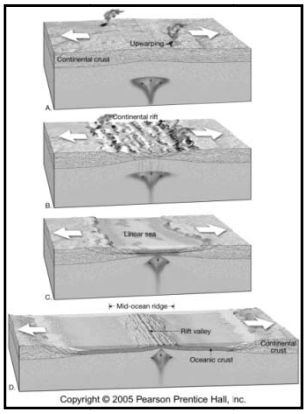
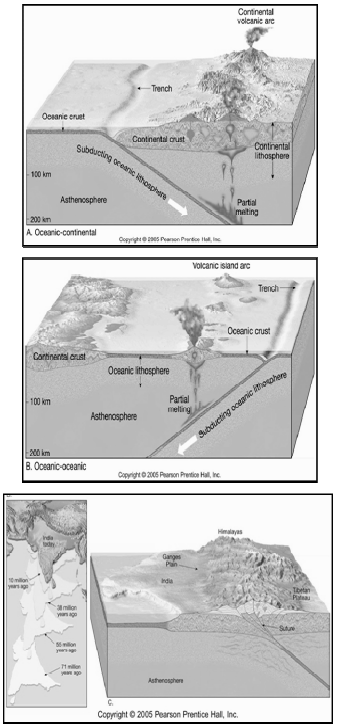


Fig. 12: Divergent boundaries leading to formation of oceanic lithosphere



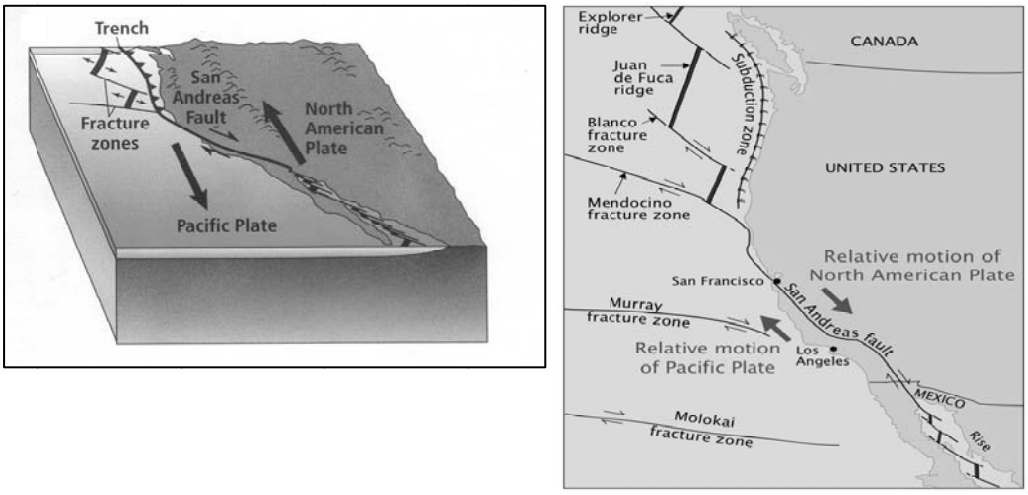
Convergent Boundary: Convergent boundary causes movement of plates together and it causes for creation of either *'subduction zones'* or *'collision zone'* (Fig. 13). *Subduction zone* may form continental volcanic arc (example: Andes, Cascades) if at least one side has denser oceanic material or volcanic island arc (example: Japan) and trenches if both the sides have oceanic materials whereas the *Collison zones* form mountain range (example: Himalayas) where both sides of a convergent boundary consist of continental (buoyant) material.

Fig. 13: Different types of convergent boundaries



Transform fault boundary: Most transform boundaries are present in the ocean moving the plates sideways past each other (example: San Andreas Fault where the North American and Pacific plates moving past each other (Fig. 14).

Fig. 14: Transform fault boundaries

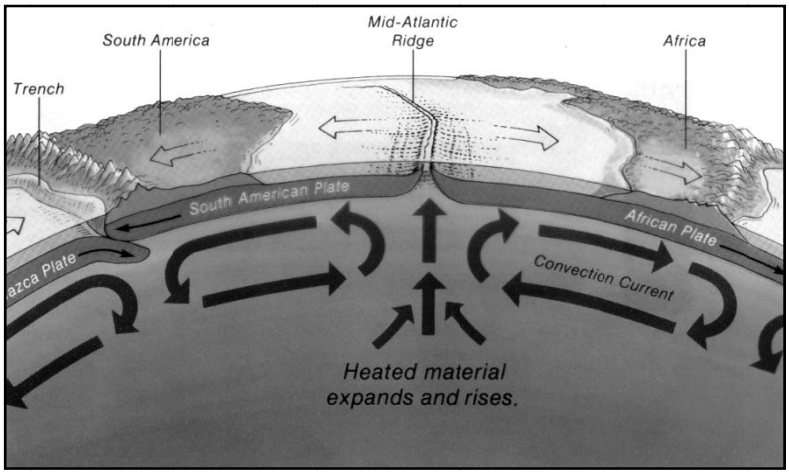


Scientists eventually succeeded to elucidate the reasons behind the plate movement by gaining detailed knowledge about seafloor and suggested two related ideas namely **'Slab pull'** and **'Mantle convection'** which are widely accepted for explaining drifting of plates.

Slab pull: Denser, colder plate sinks at subduction zone, pulls rest of plate behind it.

Mantle convection: Hotter mantle material rises beneath divergent boundaries and cooler material sinks at subduction zones (Fig. 15). As such, plate movement, earthquakes, and volcanic eruptions are due to Earth's loss of internal heat.

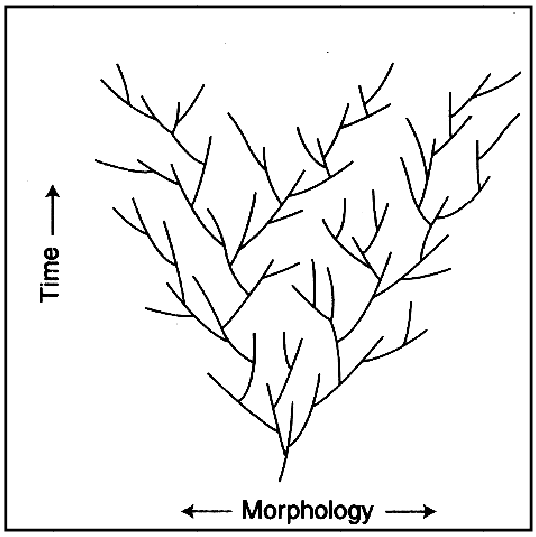
Fig. 15: Convection current



Plant fossil record in deciphering evolutionary history and mass extinction event: Two evolutionary theories Phyletic gradualism and Punctuated equilibrium are established based on fossil record.

Phyletic gradualism (Darwin, 1859): Evolutionary change results from descent with modification and accomplished by 'natural selection'. Species are therefore temporary stages in continuing gradual change. Ecological interactions are ultimate driving mechanism behind macroevolution. So, this theory supports the view that speciation is a gradual and continuous process (Fig. 16). "If variations useful to any organic being do occur, individuals thus characterized will have the best chance of being preserved in the struggle for life; and from the strong principle of inheritance they will tend to produce offspring similarly characterized"

Fig. 16: Phyletic gradualism

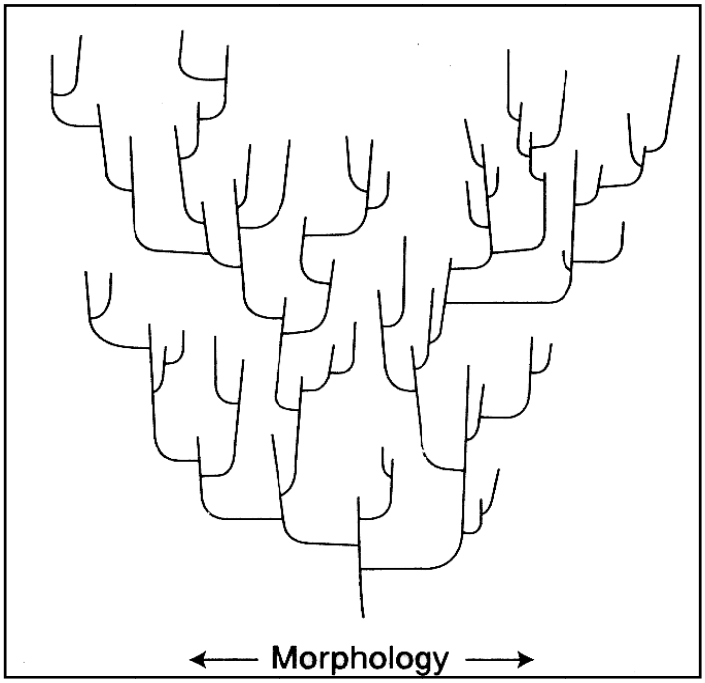


Punctuated equilibrium (Eldredge & Gould, 1972):

Geological record does not indicate a long sequence of continuous intermediate species from the parental to the descendant species as expected if the phyletic gradualism was occurring. Rather there are long periods where there appears to be little morphological change or speciation, followed by periods of relatively rapid change (Fig. 17). This pattern of abrupt speciation in the geological record has been attributed to gap in the fossil record- the 'breaks' could represent periods of rapid speciation. A phyletic line that was usually in stasis disturbed only rarely by rapid and episodic events of speciation. There are relatively rapid periods of speciation interspersed by long periods where there appears to be little morphological change or speciation, referred to as stasis.

Fig. 17: Phyletic gradualism

Cone of increasing diversity, but with vertical branches representing periods of stasis, interspersed by horizontal lines representing periods of rapid change



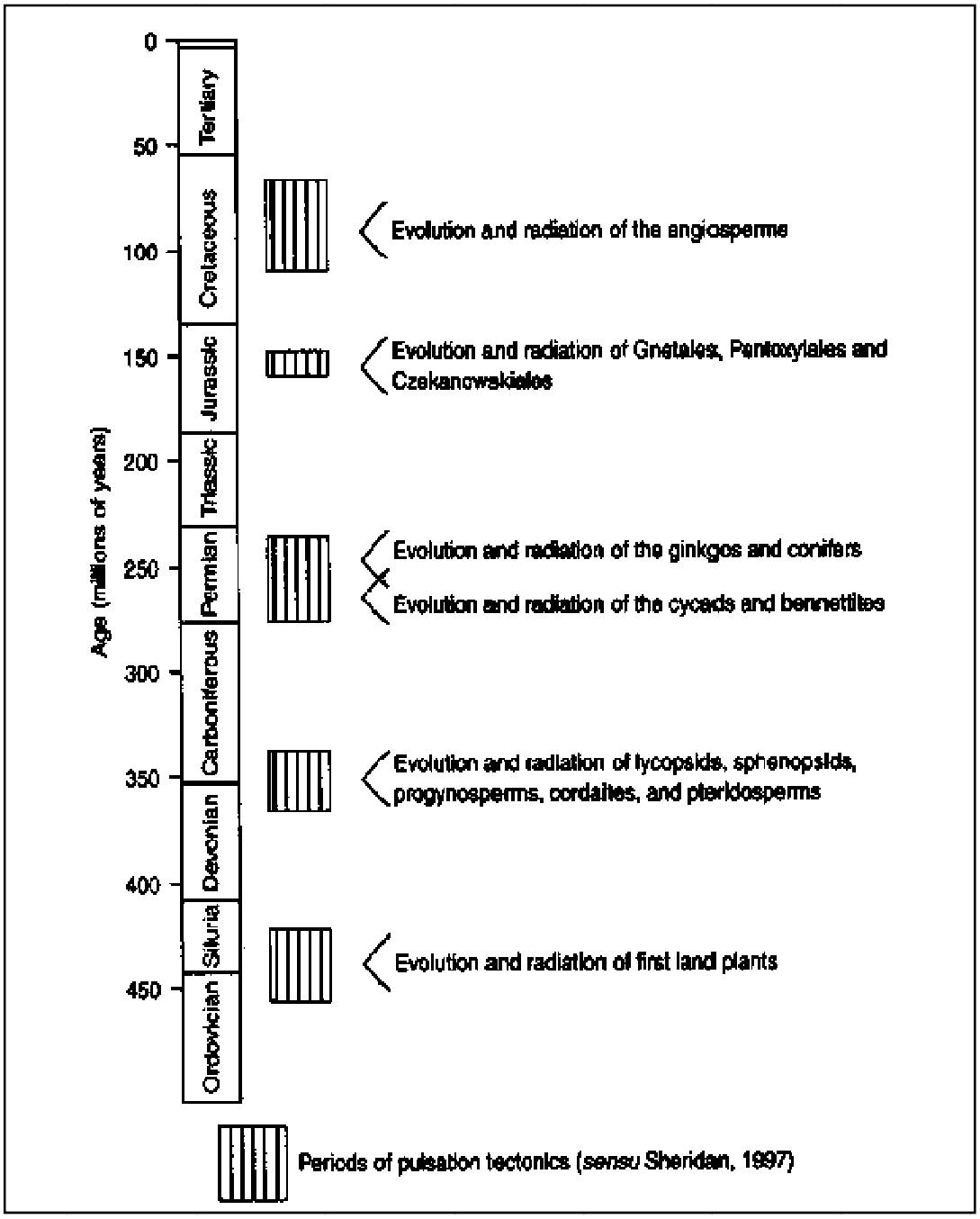
Mass extinctions (physically or biologically mediated) are the punctuating events leading to the apparent sudden speciation events. It effectively wipes the slate clean by clearing away the accumulated evolution of a lineage, and thereby allow a new lineage to evolve rapidly to fill the niche. Evolutionary time is a hierarchical system of distinct tiers, with the uppermost tier of mass extinctions representing the ultimate driving mechanism behind macroevolution

Recent survey concluded that the 'palaeontological evidence overwhelming supports a view that speciation is sometimes gradual and sometimes punctuated and that no one mode characterizes this very complicated process in the history of life'. Existence of living fossils (*Ginkgo biloba*) show morphological 'stasis' since the Jurassic (~200 Ma) and supports punctuated equilibrium theory. It is established that changes in the environment are largely responsible for the radiation and extinction of taxa (Fig. 18) for which **Turnover-pulse hypothesis is** proposed.

Turnover-pulse hypothesis

Evolution is normally conservative, and speciation does not occur unless forced by changes in the physical environment (Vrba 1985, 1993; Bennett 1990, 1997) So, it supports the concept of punctuated equilibrium suggesting that periods of speciation are largely a function of changes in the physical environment, especially climatic change, possibly of tectonic origin, but most probably of astronomical origin.

Fig. 18: Periods of pulsation tectonics in plant fossil records indicating evolution and radiation of major plant groups (Sheridan, 1997)



The model also proposes that extinction may result directly from climatic change or from ecological interactions among newly associated species. In broader scale, two factors are responsible which are Orbital variations and Plate tectonics.

The patterns of major events occurred in geological past (Table 1), evolutionary history (Fig. 19) and their changes (Fig. 20) are best explored through plant fossil records.

Fig. 19: Major Evolutionary history through geological time scale

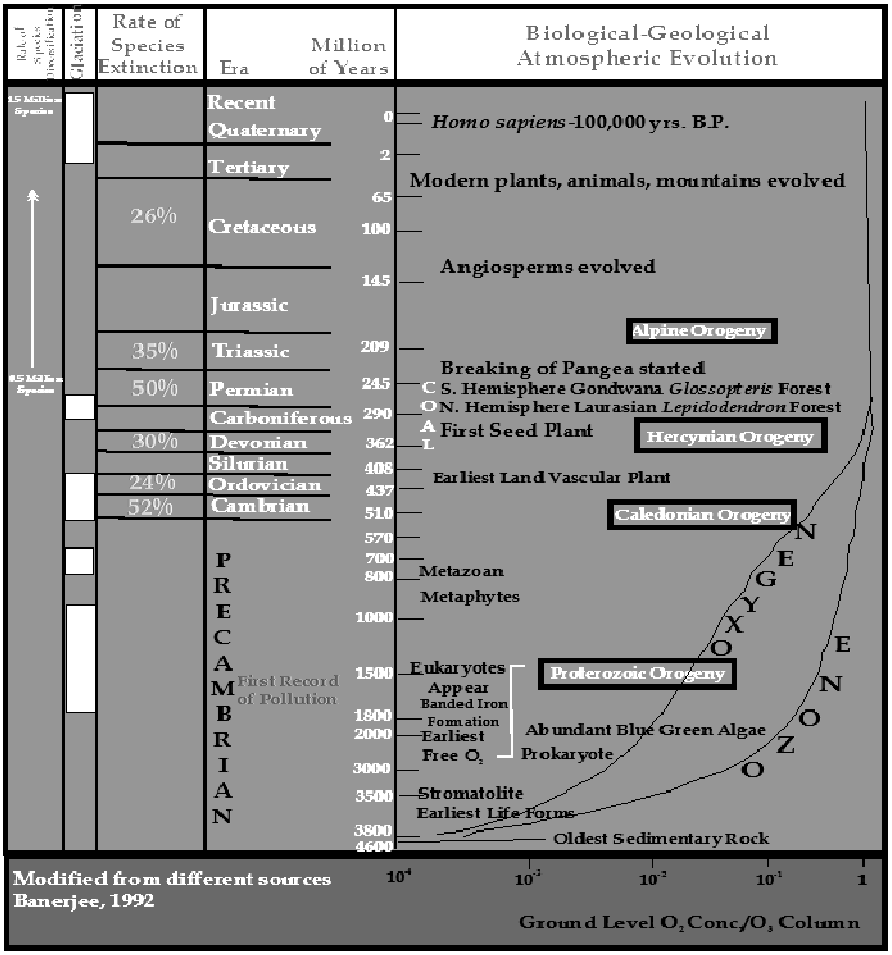


Fig. 20: Patterns of evolutionary change in the plant fossil record

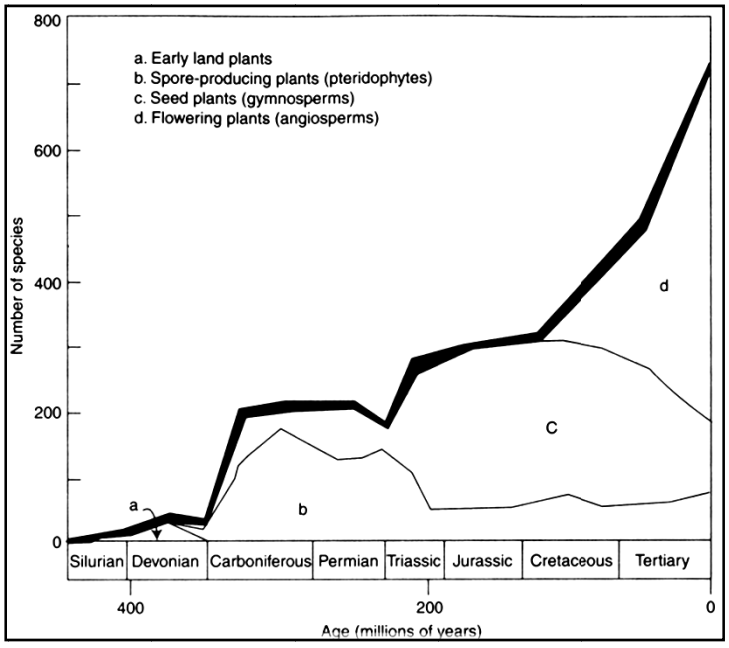


Table 1: Major events in evolutionary history occurred in geological past

Geological Period	Major events in evolutionary history
Quaternary	Modern humans
Tertiary	Primitive humans & modern mammals
Cretaceous	The first flowering plants; placental mammals
Jurassic	Giant dinosaurs & first birds
Triassic	Diversification of ferns & conifers, first dinosaurs & small mammals
Permian	Diversification of glossopterids & reptiles
Carboniferous	The first conifers and reptiles; seed ferns, arborescent lycopods and calamites
Devonian	The first amphibians, diversification of vascular plants
Silurian	First vascular plants & breathing animals
Ordovician	First vertebrates
Cambrian	First trilobite, abundant marine invertebrates & BGA
Precambrian	Primitive life forms

Mass extinction

Mass extinction is a real catastrophe brought about by extraordinary environmental factors & involves substantial biodiversity losses that are global in extent, taxonomically broad & rapid. It describes a relatively short intervals of geological time when a high proportion of diverse and geographically wide spread taxa underwent extinction. It differs from normal 'background' species level extinction in that it is characterized by the relatively rapid extinction of groups of organisms, usually at a higher taxonomic level, that is families and even orders. There is different earth bound and extra-terrestrial causes behind the mass extinction event through environmental changes like shock-waves, heat-waves, impact winters (long periods of darkness shutting down photosynthesis), super-acid rains, toxic oceans, super waves & super floods etc.

Earth bound causes (physical & biological):

- Global cooling and glaciations
- Massive volcanism
- Reduction of salinity & Oxygen in the ocean
- Sea-level changes

Extraterrestrial causes:

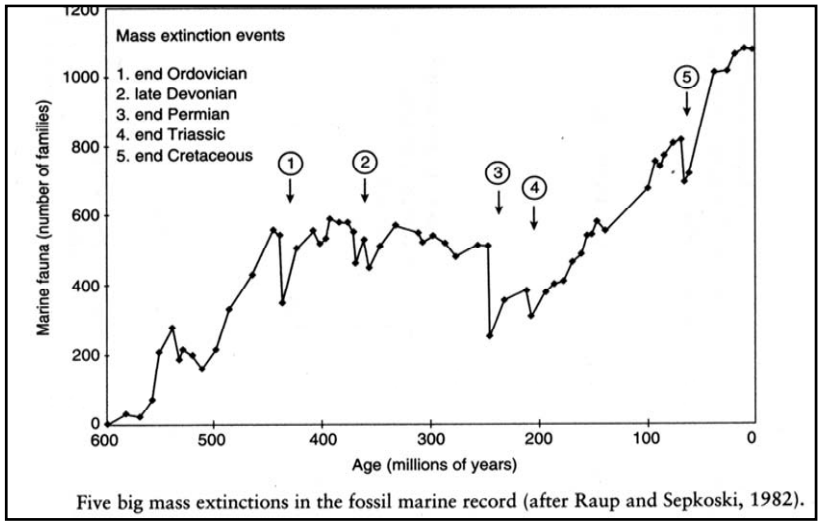
- Impacts from meteorites
- Comet storms
- Radiation from supernovae
- Large solar flares

Fossil study (mainly marine fossil record) is one of the reliable methods for establishing mass extinction event. Five major extinction events (Table 2; Fig. 21) are recorded in geological history.

Table 2: Magnitude in the major mass extinction (Erwin 1993)

Geological Period	Geological Time	Percentage (%) of family extinction	Percentage (%) of generic extinction
End Cretaceous (K-T extinction)	65 Ma at the Cretaceous-Tertiary transition	17	50
End Triassic	206 Ma at the Triassic-Jurassic transition	23	48
End Permian (P-T extinction)	248 Ma at the Permian-Triassic transition	57	83
Late Devonian	364 Ma near the Devonian-Carboniferous transition	19	50
End Ordovician	443 Ma at the Ordovician-Silurian transition	27	57

Fig. 21: Five mass extinction events in the fossil record



Five big mass extinctions in the fossil marine record (after Raup and Sepkoski, 1982).

Plant fossils in analysis of palaeoenviroment, and palaeoclimate: Environment is the natural environment encompasses all living and non-living things occurring naturally on Earth or some region thereof. The biophysical environment is the biotic and abiotic surrounding of an organism, or population, and includes particularly the factors that have an influence in their survival, development and evolution.

Palaeoenvironment is the past environment of an area during a given period of its history. Paleoenvironmental records are derived from a wide variety of natural archives, such as:

- Fossils both mega and micro
- Tree rings
- Lake and ocean sediments
- Wind-blown deposits
- Coral
- Ice cores
- Historical documents

Plant fossil is one of the most reliable tools for deciphering past environment because of their ecological and geographical specificities. In their structure & distribution, extant organisms often reflect the environmental composition in which they grow and reproduce. In the same ways as those of the present, we make assumption that organism of the past became adapted to their environments. As for example-

- the presence of growth rings in petrified fossil wood suggests seasonal variations in the availability of water usually accompanied by temperature changes in the palaeoenvironment.
- fossil woods lacking growth rings suggest an equable environment with a continuous supply of water and a more uniform temperature regimen.
- thick cuticles and sunken stomata of fossil leaves suggest lack of available water
- roots and stems with spongy tissues indicate an aquatic or swamp environment.

Information of this kind gleaned from the morphology and anatomy of the fossil plants provides part of the basis of palaeoecology and palaeoclimatology. Chemical, isotopic, and ecological analyses of these records have demonstrated that the natural climate system has varied locally and globally over a far greater range.

Climate is the statistical data of temperature, humidity, atmospheric pressure, wind, rainfall, atmospheric particle count and numerous other meteorological elements in a given region over long periods of time. It can be contrasted to weather, which is the present condition of these same elements over periods up to two weeks.

Paleoclimate is the past climate and it changes taken on the scale of the entire history of Earth. It uses a variety of proxy methods from the Earth and life sciences to obtain data previously preserved within rocks, sediments, ice sheets, tree rings, corals, shells, mega & microfossils. It then uses these records to determine the past states of the Earth's various climate regions and its atmospheric system. Studies of past changes in the environment and biodiversity often reflect on the current situation, and specifically the impact of climate on mass extinctions and biotic recovery.

Few basic approaches used by paleobotanists to reconstruct ancient climate from fossil assemblages are:

- Nearest living relative (NLR) or the coexistence model
- Leaf Physiognomy: Leaf Area & Leaf Margin Analysis
- Palaeodendrology: Tree ring analysis
- Stomatal Index

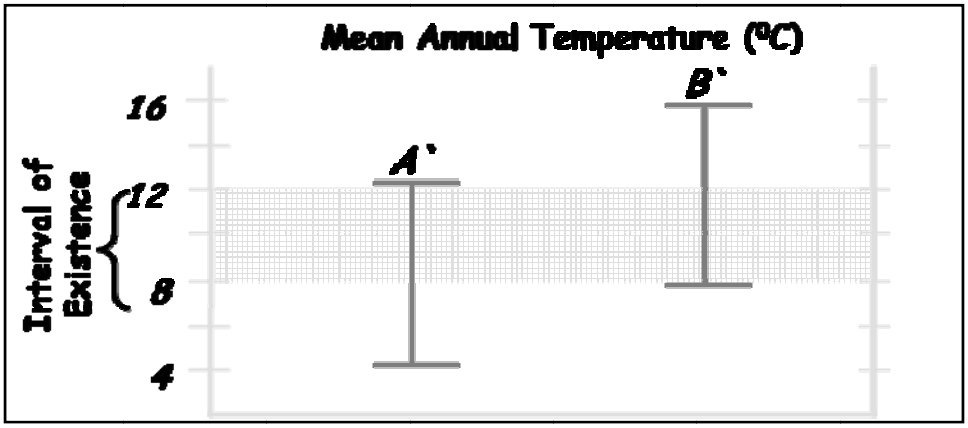
Nearest living relative or the coexistence model

For a given fossil flora and a given climatic parameter the climatic interval in which all nearest living relatives (NLR) of the fossil flora can co-exist. It assumes that fossil plant taxa have similar climatic requirements as their nearest living relatives. The coexistence approach (Mosbrugger and Utescher, 1997) is an efficient and reliable method for quantitative terrestrial palaeoclimate reconstructions from the Cenozoic palaeobotanical record.

Suppose a fossil flora with two taxa, A and B, is given and that the nearest living relatives of these fossil taxa, A' and B', are known, along with their climatic requirements (Fig. 22). It is assumed that the modern taxa A' and B' require a mean annual temperature between 6-12^{0}C and 8-16^{0}C, respectively. Obviously, there exists a mean annual temperature interval between 8°C and 12°C in which A' and B‘ can (or could) coexist (N.B.: it is not required that A' and B' do really coexist in a modern phytocoenosis). According to the coexistence approach this interval of coexistence is taken as the best estimate of the mean annual temperature under which the fossil taxa A and B once lived. In a

similar way estimates (coexistence intervals) can be obtained for other climatic parameters, such as temperature of the coldest and warmest month or mean annual precipitation, etc.

Fig. 22: Graphical representation of co-existence approach for deciphering past climate



The list of climate parameters considered for co-existence model is

- MAT [Mean Annual Temp (^{0}C)]
- WMT [Mean temp of the warmest month (^{0}C)]
- CMT [Mean temp of the coldest month (^{0}C)]
- RH [Mean relative humidity (%)]
- PE [Potential evaporation (mm)]
- MAP [Mean annual precipitation (mm)]
- MMaP [Mean maximum monthly precipitation (mm)]
- MMiP [Mean minimum monthly precipitation (mm)]
- MWP [Mean precipitation of the warmest month (mm)]
- AI [Aridity index MAP/PE)]

Leaf Physiognomy: Leaf Area & Leaf Margin Analysis

Analyses of leaf physiognomy (characteristic external features of extant vegetation) have been used to obtain more precise information, which in turn can be extrapolated when preparing reconstructions of climatic and vegetational changes of the past. Wolf (1979) established a classification of vegetational types based on the physiognomic characteristics of extant angiosperm leaves that occur within the temperature parameters.

Three categories of temperature parameters

- microthermal vegetation growing within a range of -5^{0}c to 13^{0}c
- mesothermal vegetation growing within a range of 13^{0}c to 20^{0}c
- megathermal vegetation growing within a range of 20^{0}c to 30^{0}c

A correlation exists between leaf size and decreasing temperatures and /or precipitation which are as follows:

Serrate (toothed) leaf margins: are typical of humid microthermal vegetation. Show a marked decrease in abundance in forests where higher temperatures prevail (replaced by entire margin species)

Thick leaf texture: is typical of evergreen angiosperm that predominate in megathermal and mesothermal vegetation where precipitation may be limited

Usually deciduous plants with thin leaves: predominate in microthermal or successional mesothermal vegetation

Evergreen leaves in humid environments: usually have attenuated apices (drip-tips)

Compound leaves: tend to occur on plants that are frequently associated with deciduous plants.

Narrow leaves: are often found on plants inhabiting stream banks where the substrate is disturbed

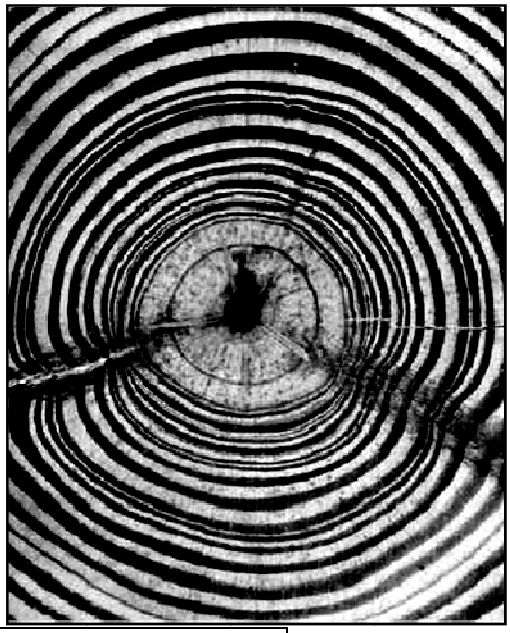
Broader heart-shaped leaves, usually with palmate venation: are produced by lianus, sprawling shrubs in successional vegetation, or colonizers in open areas of the forest

Lobed leaves: are often found on successional or understory species

Palaeodendrology

It is the branch of paleobotany that studies fossil trees rings (Fig. 23). Tree rings are an important source of information about past climates and fossil environments. Fossil tree rings are unique source of detailed information about seasonality, annual growing conditions, water availability, limiting temperatures and forest productivity in the geological past.

Fig. 23: Fossil tree ring



Stomatal index (SI) study

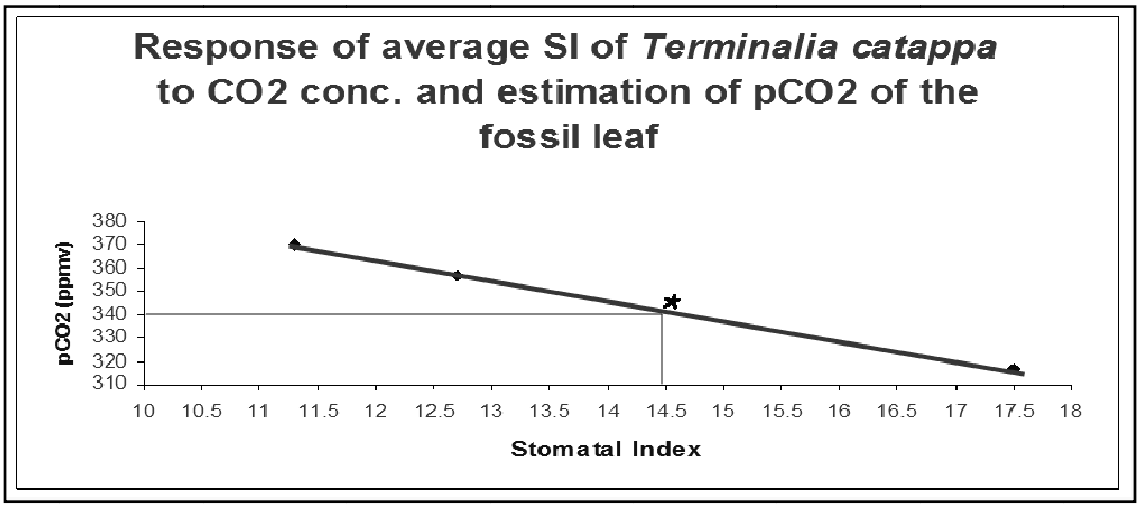
Stomatal index study is utilized for determining the pCo_2 Concentration by using the principle '**Stomatal frequency is inversely proportional to atmospheric CO_2 conc.'.** The pCO_2 could be estimated by plotting the data of extant SI and corresponding CO2 conc. in graph (Table 3; Fig. 24).

Table 3: Stomatal Index (SI) & pCO_2 data for extant and fossil leaves of *Terminalia catappa* *(after Van der Burgh et al., 1993; Mehrotra et al., 2003)*

Extant Leaf	**Stomatal Index (SI)**	**CO_2 conc (in ppmv)**
2002	11.3	370
1992	12.7	357
1960	17.5	317
Fossil Leaf	**14.5**	**342 [pCO_2 conc (in ppmv)]**

Fig. 24: pCO_2 estimation through plotted data of extant SI and correspondence CO_2 conc.

Determination of past climatic data from various sources is prerequisite in modern day aspect for climatic simulation study for future trend analysis of climate.



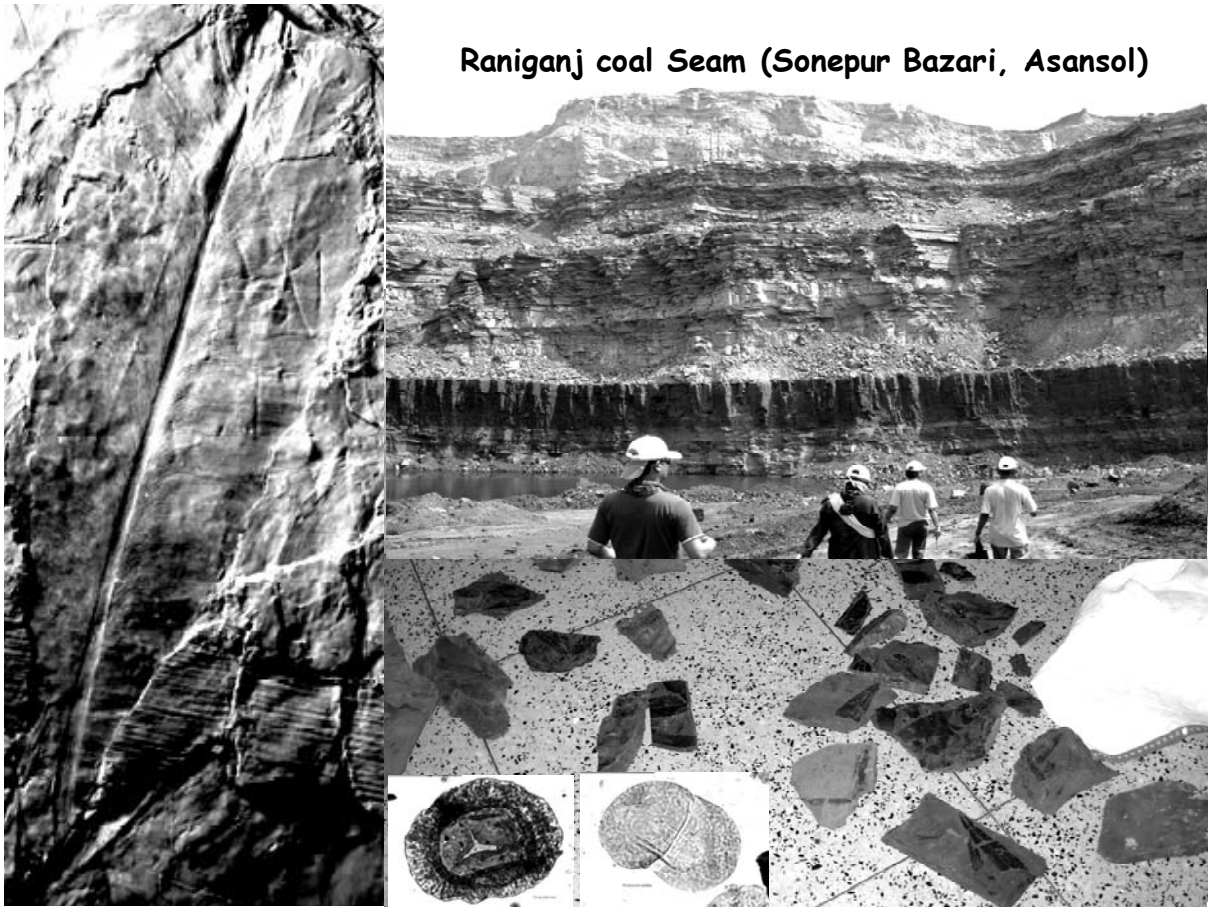
Plant fossil in determining palaeoecology & energy resources: Palaeoecology is the study of the interrelationships between ancient organisms and the palaeoenvironemnts in which they lived. Fossil fuels in form of solid (peat, lignite, coal), liquid (petroleum), gas, and other hydrocarbons, limestone, algal stromatolite, phosphorites, diatomaceous Earth etc. are the different energy resources i.e. biogenic substances fossilized in different ecological conditions in geological past. Fossil fuels are, in effect, stored ancient sunlight through carbon captured by plants and stored by geology and we now rely on them for so much of our energy needs.

It was during the Cool, wet and glacial Carboniferous Period that an unusual and fortuitous set of ecological conditions prevailed which began a process of mass sequestration of terrestrial carbon, resulting in the oil, gas and coal deposits we enjoy burning so much today. Thus, coal deposits were formed around 300 Ma ago in a geological period known as the **CARBONIFEROUS** (meaning coal bearing) in terrestrial ecological conditions through fossilization of *Lepidodendron* flora in northern hemisphere and *Glossopteris* flora in southern hemisphere continents.

Coal is older than petroleum oil and gas, which were deposited in organic rich marine strata of Jurassic and Cretaceous age (65-200 Ma ago). Petroleum is made from the remains of phytoplanktons and zooplanktons which fell to the bottom of seas where this is little or no oxygen. The organisms were buried under sediments through repeated ice age style rises and falls of sea level flooding and then retreating from coastal swamps and there slowly cooked at high temperature and pressures to produce oil. The higher the temperature and pressures and the longer the process, the lighter the oil becomes as it continues to evolve chemically, some turning into associated natural gas.

Being the fossilized product, fossil fuel rich palaeoecological conditions could easily be identified through fossil study. As for example study of *Glossopteris* flora (Fig. 25) is important in coal exploration in Gondwanaland continents including India.

Fig. 25: *Glossopteris* fossils explored from an Indian coal deposits



Fossil study in determining plant animal interaction: Plant animal interaction in geological past is determined through fossil study and it is important to infer the co-evolution of these two biological organisms in geological past.

Discovery of new fossils may change the conventional ideas of taxonomists and evolutionary biologists: Reconstructed Plant *Archaeopteris* of Devonian age introduced a new group-Progymnosperms by Charles Beck in 1960 after the discovery of fossil of *Callixyon* wood (like gymnosperm) and *Archaeopteris* leaf (like fern) connected organically.

Palaeogenetics and Palaeophytochemistry: Study of chemical fossils and isolation of a-DNA developed new branches namely 'palaeophytochemistry' and 'palaeogenetics' respectively for exploring new vistas in phylogenetic and climatic research.

Study of sub fossils from Archaeological sites: It helps to detect the Socio-cultural evolution of ancient people and the Agricultural practices and other uses of plants (Fig. 26).

Fig. 26: Charred seeds recovered from archaeological site



4. Preservation of plants as fossils: Definition; taphonomy; environment for fossilization; modes of preservation; types; major rock types, rock cycle and rocks containing Fossils; systematics, reconstruction and nomenclature

Definition

The word 'Fossil' is derived from the Latin verb *'fodere'* which means *'to dig'*. Originally it described to anything one might remove from the Earth. Subsequently, it changed to the remains of whole or part of an organism or the evidence of former existence of an organism preserved in the consolidated sediments of the Earth. As such, the definition of fossil is **"Any direct** (*actual or imprint of body of organism as a whole or part)* **or indirect** *(tract, trail, boring, teeth mark, coprolite etc.)* **evidences of the life forms of geological past which are predominantly found in sedimentary rocks".**

Taphonomy

The process for being a fossil from a living biological organism is apparently a simple, natural phenomenon though in true sense it is an elaborate and complicated process, scientifically termed as **'Taphonomy'**.

Taphonomy involves sequential steps of biological, environmental, and sedimentological factors, namely **'Necrology'** (death/abscission/decay of organisms), **'Biostratinomy'** (burial in the sediment), **'Diagenesis'** (microbial diagenesis, or other physico-chemical changes), and '**Lithification'** (post burial changes & mineralization) (Fig. 27). The formation of a fossil begins after the death of a whole organism or any of its part(s). Gradually its body sinks through the depth of enclosed water bodies of freshwater or marine environments (Basin). Ultimately, it settles down into the bottom of the basin. The soft body parts decay quickly by microbial diagenesis or are eaten by scavengers leaving behind the hard, skeletal parts. In due course of time, the sediments comprising of sand, mud, and other debris deposits on this and continue to accumulate year after year, compressing the successive layers. The compacted sediments are gradually changed into sedimentary rocks, and finally the biological remains harden into fossils.

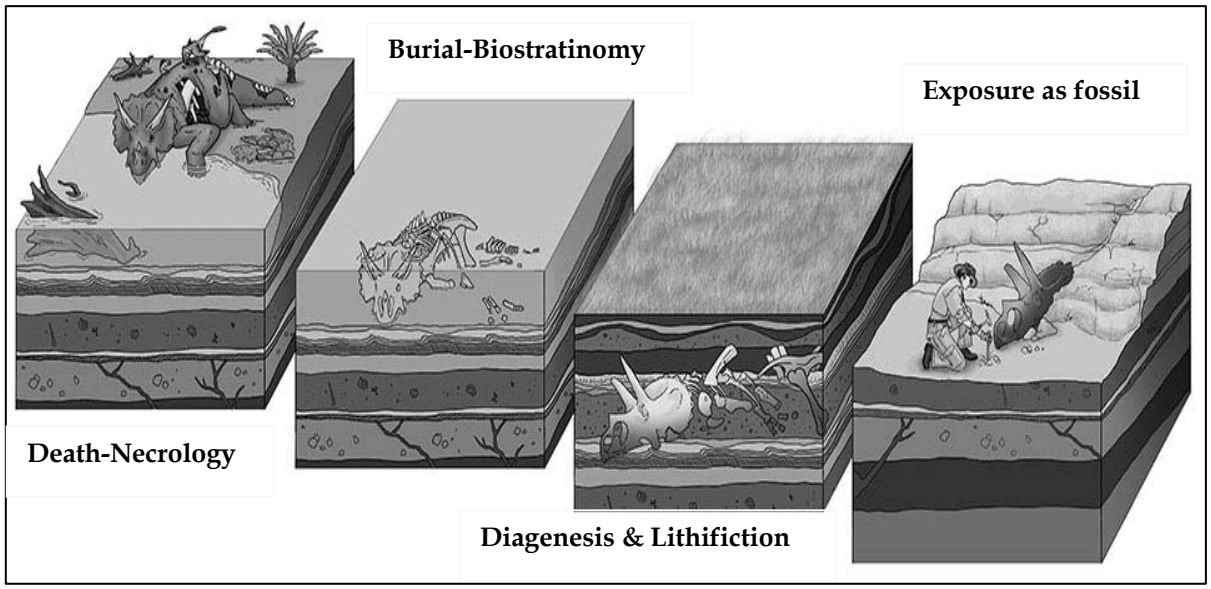
Environment for fossilization

The fossilization process is dependent on a variety of internal (structural) and external (environmental) factors.

Internal (structural) factors: The tissue systems of any vascular plant are made up of different types of soft and hard cells like parenchymatous, collenchymatous, sclerenchymatous, vascular etc. Among these, the harder tissues like bark, wood, sclerenchyma and cutinized cells are usually better preserved than the softer tissues like parenchyma.

External (environmental) factors: The external factors are both mechanical and biological. The mechanical action of wind, water, rolling stones etc. influence greatly the extent of preservation. The microorganisms are responsible for the decay and decomposition of the dead plant bodies that can escape them by suitable environment like-

Fig. 27: Step wise process for fossilization
(source: http://blog.statemuseum.nd.gov/blog/life-of-fossil)



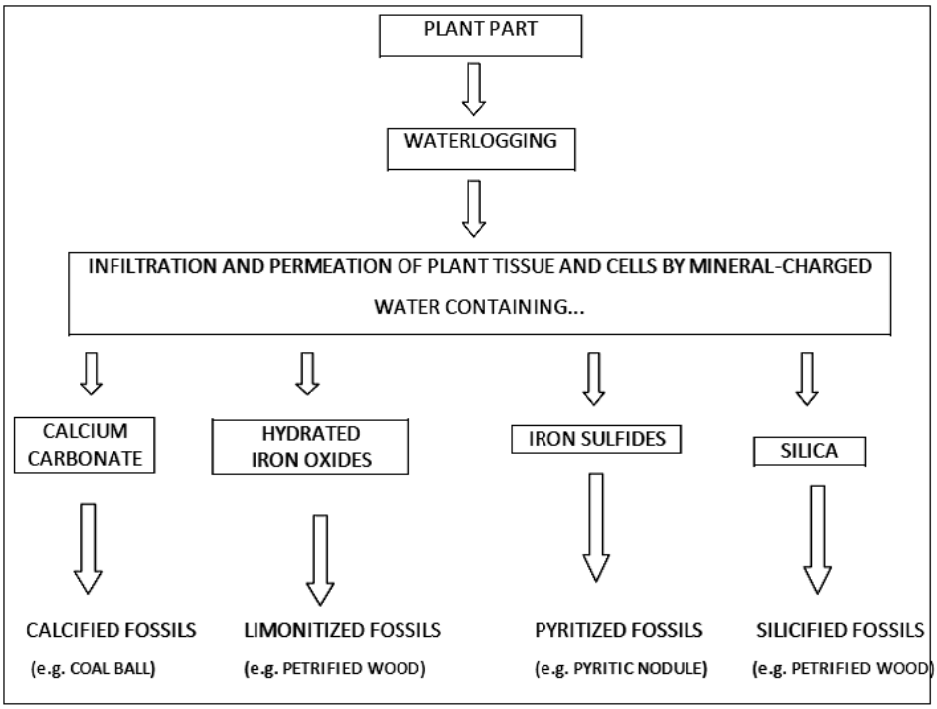
- ***Enclosed or protected water* (small lake, swamp etc):** the dead organism should remain in undisturbed condition
- ***Rapid burial*:** to avoid first microbial decomposition
- ***Low O_2 content* (e.g., anaerobic sediment):** to check aerobic decomposition because most decomposers (e.g., fungi, most decomposing bacteria and invertebrates) require oxygen for metabolism.
- ***Acidity or Low pH*:** limits the activity of decomposers
- ***High concentration of toxic substances*:** to retard decay process
- ***Protection against high wind*:** to prevent mechanical destruction of organisms and from being carried away from its place of origin
- ***Absence of strong water current*:** the wave action and rolling boulders cause fragmentation of the organism
- ***Higher depth of water*:** activity of aerobic decomposition is restricted at higher depth; afford good protection from wave action
- ***Fine textured sediments*:** allow lesser amount of O_2 in inter-grain space retarding the process of aerobic decomposition
- ***Ample source of particulate sedimentary material*:** may reduce the chance of long exposure of the organisms to the decomposer and /or scavenger

Modes of Preservation (Schopf 1975)

James M. Schopf (1975) stated that the processes of geologic preservation are important for understanding the organisms represented by fossils and the interpretation of fossils are influenced by modes of their preservation. He distinguished four modes of preservation namely **(1) Cellular permineralization ("petrifaction")** preserves anatomical detail, and, occasionally, even cytologic structures **(2) Coalified compression ("impressions")**, best illustrated by structures from coal but characteristic of many plant fossils in shale, preserves anatomical details in distorted form and produces surface replicas (impressions) on enclosing matrix **(3) Authigenic preservation (cementation)** replicates surface form or outline (molds and casts) prior to distortion by compression (4) **Duripartic (hard part) preservation,** characteristic of fossil skeletal remains, predominantly animal.

(1) Cellular permineralization ("petrifaction"): This is the most informative fossil as because anatomical details are preserved through this mode of fossilization (Fig. 29). Here, infiltration of cells and intercellular spaces by mineral matrix occur at or very soon after deposition. Depending upon infiltrated minerals it may be of Silicified (silica), Calcified, Pyritized, Limonitized etc. (Fig. 28). Cryo-preservation is another form of permineralization, where tissue becomes permeated microcrystalline ice. However, this form of permineralization tends to contain only relatively recent fossils; for example, the Pleistocene frozen mammoths found on the plains of Siberia and dating back to approximately 10,000 years ago.

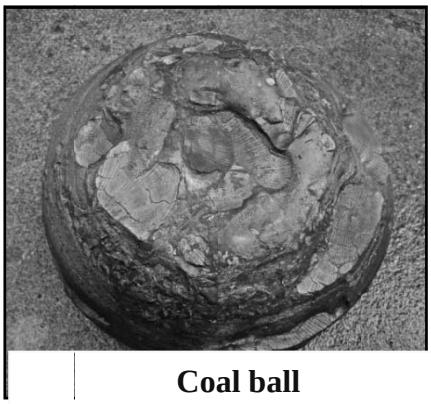
Fig. 28: Different types of permineralized fossils



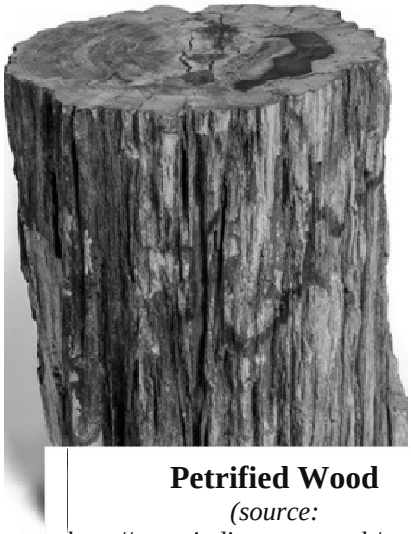
Most common types of permineralized plant fossils

"Coal balls" are very distinctive calcified permineralized fossil which vary considerably in size, but are usually spheroidal in shape, *c*. 10-20 cm radius, and composed of numerous plant fragments incorporated within a Calcium carbonates matrix (Fig. 29). They are found exclusively in coal deposits of Carboniferous age (~354-290 Ma) in Europe and North America, and of Permian age (~290 Ma) in China.

Fig. 29: Fossils preserved through Cellular Permineralization mode



Coal ball
(source: https://www.flickr.com/photos/28709338@N04/16224790745)



Petrified Wood
(source: http://www.indigenous.co.uk/contemporary-furniture/Petrified-wood-

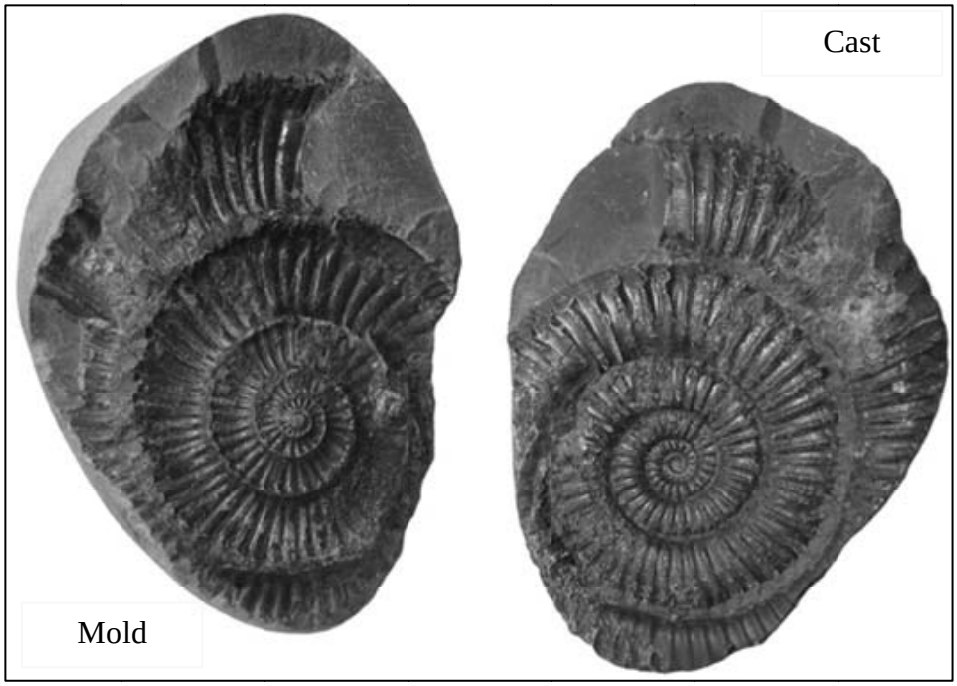
(2) Coalified compression ("impressions"): This mode comprises softening of cell walls, collapse of internal cell spaces, loss of volatiles matters like, gas, moisture, soluble, and finally consolidation of nonmineralized altered residues in an anoxygenic environment which in consequence, it becomes coaly deposits due to lithification. Splitting commonly yields one coalified part which becomes compression, and the counterpart becomes impression (Fig. 30).

Fig. 30: Fossils preserved through Coalified Compression mode



(3) Authigenic preservation (cementation): Authigenic preservation involves early cementation of dead body parts by minerals like iron and carbonate compounds during the soft-mud stage soon after burial which eventually preserves the surface of buried organic parts. Traces of internal organization usually are lost, and most tissues are degraded and thus it acts as 'Mold' and occasionally the hollow internal part is filled with bulk materials which form the 'Cast' (Fig. 31).

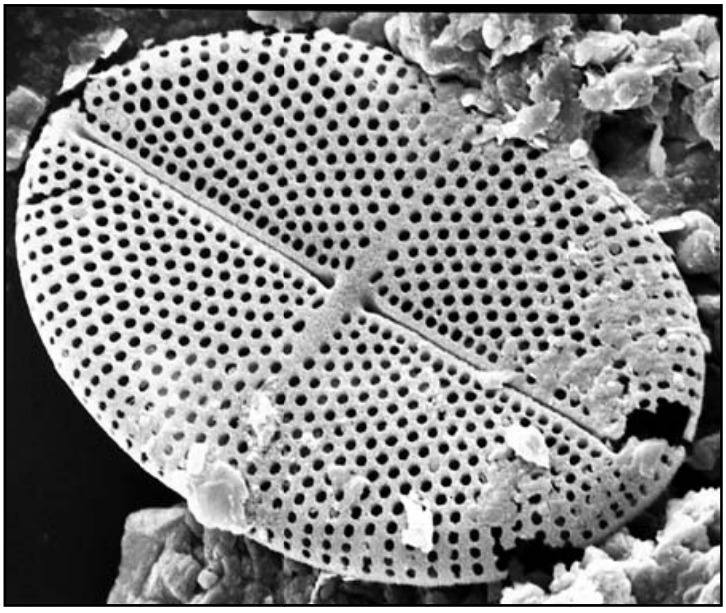
Fig. 31: Cast and mold as preserved through authgenic preservation



(4) Duripartic preservation (Hard part): The hard parts of both plants and animals are most readily preserved and form the most abundant class of fossils. Certain parts of organisms are durable because of their resistance to decay and oxidation and resistant to physical change. Among plants, the "skeletal" parts of lime-precipitating algae, coccoliths, and diatom frustules are usually fossilized through this mode (Fig. 32).

Fig. 32: Fossils preserved through Duripartic Preservation mode

(source: http://www.ucl.ac.uk/GeolSci/micropal/images/diat/diat006.gif)

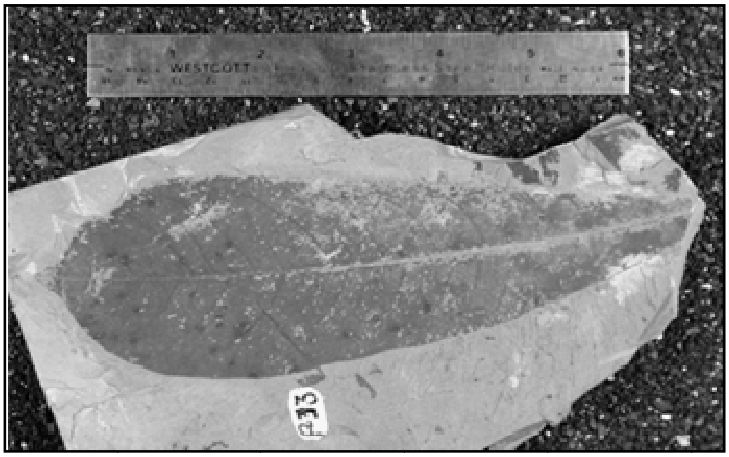


Exceptional preservation: unaltered forms

Besides, above mentioned modes, there are three most common deposits namely, lake sediments, amber and packrat middens from where organic remains have been recovered almost in unaltered fossilized forms as because here burial occur in microbial inhibited, or at least severely restricted environment.

Fig. 33: Fossil leaf of *Magnolia* from Miocene lake sediments (Clarkia beds) in Idaho, U.S.A.

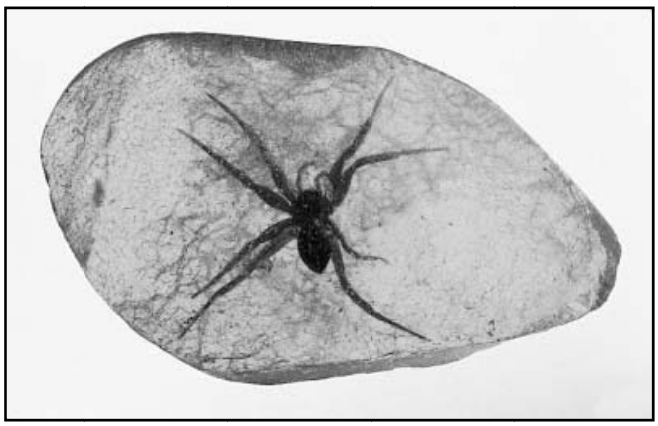
(http://www.webpages.uidaho.edu/tertiary/images/dirtwo/magnolia.jpg)



Many exceptional plant fossils have been recovered from lake sediments, where burial has been rapid and the environment at the bottom of the lake anoxic. An example of this type of burial includes the Clarkia beds in Idaho, northern U.S.A. where thousands of early Miocene fossil leaves (~17-20 Ma) have been recovered. Some of these leaves are in such an exceptional state of preservation that it is possible to extract strands of ancient DNA fossil leaf of *Magnolia latahensis* (Golerberg *et al*., 1990; Soltis et al., 1992) (Fig. 33).

Fig. 34: Exceptional preservation of spider within fossilized resin (Amber)

(source: https://www.pinterest.com/pin/493003490432865299/)



Amber is a fossilized plant resin and when the resin is formed, any animal or plant fragments (e.g. small flowers, leaves, seeds, and insects) that fall into it are incorporated due to its sticky nature. Oligocene fossil leaves contained in amber (~24-34Ma) have been recovered from several localities, and ancient DNA has been extracted successfully from the extinct plant ***Hymenaea protera*** contained in fossil amber (Fig. 34) from the La Toca mines, Dominican Republic.

The most unusual preservation medium for unaltered fossil plants are packrat middens found in the arid areas of south-eastern North America, the Middle East and South Africa. The middens provide shelters/nests for the animals, are constructed of plant material (Fig. 35) that they collected, and which became encased in crystallized urine (amberat). The amberat combined with the extremely dry environment, provides an exceptional preservation medium.

Fig. 35: Piece of Packrat Midden showing well defined sub-fossilized plant remains
(source: https://sites.google.com/site/joywardlab/research/ice-age-plant-physiology/wells-packrat-collection)



Types of Fossils

Depending on the modes of preservation (Schopf, 1975), namely, cellular permineralization, compression-impression, authigenic, and duripartic, we can get either external or internal characteristics of fossilized organisms. Eventually, the recovered fossilized forms are the basis for segregation in different types (Frey 1975; Hayes 1983, Bateman 1991) which are as follows:

Body fossil: Actual or imprint of body of organism or part is called as 'Body Fossil'. Depending on size, it is divided into macro or *mega (body fossil beyond the size range of 200 µm)* (Fig. 36) and micro *(body fossil within the size range of 200 µm)* (Fig. 37) fossils.

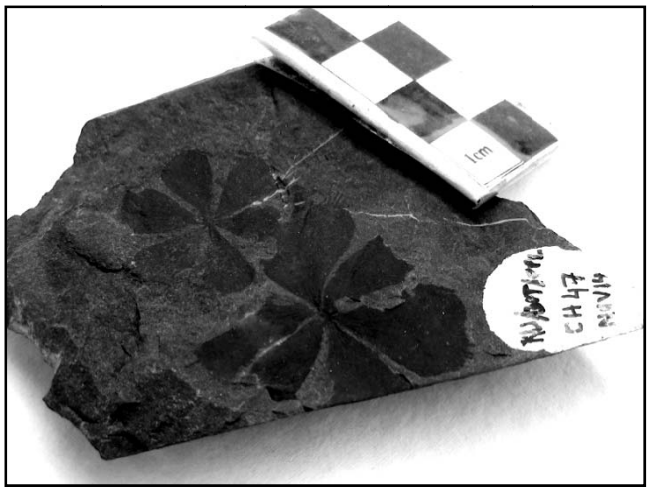


Fig. 36: Body-Megafossil of *Sphenophyllum* leaf

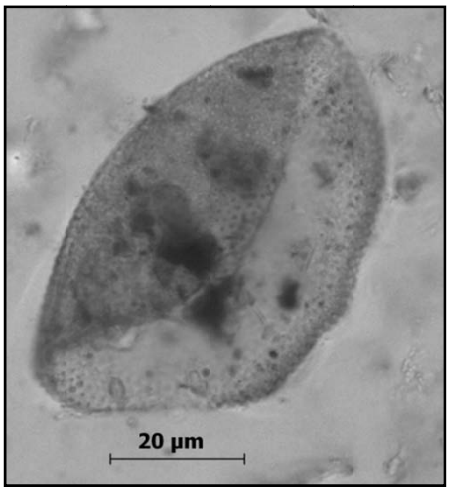


Fig. 37: Body-Microfossil of Palm pollen grains

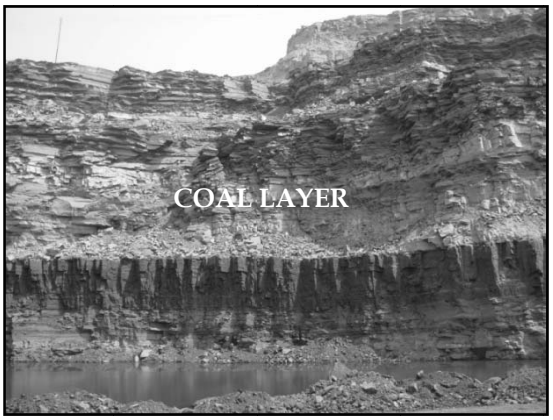
Ichnofossil or trace fossil: It is the relic of activity or movement pattern *(footprint, track, burrow, trail, fecal matter etc.)* of organism of geological past providing indirect evidences of life (Fig. 38).

Fig. 38: Ichnofossil: Track of *Eohippus* sp.
(source: http://www.fossilmall.com/Western-Fossils/Ichno7/ICH007e.jpg)



Chemical fossil: If different types of chemical namely, kerogen, amino acids, lignin etc. are found in fossilized forms are categorized in chemical fossil type.
Organic molecules: Several bio-molecules like DNA, RNA etc. may be preserved as fossils.
Organic deposits: Solid (lignite, coal etc.), and liquid (petroleum etc.) hydrocarbons are the fossilized form of organic remains of past plant (Fig. 39) and animal organisms.

Fig. 39: Coal layer-fossilized as organic deposits



Subfossil: Sometimes incomplete diagenesis process forms subfossils.

Fig. 40: Peaty clay deposition remains in subfossil state



Pseudofossil: Occasionally, due to mineral deposition some rocks may mimic fossilized structure (Fig. 41) of any organism which are not a fossil in true sense. These forms are termed as pseudofossils.

Fig. 41: Pseudofossil- Iron oxide deposition on rock looks like a plant

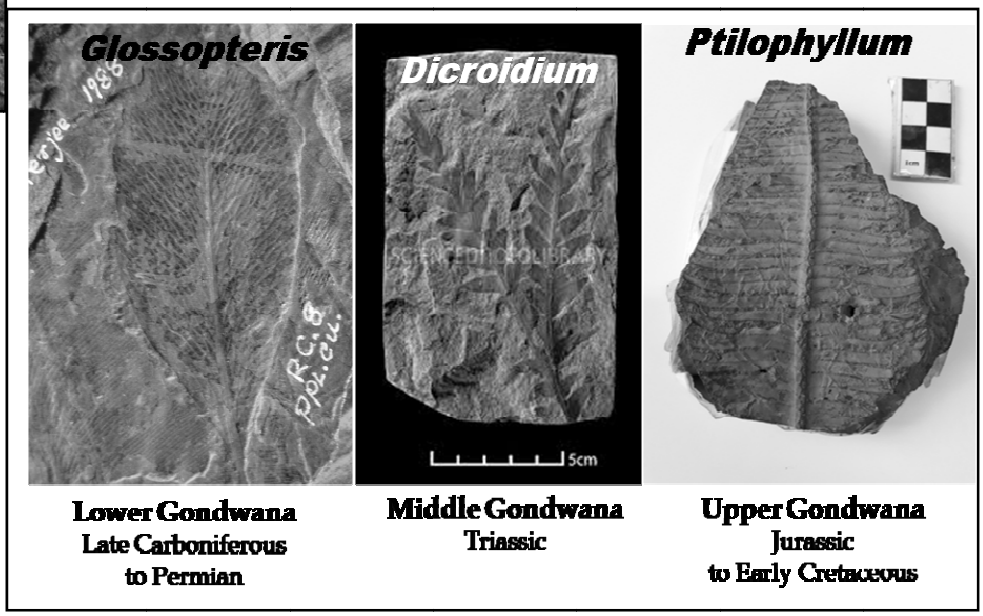


In addition to above mentioned fossil types, Index fossil is another well-known type which is categorized by the palaeontologists on basis of the application of the fossil.

Index fossil: The index fossil is defined as *"a fossil which is useful for dating and correlating the strata in which it is found"*. An index fossil should be easily recognizable and abundant in the strata having a wide geographic distribution (broad range of geographical regions) and a narrow geological distribution (short range through geological timescale). Example: *Glossopteris* (Fig. 42, 43) is a prevalently used as an index fossil which is found abundantly in Upper Carboniferous to Early Triassic in the present-day countries (Australia, Africa, South America, Madagascar, Indian sub-continent, New Guinea, New Zealand, and Antarctica) which were belonging to the supercontinent Gondwanaland. *Dicroidium* and *Ptilophyllum* are also well-known index fossils (Fig. 43).



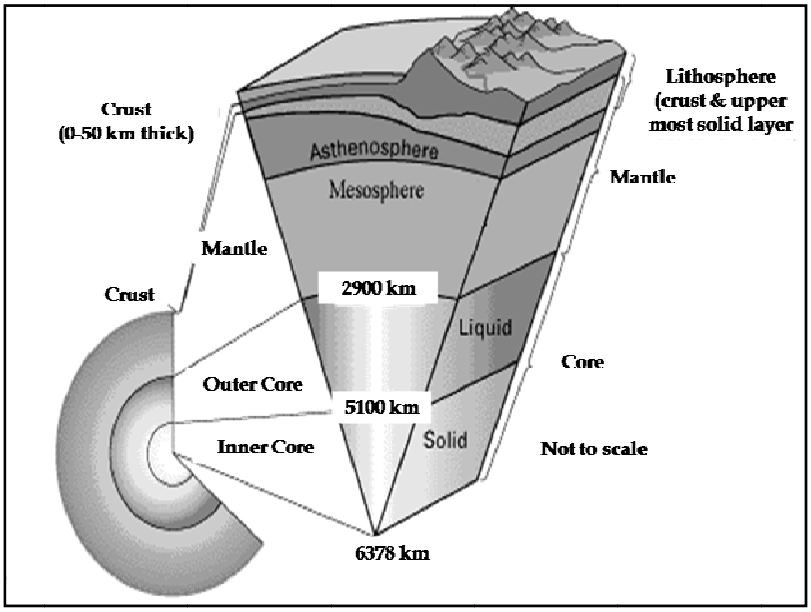
Fig. 42 & 43: Some well known index fossils of Gondwana sediments



Rock type, rock cycle and fossiliferous rock

Rock is a naturally occurring solid aggregate of minerals. The Earth's outer solid layer, the lithosphere is made up of rock (Fig. 44).

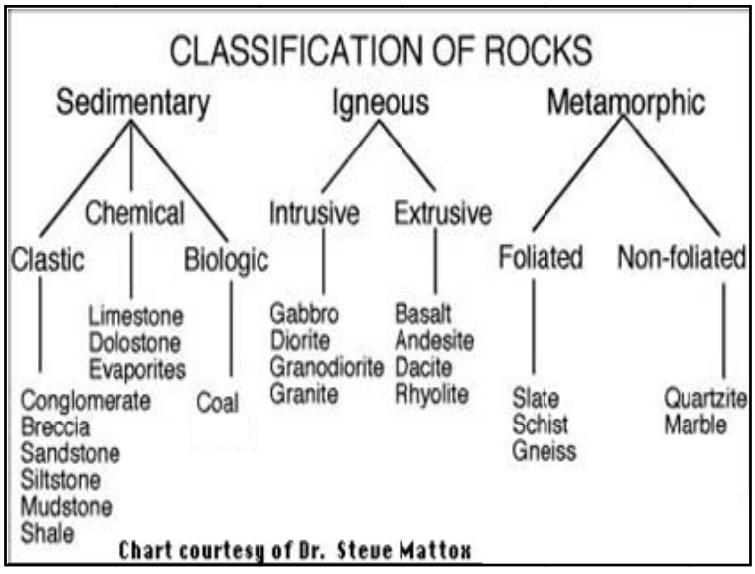
Fig. 44: Lithosphere of Earth containing of rock



Rock Type

Basic rock types are mainly three those that form from melt (**igneous** rocks), those that are deposited from air or water (**sedimentary** rocks), and those that have formed by "cooking" or otherwise altering another rock (**metamorphic** rocks). Sedimentary rocks form by breaking down other kinds of rocks into small particles and washing or blowing them away; metamorphic rocks form from other rocks and igneous rocks form by melting other rocks. These three basic types are again classified into different forms by their origin (Fig. 45).

Fig. 45: Classification of Rock



Igneous rock: Igneous rocks (from the Greek word for fire) form from when hot, molten rock crystallizes and solidifies. The melt originates deep within the Earth near active plate boundaries or hot spots, and then rises toward the surface. Igneous rocks are divided into two groups, intrusive or extrusive, depending upon where the molten rock solidifies.

Intrusive Igneous Rocks: Intrusive, or plutonic, igneous rock forms when magma is trapped deep inside the Earth. Great globs of molten rock rise toward the surface. Some of the magma may feed volcanoes on the Earth's surface, but most remains trapped below, where it cools very slowly over many thousands or millions of years until it solidify. Slow cooling means the individual mineral grains have a very long time to grow, so they grow to a relatively large size. Intrusive rocks have a coarse grained texture. Example: Granite

Extrusive Igneous Rocks: Extrusive, or volcanic, igneous rock is produced when magma exits and cools above (or very near) the Earth's surface. These are the rocks that form at erupting volcanoes and oozing fissures. The magma, called lava when molten rock erupts on the surface, cools and solidifies almost instantly when it is exposed to the relatively cool temperature of the atmosphere. Quick cooling means that mineral crystals don't have much time to grow, so these rocks have a very fine-grained or even glassy texture. Hot gas bubbles are often trapped in the quenched lava, forming a bubbly, vesicular texture. Example: Basalt

Sedimentary Rock: Rock formed from weathering, erosion, deposition and compaction of rock material through agents like wind, water, ice and chemical precipitation. Sedimentary rocks cover about three-quarters of the land surface**.**

Clastic or detrital: These are formed from the cemented sediment grains that are fragments of preexisting rocks i.e. Shale, Conglomerate etc. Shale is the most common sedimentary rock.The type of Detrital or Clastic Sedimentary Rocks is dependent on sediment Particles (Table 5).

Table 5: Clastic or Detrital Sedimentary Rock

Diameter (mm)	**Sediment**		**Clastic or Detrital Sedimentary Rock**
256+	Boulder	Gravel	Breccia (angular particles) Conglomerate (rounded particles)
64-256	Cobble		
4-64	Pebble		
2-4	Granule		
1/16-2	Sand		Sandstone
1/256-1/16	Silt	Mud	Siltstone (mostly silt)
Less than 1/256	Clay		Shale or mudstone (mostly clay)

Chemical: are deposited by precipitation of minerals from solution

- Limestone: CaCO3; Dolomite: CaMg(CO3)2;
- Evaporites: evaporation of seawater or a saline lake e.g. gypsum (CaSO4.2H2O); Chert: entirely of silica

Biologic or organic: This is composed or organic carbon compounds formed by fossilized biological organisms. For example-Coal

Metamorphic rock: Metamorphism refers to change to existing rocks that take place in Earth's interior due to heat and pressure. The changes may be new texture, new minerals assemblages, or both. Transformations occur in the solid state (i.e. rock does not melt). The changed new rock is metamorphic rock.

REGIONAL METAMORPHISM: metamorphism takes place at considerable depth at underground so affects a large area and it is results from plate tectonics. It is almost always foliated. "Foliated" rocks contain much mica and other rocks that produce layering or banding. Example: Slate, Gneiss etc.

CONTACT METAMORPHISM: mostly takes place not too far beneath earth's surface (less than 10 km) i.e. affects rocks on a local scale, such as "baking" of sedimentary rocks adjacent to an intrusive contact. This type is mostly non foliated in nature.

Example: Marble (from limestone), Dolomite marble (from dolomite), Quartzite (from sandstone), Hornfels (from shale and basalt) etc.

Rock Cycle:

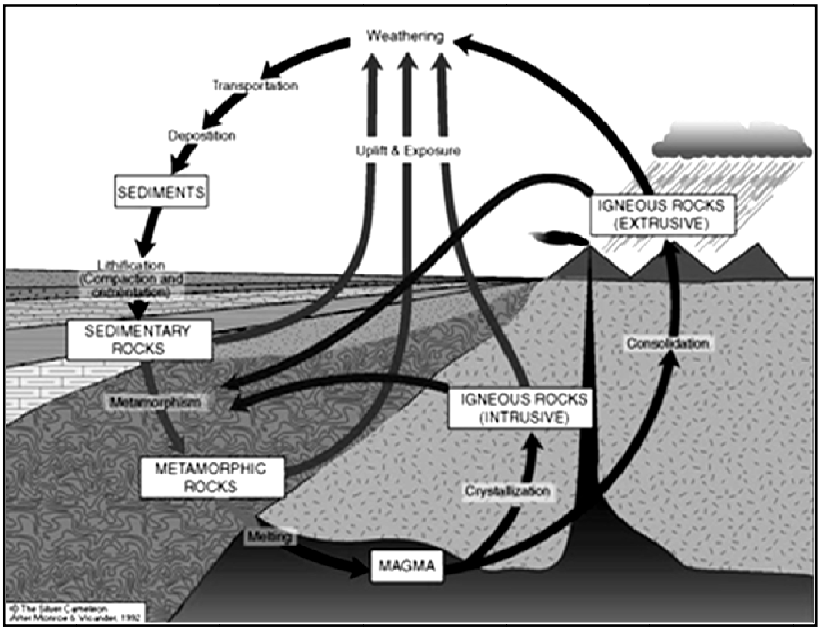
The rock cycle is a fundamental concept in geology that describes the dynamic transitions through geologic time among the three main rock types: sedimentary, metamorphic, and igneous. So the rock cycle is an illustration that explains how the 3 rock types are related to each other and how processes change from one type to another over time (Fig. 46). The original concept of the rock cycle is usually attributed to James Hutton, father of geology from the eighteenth century. Each type of rock is altered or destroyed when it is forced out of its equilibrium conditions. An igneous rock such as basalt may break down and dissolve when exposed to the atmosphere or melt as it is subducted under a continent. Igneous rock can change into sedimentary rock or into metamorphic rock. Sedimentary rock can change into metamorphic rock or into igneous rock. Metamorphic rock can change into igneous or sedimentary rock.

Igneous rock forms when magma cools and makes crystals. Magma is a hot liquid made of melted minerals. The minerals can form crystals when they cool. Igneous rock can form underground, where the magma cools slowly. Or, igneous rock can form above ground, where the magma cools quickly. When it pours out on Earth's surface, magma is called lava. Yes, the same liquid rock matter that you see coming out of volcanoes.

On Earth's surface, wind and water can break rock into pieces. They can also carry rock pieces to another place. Usually, the rock pieces, called sediments, drop from the wind or water to make a layer. The layer can be buried under other layers of sediments. After a long time the sediments can be cemented together to make sedimentary rock. In this way, igneous rock can become sedimentary rock.

Inside Earth there is heat from pressure. There is also heat from radioactive decay and this heat bakes the rock. Baked rock does not melt, but it does change. It forms crystals. If it has crystals already, it forms larger crystals. Because this rock changes, it is called metamorphic. Metamorphosis can occur in rock when they are heated to 300 to 700 degrees Celsius. When Earth's tectonic plates move around, they produce heat. When they collide, they build mountains and metamorphose the rock. The rock cycle continues. Mountains made of metamorphic rocks can be broken up and washed away by streams. New sediments from these mountains can make new sedimentary rock. The rock cycle never stops.

Fig. 46: Rock Cycle

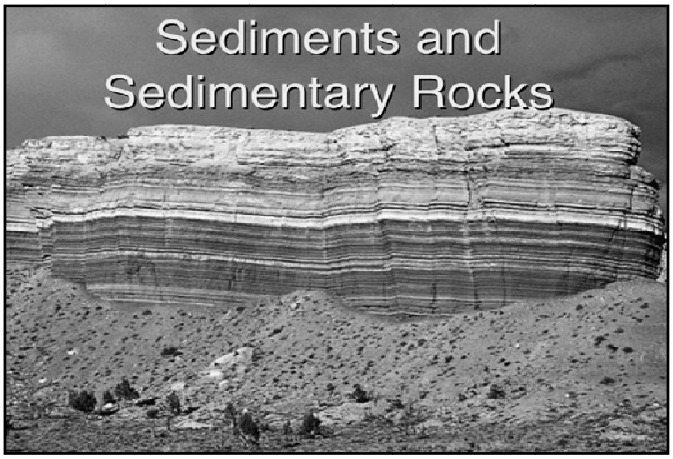


Fossiliferous

Rock

Most favoured site for fossil occurrence is sedimentary rock (Fig. 47) as because here layering of deposition occurred. Though fossils are most commonly found in sedimentary rocks, but all sedimentary rocks are not necessarily fossiliferous. Here are the three main reasons: Few if any creatures or plants larger than microscopic forms lived in those environments because it was toxic or otherwise unsuitable for things to live in; there was an incredible amount of biological activity such as the that of soft-bodied, bottom dwelling organisms that continually turned over the sediments and prevented things from getting trapped and preserved in the forming sediments; subtle heating of the strata by nearby volcanic activity, or altering of the rocks by chemicals, one or both destroying the fossil evidence, but not enough metamorphosis to alter the appearance of the deposits.

Fig. 47: Sedimentary rock as most favoured fossiliferous Rock



Systematic, nomenclature, reconstruction

Systematic, nomenclature

The beginning of the nomenclature of fossil plants is appeared in the year 1820 by Sternberg who first validly described *Lepidodendron obovatum* for a bark fragment. This date is chosen as the starting point for palaeobotanical nomenclature but since the publication of the Vienna Rules (1906) it has been accepted that taxonomic nomenclature of plant fossils should follow essentially the same procedure as used for living plants. But the issue was properly addressed in the year 1950 at Stockholm Congress, where the International Association for Plant Taxonomy was established, and the Rules were recognized as the International code of botanical nomenclature (Stockholm Code 1952).

Very rarely, the palaeobotanists unearth a whole plant as fossil (fossil plant) rather they generally find detached plant parts of leaf, stem, flower, spore, pollen grains etc. (plant fossils) as fossilized through various modes of preservation. Cleal and Thomas (2010) very clearly explained two confusing concepts – **'plant fossils'** and **'fossil plants'**. They clarified that the *"plant fossils are the physical objects taken out from the ground that reveal evidence of long dead plants whereas fossil plants were the plants from which these fossilized remains were derived"*. As such, 'plant fossils' represent just fragments of the plants in which aspects of the original tissue have been at least partly lost through fossilization and they are now biologically inert objects but originated from living organisms. In contrast, 'fossil plants' were whole living organism, which in principle could be treated in the same way as living plants. As such, due to this non-entirety discovery of fossil plant, it becomes impossible to name the 'fossil plants' and 'living plants' in exactly in the same manner.

If the characteristics of the discovered plant fossils are alike to the modern plants, some taxonomists suggest that in spite of this similarity the generic name of the fossils should have a different name by

adding appropriate suffix (*odendron* for stem-*Lepidodendron*; *oxylon* for wood-*Araucarioxlon*; *opteris* for leaf-*Glossopteris* and so on) or prefix (*Rhizo* for wood belongs to root; *Cormo* for stem; *Clado* for branch wood and so on) whereas other palaeobotanists prefer to use same generic name as living plant with a different species designation in the binomial.

The naming of the 'fossil plant' would only be possible after reconstruction of the whole plant from detached plant fossils. By using the rule of priority, the earliest validly published generic name applied to any one of its parts might be applicable for naming of whole fossil plant or investigator can choose a new binomial for the reconstructed fossil plant. If the whole plant is known, there is no nomenclatural difficulty in naming the parts but if there is no inevitable indication as to where a detached plant fragment belongs, it is necessary to establish a term by which one may refer to it, until and if ever its real position becomes known. This is the background for the establishment of various different nomenclatural measures over the years for inclusion within the *code* to accommodate fossils which were started from "form and organ genera" in the Stockholm Code (1952), and subsequently "morphotaxa" in the St. Louis Code (2000), and "fossil taxa" in the Vienna Code (2006).

Since the names of the species, and consequently of many of the higher taxa of fossil plants are usually based on fragmentary specimens, and since the connection between these organs can only rarely be proved, organ genera and form genera are distinguished as taxa within which species may be recognized.

Organ-genus: An organ-genus is a genus assignable to a family whose diagnostic characters are derived from single organs of the same morphological category or from restricted groups of organs connected together. Organ genera based on detached parts may be distinguished not only by morphological characters, but also by reason of different modes of preservation.

Form-genus: A form-genus is a genus not assignable to a family, but it may be referable to a taxon of higher rank and it is maintained for classifying fossil specimens that lack diagnostic characteristics indicative of natural affinity but which for practical reasons need to be provided with binary names. Form genera are artificial in varying degree.

Examples: **organ-genera:** *Lepidocarpon* Scott (Lepidocarpaceae), *Mazocarpon* (Scott) Benson (Sigillariaceae); **form-genera:** *Dadoxylon* Endl. (Coniferopsida), *Stigmaria* Brongr. (Lepidophytales and Lepidospermales)

Morphotaxa: In St. Louis Congress Code (1999), a new nomenclatural concept, **'morphotaxa'** was adopted replacing the two old terms 'organ genus' and 'form genus'. The 'morphotaxa' is ***"a fossil taxon which, for nomenclatural purposes, comprises only the parts, life history stages, or preservational states represented by the corresponding nomenclatural type"***.

Fossil taxa: The Vienna Code (2006) referred one more term **'fossil taxa'** in addition to morphotaxa for classifying plant fossils. Fossil taxa are any taxon whose type is a fossil. The International Code of Nomenclature (ICN) for algae, fungi, and plants (Melbourne Code 2011) adopted a set of proposals by which the concept of 'morphotaxa' was abandoned (Melbourne Code 2011) and 'fossil taxa' was only retained which is defined as ***"A taxon (diatom taxa excepted) the name of which is based on a fossil type"***. A fossil-taxon comprises the remains of one or more parts of the parent organism, or one or more of their life history stages, in one or more preservational states, as indicated in the original or any subsequent description or diagnosis of the taxon"

Recent views regarding Nomenclature: The Nomenclature Section of ICN (2011) adopted a set of proposals (for details see Cleal & Thomas in Taxon 59: 261–268; 312–313. 2010) by which the concept of morphotaxa is abandoned.

Art. 1.2 A taxon (diatom taxa excepted) the name of which is based on a fossil type is a fossil-taxon. A fossil-taxon comprises the remains of one or more parts of the parent organism, or one or more of their life history stages, in one or more preservational states, as indicated in the original or any subsequent description or diagnosis of the taxon (see also Art. 11.1 and 13.3).

Art. 11.1 Each family or taxon of lower rank with a particular circumscription, position, and rank can bear only one correct name, special exceptions being made for nine families and one subfamily for which alternative names are permitted (see Art. 18.5 and 19.8). However, the use of separate names is allowed for fossil-taxa that represent different parts, life-history stages, or preservational states of what may have been a single organismal taxon or even a single individual (Art. 1.2).

Art. 13.3 For nomenclatural purposes, a name is treated as pertaining to a non-fossil taxon unless its type is fossil in origin (Art. 1.2). Fossil material is distinguished from non-fossil material by stratigraphic relations at the site of original occurrence. In cases of doubtful stratigraphic relations, and for all diatoms, provisions for non-fossil taxa apply.

Usual suffixes/prefixes used for nomenclature

- Leaf/Fronds : -phyllum (*Sphenophyllum*)
  -pteris (*Archaeopteris*)
- Secondary Xylem : -oxylon (*Araucarioxylon*)
- Microsporangiate str : -theca (*Glossotheca*)
- Ovulate structure : -carpon (*Lepidocarpon*)
  -sperma (*Archaeosperma*)
- Cone : -strobus (*Lepidostrobus*)
- Seed : -testa (*Pachytesta*)
- Spores : -spora (*Lycospora*)
  -sporites (*Laevigatosporites*)
- Pre-pollen, Pollen :-pollenites (*Palmaepollenites*)

Reconstruction: Three well known forms of evidences are present for reconstruction of a fossil which are

- Actual attachment
- Similar anatomical characters
- Frequency of association

Actual attachment

Charles Beck (1960) reconstructed *Archaeopteris*: actual attachment of Late Devonian free sporing fern like frond *Archaaeopteris* + *Callixylon* stem bearing gymnospermous secondary wood characters

Similar anatomical characters

Oliver and Scott (1904) reconstructed *Lyginopteris oldhamia:* Similar anatomical feature, large capitate gland, on the surface of dispersed stems (*Lyginopteris*), leaves (*Sphenopteris*) and cupulate ovule (*Lagenostoma*)

Frequency of association

Reconstruction of the plant *Medullosa*: Repeated occurrence of pteridopsermous stem *Medullosa*, foliage of *Alethopteris*, seeds of *Pachytesta* and pollen bearing structure *Bernaultia* as dispersed state in assemblage

5. Geologic Time: Geologic timescale, relative *vs*. numerical age, physical and biological principles for defining relative and numerical age

Dating is a method for age determination of rock strata and fossils. Earth scientists generally consider two methods for the purpose: Relative dating and Absolute dating and by combining these two methods they developed a standard geological time scale.

Geological Time scale

Geological timescale is a hierarchical scale in which the 4.6 Byrs history of the Earth (since the Earth Formation) is divided into time units of varying duration i.e. eons, eras, periods, and epochs. It is necessary for establishing the history of geological events and for determining the rates of geological processes.

Geological time scale is necessary for establishing the history of geological events and for determining the rates of geological processes

Relative dating: It is a method of sequencing events in chronological order (i.e. in the order they happened through geological time scale) without exact dates. Relative dating method is based on fossil stratigraphy (biostratigraphy) and this is the basis for formation of geological time scale initially (Fig.). Three major Era boundaries are identified; two of which are defined by mass extinctions in the animal record and the remaining one by the rapid expansion of multicellular animals. The era boundaries are-

- **Mesozoic-Cenozoic** *(c. 65 Ma, dinosaur died out along with 75% of marine invertebrates)*
- **Palaeozoic-Mesozoic** *(c. 248 Ma, extinction of upto 96% of marine species)*
- **Precambrian-Paleozoic** *(c. 543 Ma, major increase in numbers of multicellular animals with hard parts soon after the boundary-Cambrian Explosion)*

Principles of Relative dating: Different pprinciples should keep in mind for Relative dating which are as follows:

Uniformitarianism: The theory that changes in the earth's crust during geological history have resulted from the action of continuous and uniform processes.

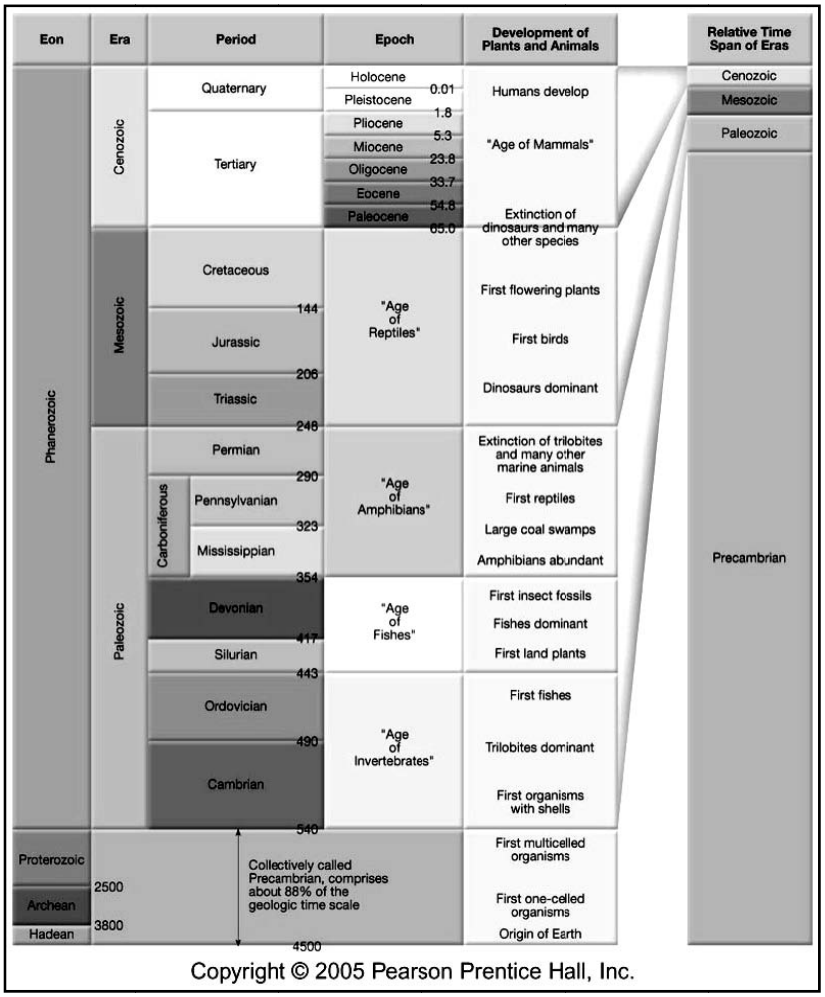
Superposition: In an undisturbed sequence the oldest rock is at the bottom and each successive layer is younger. As layers accumulate through time, older layers are buried beneath younger layers

Original Horizontality: Sediment particles settle to the bottom of a body of water in response to gravity and thus, sedimentary rocks are deposited in horizontal layers

Lateral continuity: Layers of sediment extend in all directions horizontally for some distance over Earth’s surface—from a few meters to hundreds of kilometers, depending on the conditions of deposition. Continuity may be interrupted unless a river erodes them or an earthquake moves them

Faunal Succession: This principle is attributed to William Smith, an English engineer in the late 1700s. Smith noticed that the kinds of fossils he found changed through a vertical succession of rock layers, and furthermore, that the same vertical changes in fossils occurred in different places. The observation that fossils change in a consistent manner through stratigraphic successions can be extended to the entire world.

Fig. 48: Standard Geological time scale (*fossil and correlation)

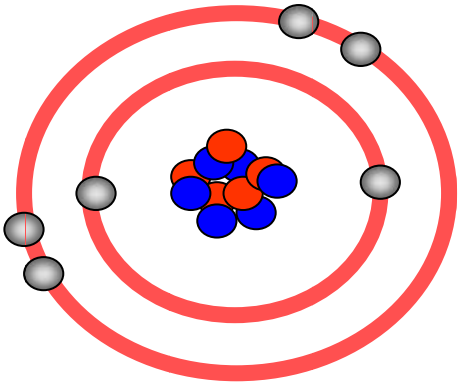


Cross cutting relationships: A vein of rock (intrusion) or a fault is younger (more recent) than the rock it cuts across.

Absolute dating: It is a method of dating of rocks and fossils by using techniques especially radiometric dating to determine their actual numerical age. The initially developed geological time scale is later on calibrated by radiometric method and eventually standard geological time scale was formed (Fig. 48).

The technique of radiometric dating was developed early in the 20th century after the discovery of radioactive isotopes n the laboratories of Cambridge University, England. The frequently used radioactive isotopes for absolute age determination of rock and fossils are Uranium-238, Uranium-236, Thorium-232, Potassium-40, Carbon-14. Uranium and Thorium are mostly found in igneous rocks whereas Potassium-40 is component of some sedimentary rocks and Carbon-14 is utilized to date the past organic remains that are fossils.

Fig. 49: Atomic nucleus



The atom is a basic unit of matter that consists of a dense central nucleus surrounded by a cloud of negatively charged electrons (Fig. 49). Electrons have virtually no mass; present usually in orbit around the nucleus, and also in the nucleus as part of a neutron. The atomic nucleus contains a mix of positively charged protons and electrically neutral neutrons. Neutron is a proton with an electron inside of it; thus it is electrically neutral.

Atomic Number: Number of Protons; **Atomic Mass Number:** Combined No.s of Proton & Neutron

Not all atoms of the same element have the same number of neutrons. These variable forms of the same element are called

isotopes. Most isotopes are stable; some are unstable and spontaneously decay to a more stable form. It is the process by which the radioactive substances (an unstable atomic nucleus) are spontaneously transformed into an atomic nucleus of a different element. Three (3) types of radioactive decay are present which are-

Alpha Decay: Emission of 2 protons+2 neutrons from nucleus; Loss of 2 atomic number, 4 atomic mass number

Beta Decay: Emission of fast moving electron from a neutron in the nucleus, changing that neutron to a proton; Increase of 1 atomic number, no change in atomic mass number

Electron Capture: A proton in the nucleus captures an orbiting electron, thereby converts to a neutron; Loss of 1 atomic number, no change in atomic mass number

Some elements undergo only one decay step in the conversion from an unstable form to a stable form

Rubidium 87 decays to Strontium 87 (single beta emission)

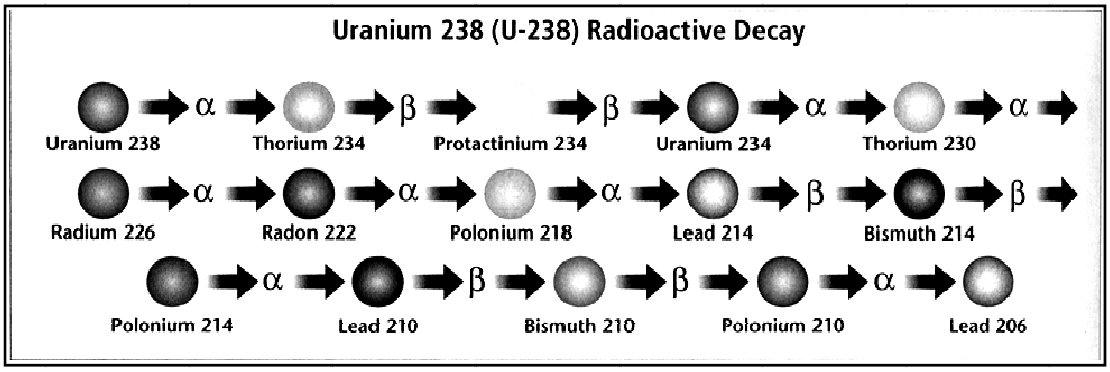
Potassium 40 decays to Argon 40 (single electron capture)

Other radioactive elements undergo several decay steps (Fig. 50)

Uranium 235 decays to Lead 207 (7 alpha, 6 beta steps)

Uranium 238 decays to Lead 206 (8 alpha, 6 beta steps)

Fig. 50: Decay step of Uranium 238

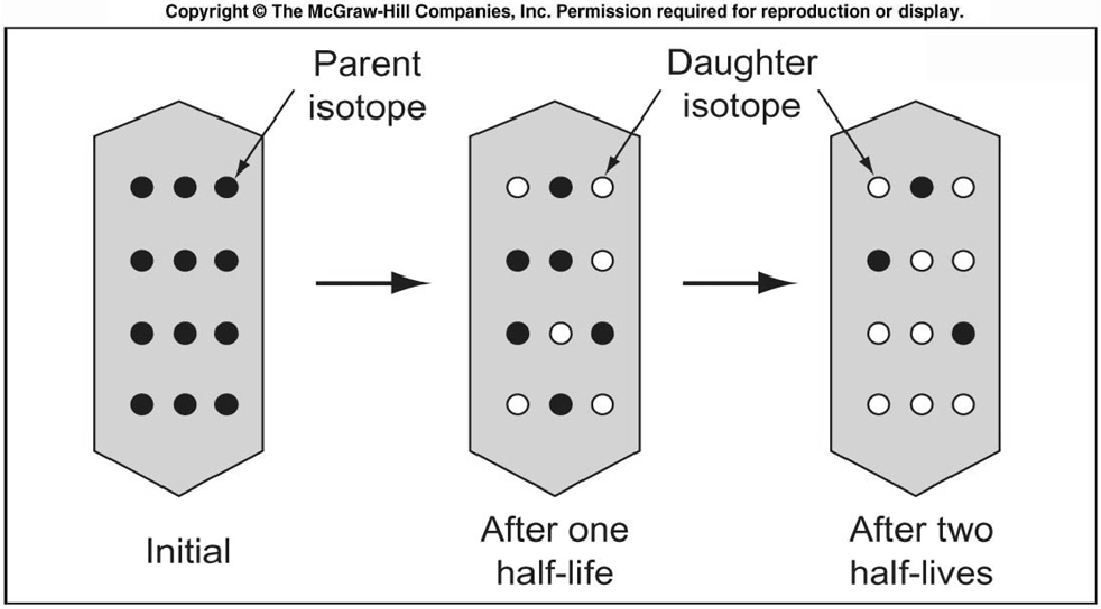


Parent Isotope: The original isotope is called parent isotope (Fig. 51) e.g. Uranium 238

Daughter Isotope: The new isotope is called daughter isotope (Fig. 51) e.g. Thorium 234

Fig. 51: Schematic representation of Parent and daughter isotope

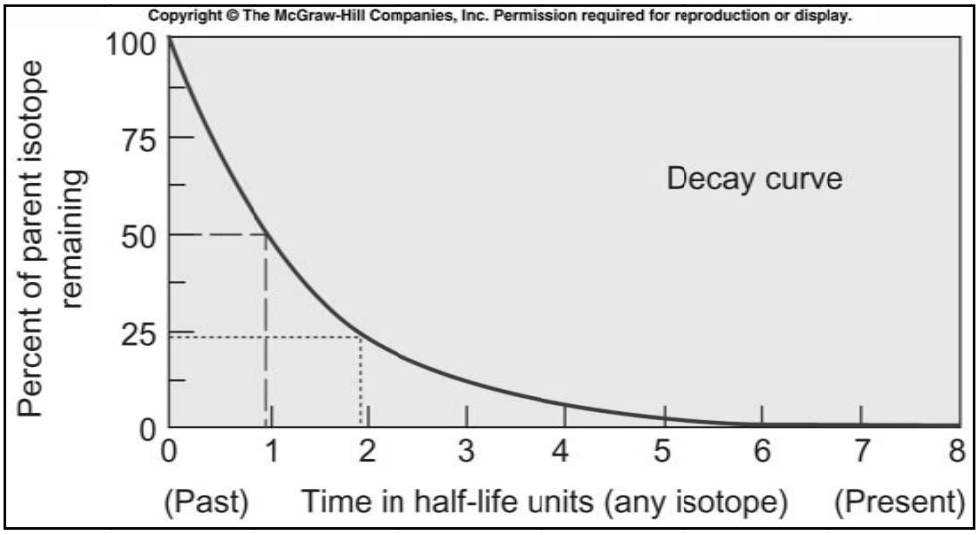
Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.



Half–life

The half life of a radioactive element is the time it takes for one-half of the atoms of the original unstable parent element to decay to atoms of a new, more stable daughter element. Since the rate of decay is constant, the ratio of parent to daughter atom is calculated to determine the age of the rock. The parent-daughter ratio is usually determined by a mass spectrometer, an instrument that measures the proportions of atoms of different masses. **Radioactive decay curve** is a simple arithmetic plot of a universal isotopic decay curve. After 1 half-life 50% of parent isotope remains; after 2 half-lives, 25% remains. So, it is geometric curve rather than a linear curve (Fig. 52).

Fig. 52: Radioactive decay curve



Radiocarbon dating method

Carbon dating was developed by a team led by Willard Libby. Carbon-14 is a radioactive isotope of carbon, with a half-life of 5,730 years, which is very short compared with other radioactive isotopes. Carbon–14, though, is continuously created through collisions of neutrons generated by cosmic rays with nitrogen in the upper atmosphere and thus remains at a near-constant level on Earth. The carbon-14 ends up as a trace component in atmospheric carbon dioxide (CO_2).

An organism acquires carbon during its lifetime. Plants acquire it through photosynthesis, and animals acquire it from consumption of plants and other animals. When an organism dies, it ceases to take in new carbon-14, and the existing isotope decays with a characteristic half-life (5730 years). The proportion of carbon-14 left when the remains of the organism are examined provides an indication of the time elapsed since its death. The carbon–14 dating limit lies around 58,000 to 62,000 years.

The rate of creation of carbon-14 appears to be roughly constant, as cross-checks of carbon–14 dating with other dating methods show it gives consistent results. However, local eruptions of volcanoes or other events that give off large amounts of carbon dioxide can reduce local concentrations of carbon–14 and give inaccurate dates. The releases of carbon dioxide into the biosphere as a consequence of industrialization have also depressed the proportion of carbon-14 by a few percent; conversely, the amount of carbon-14 was increased by above-ground nuclear bomb tests that were conducted into the early 1960s. Also, an increase in the solar wind or the Earth's magnetic field above the current value would depress the amount of carbon-14 created in the atmosphere. These effects are corrected for by the calibration of the radiocarbon dating scale.

Uranium-thorium dating method

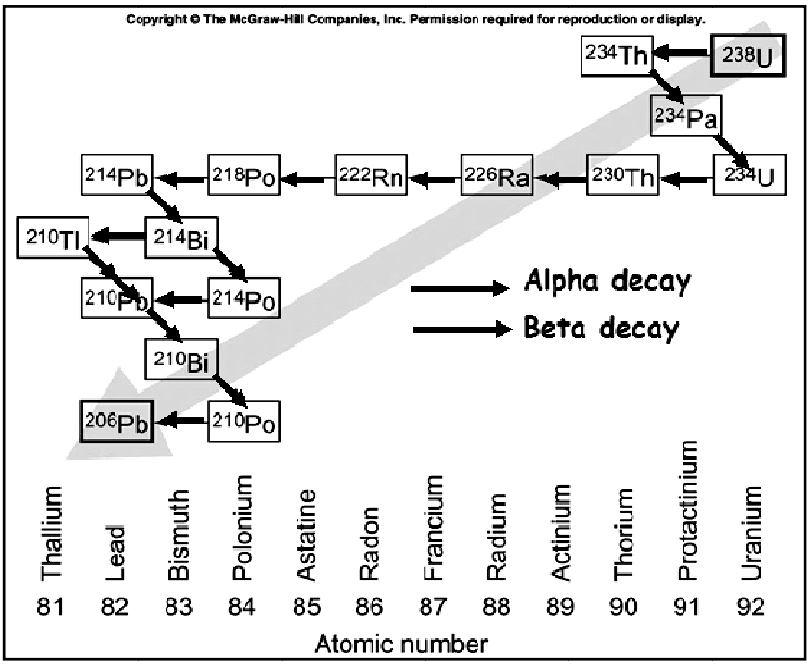
A relatively short-range dating technique is based on the decay of uranium-234 into thorium-230 (Fig. 53), a substance with a half-life of about 80,000 years. It is accompanied by a sister process, in which uranium-235 decays into protactinium-231, which has a half-life of 34,300 years. While uranium is water-soluble, thorium and protactinium are not, and so they are selectively precipitated into ocean-

floor sediments, from which their ratios are measured. The scheme has a range of several hundred thousand years.

Potassium-argon dating method

This involves electron capture or positron decay of potassium-40 to argon-40. Potassium-40 has a half-life of 1.3 billion years, and so this method is applicable to the oldest rocks. Radioactive potassium-40 is common in micas, feldspars, and hornblendes.

Fig. 53: Decay steps of Uranium 238 and Uranium 234



The relationship between time and radioactive decay of an isotope is expressed by $N=N_0e^{-\lambda t}$

N=Number of the atoms of the isotope at time t, the time elapsed

N_0=No. of atoms of that isotope present when the clock was set

The mathematical constant e has a value of 2.718 is a decay constant – a proportionately constant that relates the rate of decay of an isotope to the number of atoms of that isotope remaining.

The relationship between λ and the half life (t_{hl}) is

$\lambda = \ln 2/t_{hl} = 0.693/t_{hl}$

Replacing λ in the first equation and converting that equation to natural logarithmic (to the base e) form, we get

$t=t_{hl}/.693 \ln N/N_0$

N/N_0 is the ratio of parent atoms at present to the original number of parent atoms.

6. Early Life: The origin of earth, earliest environment, theories on origin of life, evidences for the origin of life - prokaryotes, evolution of eukaryotes and fossil records, diversified life - algae and fungi.

The origin of earth, earliest environment, theories on origin of life

Earth is a system of interconnected components that interact and affect each other in many ways.

Principal subsystems:

Atmosphere **:** Gases that envelop Earth

Biosphere **:** All of the living or once living material on Earth

Hydrosphere **:** Water on or near Earth's surface

Litho or Geosphere **:** Rock & other inorganic Earth material that make up the bulk of the planet

Earth's interior **:** Mantle and Core

The complex interaction among these subsystems result in a dynamically changing planet in which matter and energy are continuously recycled into different forms

There are 3 options for Origin of the Cosmos that is

Option 1: Someone made the universe (Big Bang or creationist theory)

Option 2: The universe made itself (Inflationary or evolutionist theory)

Option 3: The universe has always been here (Steady State Theory)

Scientists now know that the cosmos had a beginning; therefore only options 1 and 2 are viable

The Big Bang/Nucleosynthesis: The singularity at the beginning of the universe.

- 13.7 billion years ago→Mass of universe→size of head of a pin (singularity)
- Unimaginable release of energy (an expansion not a traditional explosion)→trillions of degrees
- Nobody knows how/why it happened
- Created space and time
- Created the 4 main forces: gravity, strong nuclear, weak nuclear and electromagnetic forces.

Gravitational Force: *attraction of one body towards another;* ***Strong Nuclear Force:*** *binds protons & neutrons together;* ***Weak Nuclear Force:*** *responsible for the breakdown of an atom's nucleus and radioactive decay;* ***Electromagnetic Force:*** *combines electricity and magnetism into one force and binds atoms into molecules*

- Electrons, quarks and gluons created (building blocks for all atoms)
- Universe experienced enormous expansion
- About 300,000 years later, the Universe was cool enough for complete atoms of hydrogen and helium to form
- Early space: mostly hydrogen and helium atoms (He formed from H)
- Photons (the energetic particles of light) separated from matter and light burst forth for the first time
- During the next 200 million years, Universe continued expanding and cooling, stars and galaxies began to form, chemical makeup of the Universe changed.
- Initially Universe was 100% H & He, today it is 98% and 2% of all other elements by weight

How did such a change in the Universe's composition occur?

- Throughout their life cycle, stars undergo many nuclear reactions in which lighter elements are converted into heavier elements by nuclear fusion. When a star dies, often explosively, the heavier elements are returned to interstellar space and are available for inclusion in new stars. In this way, the composition of the Universe is gradually enhanced in heavier elements

How did earth form?

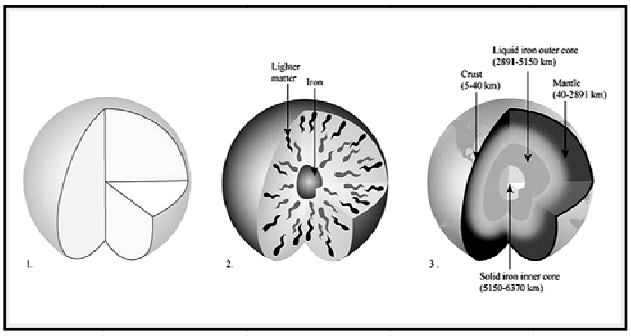
Earth is a planet of our solar system, part of the Milky Way Galaxy. **Solar Nebula Theory** is most acceptable theory for the origin of solar system-condensation and collapse of interstellar material in a spiral arm of the Milky Way Galaxy. The collapse of this cloud of gases and small grains into a counterclockwise rotating disk concentrated about 90% of the material in the central part of the disk and formed an embryonic sun-around which swirled a rotating cloud of material called a solar nebula. Within this solar nebula were localized eddies (swirling of fluid) in which gases and solid particles condensed

During the condensation process, gaseous, liquid, and solid particles began to accrete into ever-large masses-planetesimals. Some 4.6 billion years ago, various planetesimals in our solar system gathered enough material together to form Earth and other planets.

The early Earth was probably cool, of generally uniform composition and densely throughout, and composed mostly of – silicates, iron, magnesium oxides, and small amounts of all other chemical elements.

- subsequently, temperature of Earth increased by combination of meteorite impacts, gravitational compression, and heat from radioactive decay
- the temp was enough to melt iron and nickel, then the homogenous composition of Earth disappeared and was replaced by a series of concentric layers of differing composition and density, resulting in a differentiated planet (Fig. 54)
- The differentiation not only led to the formation of a crust but also contributed to the eventual formation of Earth's oceans and atmosphere

Fig. 54: Changes of homogenous composition of Earth to series of concentric layers



The earliest environments

Between 4400-3900 Ma: Oceans formed from the condensation of atmospheric water vapour Composition (high conc. of ferrous irons, dissolved CO_2 and bicarbonate, perhaps a pH as low as 6.0) and the warm temperature (between 80-1000c) of the early oceans is strongly influenced by mantle out gassing. Other gases contributing to the overall composition of the early atmosphere included hygrogen, hydrogen cyanide, formaldehyde & hydrogen sulphide – resulting from volcanic outgassing, photochemical reactions in the atmosphere, and cometary infall

~ 4200 Ma: continental crust started to form

Between 2200-1900 Ma: various sedimentary deposits require free oxygen for their formation – apparent in the geological record in form of-red-beds (sandstone and shale with red iron oxide) highly oxidized palaeosols; increasing amount of atmospheric O_2

By 1900 Ma: Early State of Continental Crust- a large single, lens-shaped body had accumulated around the equatorial belt –the supercontinent Rodinia

From 1000 Ma: Initial splitting up of Rodinia – three major parts-Laurasia (N. America, Greenland, Baltica, Siberia); East Gondwana (S. Africa, India, Most Australia, E. Antarctica); West Gondwana (S. America, W. Africa)

- Early continental crust was thin and 2-3 times more heat production than present due to heat flow from the mantle
- By 3000 Ma, heat had subsided to allow the development of a continental crust, up to 40 km in thickness
- Surface air temp is also influenced by the high level of atmospheric CH_4 and CO_2 from volcanic outgassing
- These high level of CO_2 & CH_4 also have blocked outgoing long-wave radiation, promoting greenhouse warming
- In contrast to the high levels of CO_2, early atmospheric O_2 was extremely low – before 2200 Ma the O_2 content of the air accounted for 1% of total atmospheric composition, and was probably a major limiting factors for organic evolution
- Most restrictive factor associated with this low O_2 content- lack of an ozone layer & therefore, no protection for terrestrial organisms from incoming solar radiation

Origin of Life

Life arose through chemical evolution at ~3500 million years ago. Life is a difficult concept to define.

Aristotle was the first to attempt to define life: *something that grows and maintains itself (he called this "nutrition"), and reproduces*

Austrian physicist Erwin Schrödinger (1944) defined: *life as that which resists decaying to disorder and equilibrium*

Some scientists proposed: *life is that which can reproduce itself;* however, this definition would exclude mules, which are born sterile, and would include nonliving things like fire

Some scientists suggested: *life is something that can metabolize — that is, take in energy to move or grow, and release waste* — but many nonliving things, like cars, can do that

"Life, because it is such a complex system of things with so many interacting parts, each of which is essential, it's really tough to make a definition“ ……David Wilson Deamer

- It is generally agreed that an assemblage of molecules is ‘alive’ if it can capture energy from the environment, use that energy to replicate itself, and thus be capable of evolving.
- In the living things this functions are performed by nucleic acids that carry information and by proteins, which replicate nucleic acids, transduce energy, and generate the phenotype. These components are held together in compartments-CELL-formed by lipid membranes.

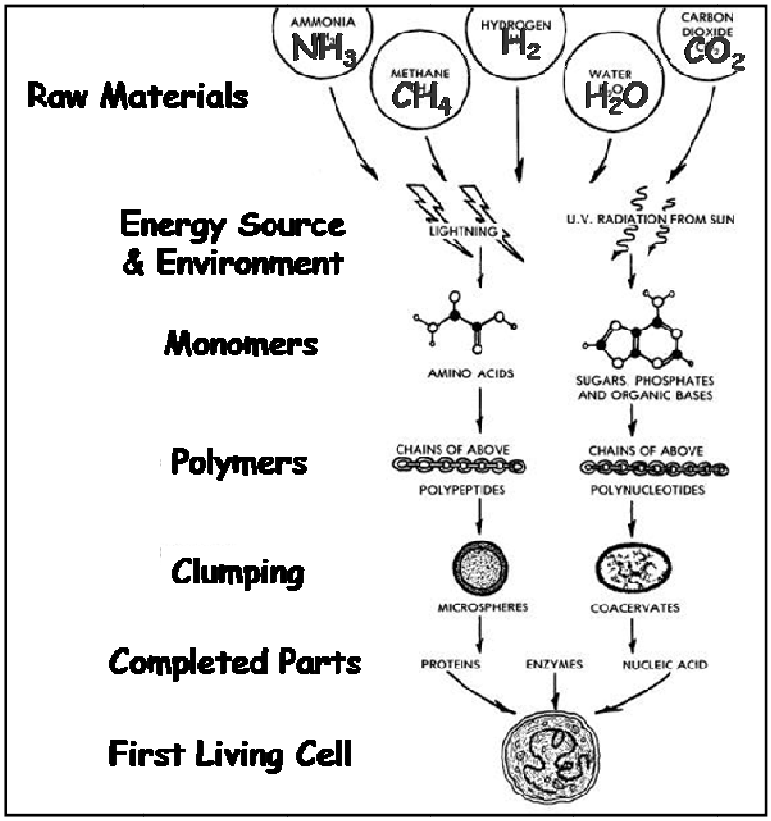
There are two contrasting theories for organic matter accumulation in the pre-biotic environment-

- **Cometary Infall *(proposed by McKay et al., 1996):*** There is increasing evidence to support this theory that meteoritic and cometary infall provided the first organic material on Earth (McKay et al., 1996)

  Provenance: Analysis of the present day meteorites on the ice beds of the Antarctic (carbonaceous chondrites) – typically contain between 1 and 4% carbon, mainly as graphite but also as much as 1% organic molecules which include hydrocarbons, amino acids, carbon, hydrogen cyanide and amphiphilic molecules

- **Terrestrial Accretion** *(proposed by Oparin & Haldane 1920; first experimentally proved by Miller 1953).* **First cells may have originated by chemical evolution involving 4 steps (Fig. 55):**
  - Abiotic (Non-biological) synthesis & accumulation of small organic molecules (monomers) → C + H = organic molecule
  - Monomers joined together to form polymers (proteins, nucleic acids)
  - Origin of self-replicating hereditary molecules (inheritance of traits) → proteins and polynucleic acids
  - Packaging of these organic molecules into cell like membrane bound droplets - protobionts (precursor to prokaryotic cell) → Aggregates of abiotically produced molecules that had an internal chemical environment differing from the external chemical environment and exhibit some of the properties associated with life (i.e. metabolism, excitability, heredity).

Fig. 55: Likely steps in the origin of Life



Evidence that supports the four-stage hypothesis for the origin of life

In 1920s, I. Oparin (Russia) and J. B. S. Haldane (Great Britain) postulated that abiotic synthesis of organic molecules is testable in the laboratory.

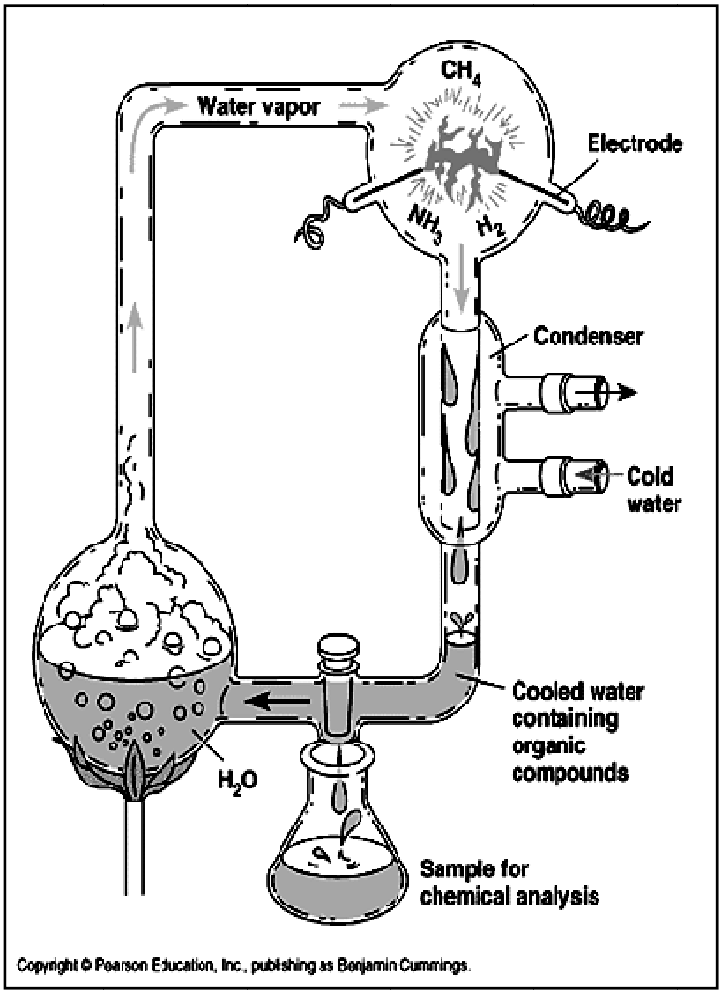
Hypothesis: Conditions on primitive earth favored chemical reactions that synthesized organic compounds from inorganic precursors. These conditions were different from present atmosphere.

As we know that earth atmosphere was

- No Free Oxygen
- More Reducing Environment than present (no free oxygen, only in form of H_2O, CH_4, NH_4, and H_2) = lots of free electrons that could be used to reduce carbon and produce organic molecules.
- Energy from lots of Lightning, UV radiation (no O_2 to block UV rays from the sun) and Volcanic Activity (heat).

This early environment was recreated in the laboratory by **Stanley Miller & Harold Urey (Fig. 56) in 1950s and tested the Oparin-Haldane hypothesis.**

Fig. 56: Urey-Miller experiment



Results of this experiment is that they were able to create Amino Acids, Urea, Simple Fatty Acids as in a week which were resultant of 15% of the conversion of carbon in the mixture to organic compounds. They concluded that **Life may have evolved in "primordial soup" of biological molecules formed in early Earth's oceans.**

The initial Miller-Urey experiment and various similar experiments succeeded in producing:

- All 20 amino acids
- Several sugars
- Lipids
- Purines and Pyrimidines

- ATP (when phosphate was added)

More recent experiments subjecting a reducing mixture of gases to a violent energy source produces:

- Formaldehyde
- Hydrogen Cyanide
- Cyanoacetylene
- All highly reactive intermediate molecules

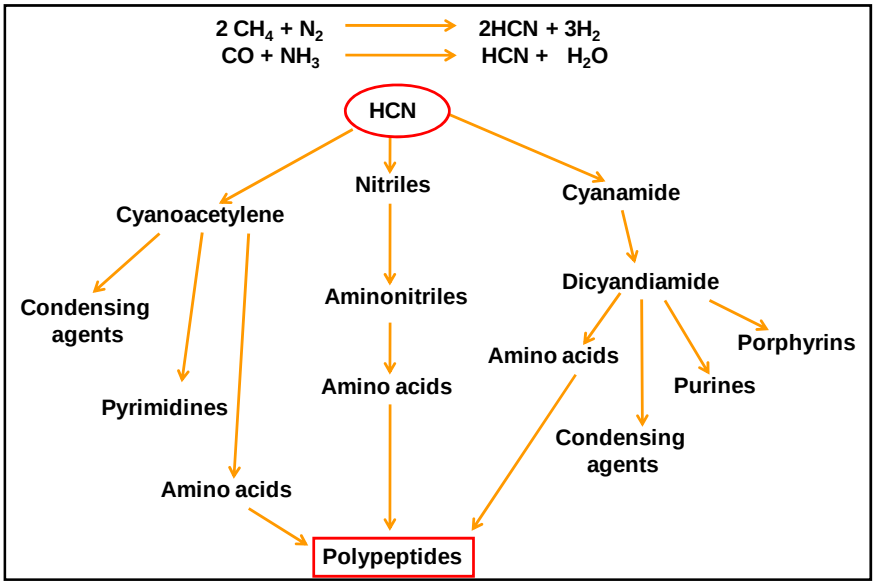
All react with water and NH_3 or N_2 to produce a variety of organic compounds:

- Amino Acids, Fatty Acids, Urea, Sugars,
- Aldehydes, Purine and Pyrimidine Bases

And it signifies the subunits for complex organic compounds.

Chemical evolution of biomolecules is supposed to produce in following pathway (Fig. 57).

Fig. 57: Chemical evolution of biomolecules



Nowadays DNA needs proteins in order to form, and proteins require DNA to form, so how could these have formed without each other? But was it the same situation during the origin of life?

Which came first- RNA?

The answer may be RNA, which can store information like DNA, serve as an enzyme like proteins, and help create both DNA and proteins. Later DNA and proteins succeeded this "RNA world," because they are more stable & efficient.

For life to originate and perpetuate, we need to have a molecule that can be used to store information and also can catalyze the synthesis of other molecules. Thomas Czech (1980) proved that

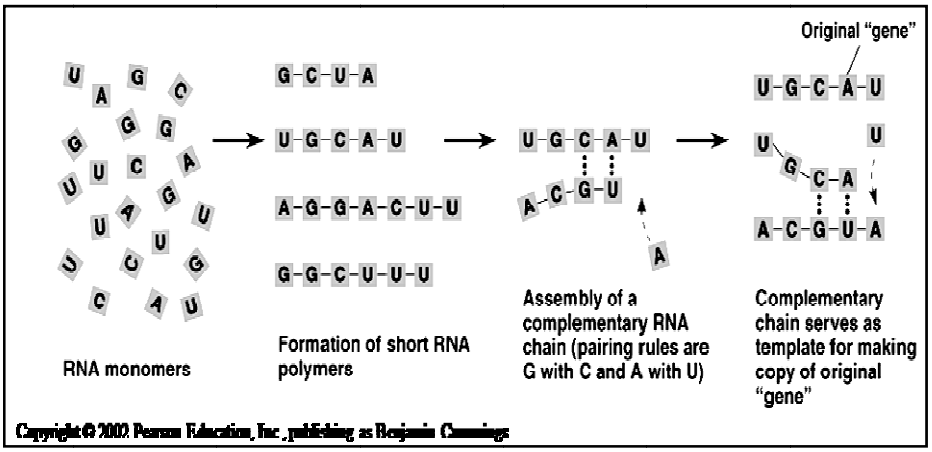
- RNA can catalyze simple reactions leading to the belief that RNA was probably the first genetic molecule to start life.
- RNA can help as a template for protein synthesis and for more RNA synthesis.
- Once proteins (enzymes) are made, they can make carbohydrates and lipids. Later on DNA evolved to be more stable molecule and proteins evolved to be more efficient enzymes.
- RNA with catalytic activity is referred to as ribozyme.

So, RNA was probably the first hereditary material which is termed as RNA World

Today some organisms such as viruses use RNA to store information.

- Short polymers of ribonucleotides can be synthesized abiotically in the laboratory (Fig. 58).
- If these polymers are added to a solution of ribonucleotide monomers, sequences up to 10 based long are copied from the template according to the base-pairing rules.
- If zinc is added, the copied sequences may reach 40 nucleotides with less than 1% error.

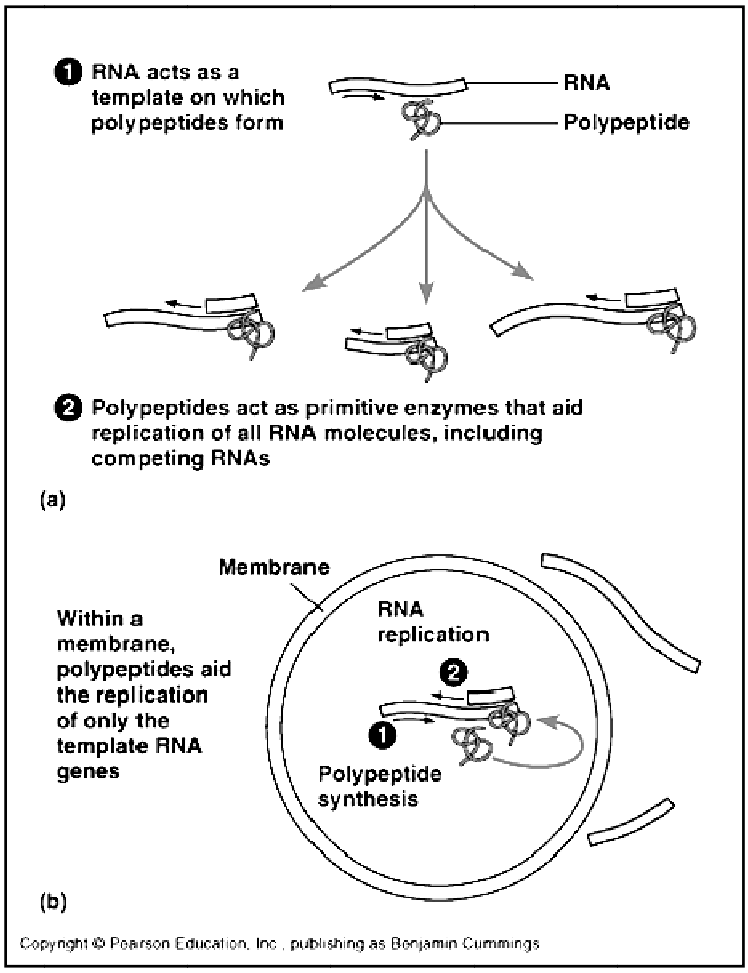
Fig. 58: Short polymer of RNA synthesis



Natural selection could refine protobionts containing hereditary information

- Once primitive RNA genes and their polypeptide products were packaged within a membrane, the protobionts could have evolved as units (Fig. 59).
- Molecular cooperation could be refined because favorable components were concentrated together, rather than spread throughout the surroundings.

Fig. 59: Protobionts containing hereditary information

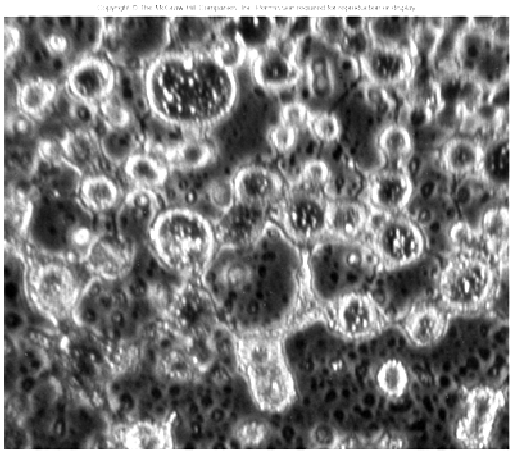


How did the membrane form?

The strongest current hypothesis for pre-biotic assembly of biologically important polymers suggests that they occurred within the boundaries of semi-permeable membranes (Fig. 60).

- Membranes were formed by aggregation of amphiphilic molecules.
- Meteorites are common sources of organic amphiphiles.

Fig. 60: Cell membrane formation



Evidences for the origin of life - prokaryotes recovered from Precambrian strata

The definite evidences of earliest life forms in the Precambrian strata were come to existence by the landmark publication of Tyler and Barghoorn (1954, 1965). A. C. Seward described the Precambrian "as an age of algae – algae with doubtful credentials." Fossil evidences of anaerobic chemo-heterotrophic bacteria, unicellular cyanobacteria are identified in the 3500 million (3.5 b.y.) year's old rocks in the Western Australia (Warrawoona Group) as the oldest structurally preserved forms of life so far recorded. Precambrian lifeforms occur principally in two types of sedimentary deposits namely 'cherts' and 'shales'.

Cherts: These rocks are composed of minute interlocking grains of silica, occurring as the mineral quartz (SiO_2), that have been deposited chemically, petrifying microscopic organisms in the place in which they live. Fossils preserved on these rocks are generally unflattened, composed of three-dimensionally preserved organic-walled cells that are thoroughly embedded in, and in filled by, the petrifying fine-grained quartz in the layers of stromalites. Stromatolites are extensive mats of microorganisms mostly comprising of cyanobacteria and green algae

Shales: This type of rocks is formed by consolidation of layers of clay or mud disseminated, along with phytoplankton and other debris, at the bottom of lakes or ocean basins. Carbonaceous microfossils of shales have been preserved by compression and flattened between thin layers of consolidated silt.

Evidences supporting the earliest life forms in the Precambrian strata are mostly recovered from the fossils sites of Onverwacht Group (Swaziland Supergroup), and Fig Tree Group of South Africa and Warrawoona Group (Pilbara Supergroup) of Western Australia

Prokaryotes dominated from 3.5 to 2 billion years ago. During this time, the first divergence occurred: Bacteria and Archaea Earliest fossil prokaryotes are mainly representative of two types of living organisms:

Blue-Green Algae (Cyanobacteria)

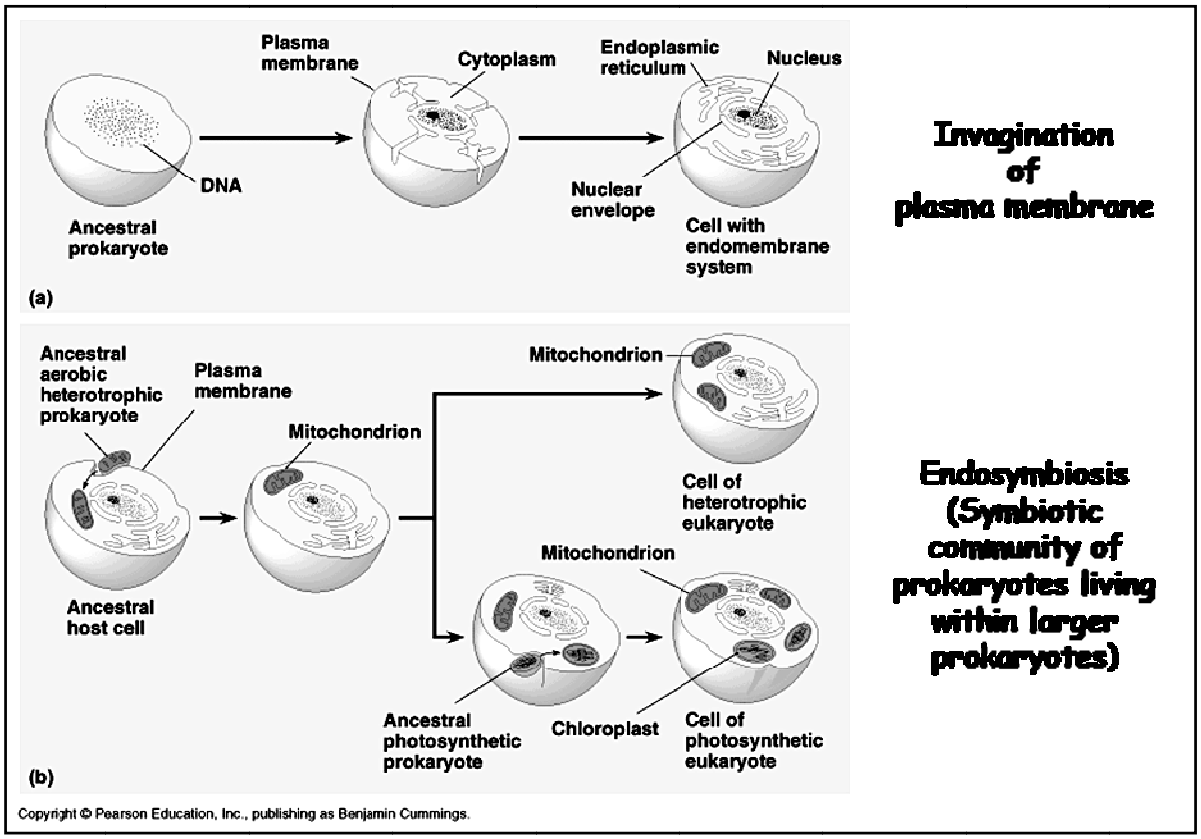
Bacteria

The oldest unequivocal remains of a diversity of microorganisms occur in the 2.0 BYO or 2000 my Gunflint Cherts of the Canadian Shield. It includes not only bacteria and cyanobacteria but also ammonia consuming *Kakabekia* and some things that resemble green algae and fungus-like organisms

Eukaryotic Origins: Endosymbiotic Theory is proposed by Lynn Margulis and her associates for describing origin of eukaryotic cell. They proposed that eukaryotes resulted from a symbiotic relationship between two or more bacteria (Fig. 61):

- Mitochondria and plastids contain their own DNA.
- Nuclear, plastid and mitochondrial ribosomal RNAs show distinct evolutionary lineages.

Fig. 61: Endosymbiotic Theory



Modern-day endosymbiotic relationships

- common among protists
- similarity between eubacteria & the chloroplasts & mitochondria of eukaryotes
- size
- inner membrane systems, enzymes, electron transport systems
- reproduction resembles binary fission
- circular DNA

Three records of early eukaryotes of particular interest to the evolution of plants

- **Oldest recorded alga *Grypania***: 2100 Ma; banded iron formations of northern Michigan, USA.
- Coiled cylindrical organisms, giant unicellular alga like modern day green algae ***Acetabularia***.
- **Bangiacean red algae**: 1200 Ma; silicifed carbonate rocks of Huntington Fm in arctic Canada.

- Unbranched, uniserate, filamentous like extant red algae ***Bangia***.
- **Cladophoralean green algae**: between 800-700 Ma; shale deposit from the Svanbergfjellet Fm on Spitsbergen (island of northern Norway).
- Multicellular, branched, filamentous thalli composed of large cylindrical cell comparable to the extant cladophoranean green algae ***Cladophora***.

7. Terrestrialization of plants: Geologic time, environment, vegetative and reproductive adaptations to land dwelling, fossil evidences - transitional plants with land adaptive features, early non-vascular land plants (bryophytes), early vascular land plants (pteridophytes).

Why so late?

What was the stimulus for their appearance?

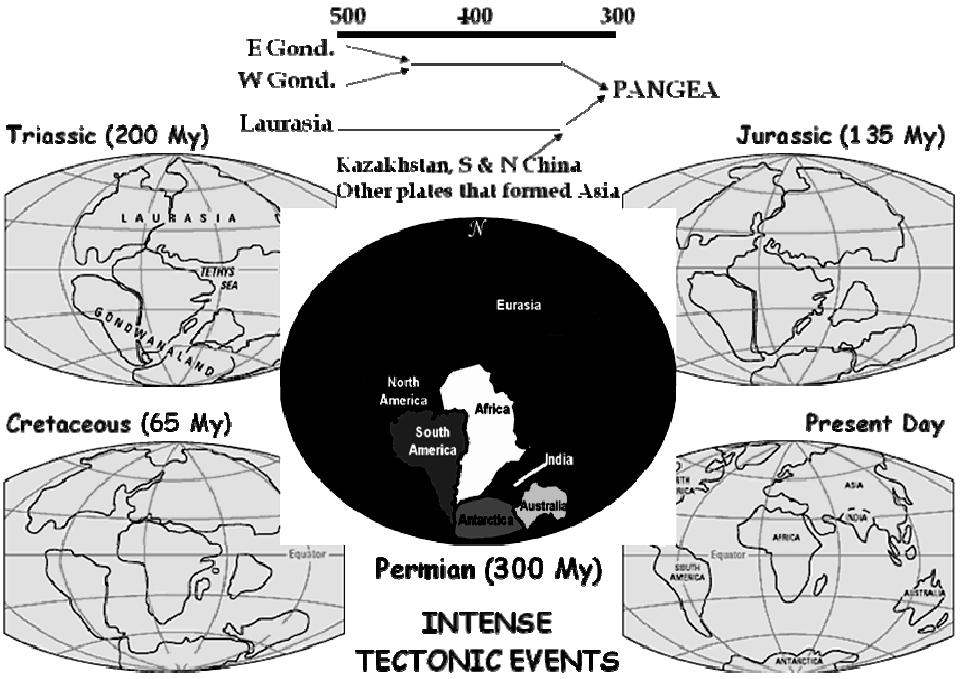
Development of essential environmental prerequisites

- Formation of new sizeable and stable near-shore environment
- Formation of soil
- Development of suitable climatic and atmospheric conditions

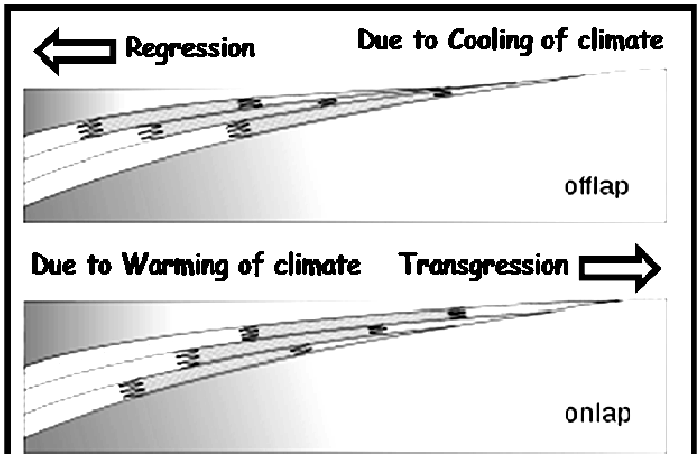
Major changes to early plants for land conquest

- Reduction of dependence on water for reproduction
- Protection against desiccation
- Formation of pore for gaseous exchange
- Anchoring mechanism
- Development of specialized cells for water and nutrient uptake

Formation of new sizeable and stable near-shore environment



Regression and transgression occur in the ice ages causing sea level change
Glaciation from approximately 440 million years ago (end of the Ordovician) led to a dramatic reduction in sea level
Melting of ice sheets during Varanger ice age in the Late Proterozoic (650-590 Ma)



Formation of soil:

Land surfaces were there but humic material or " biological available" mineral elements (e.g. N, P, Fe, S) were absent in the bare surfaces of the earliest terrestrial environments.

Various important biological and non biological processes were responsible for formation of first soils:

1. **Atmospheric element input:** addition of S and N via atmospheric transfer of volatile products from the metabolism of marine organism
   a. **Phytoplankton died→SO_2 volatized & Oxidized→transferred to terrestrial environment by dry deposition or in rain**
   b. **Fixing of atmospheric nitrogen→biologically usable through lightening strikes**

2. **Weathering by acid rain and organic acids produced by early microbial organisms:**

Cyanobacteria, non-photosynthetic bacteria, eukaryotic algae → **Secreted organic acid** → **Breaking down rock material** → **Releasing Fe & P**

How?

Chelation of Fe(III) from rock is carried out by specific organic moleccules Siderophores. These Siderophores are secreted by prokaryotic and eukaryotic organisms. These siderophores containing the Fe(III), are trhen taken up by other organisms and their breakdown results in the release of Fe.

18 times higher than present CO_2 level → **Favored increased growth of autotrophs** → **Growth of microbial mats** → **Subsequent decomposition & breakdown of mats** → **Released inorganic nutrients back into the system**

High level of atmospheric CO_2 made ppt more acidic → **Chemical decomposition of rock due to acid rain** → **Soil formation by physical disintegration of the bare mineral surfaces**

High level of atmospheric CO_2 → **Increased activity of burrowing organisms** → **Increased chemical weathering** → **Aided the soil formation process**

3. **Lichens (symbiotic association of fungus and green alga or cyanobacterium)**

Colonize-Weather Rock-Soil Formation

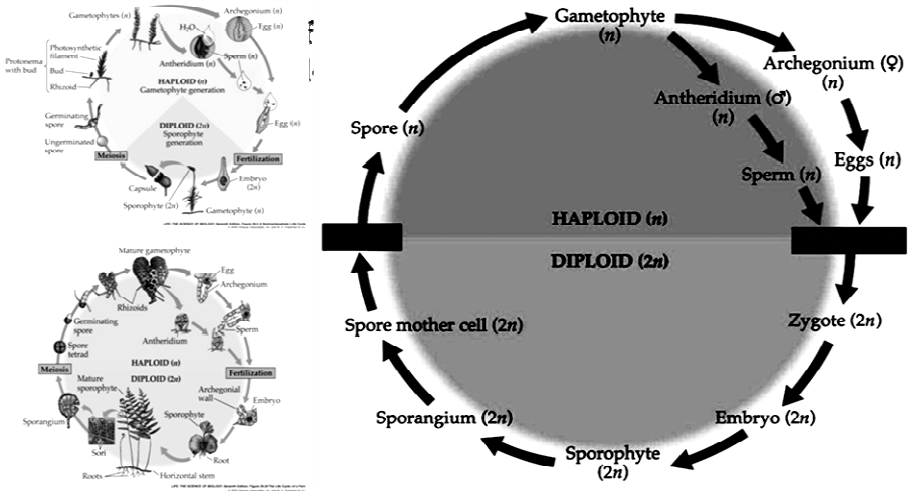
Development of suitable climatic and atmospheric conditions:

Cambrian-Early Ordovician: 18 times higher CO_2 level than present associated with high global temperature

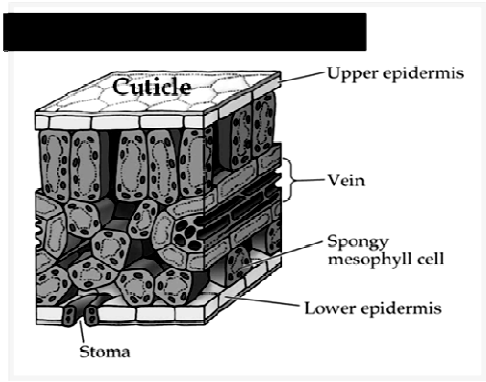
Late Ordovician (458-443 Ma): Gradual reduction of CO_2 level & global temperature because of

A. Decrease in volcanic out gassing
B. Increased burial of organic carbon
C. Late Ordovician glaciation

Reduction of dependence on water for reproduction:

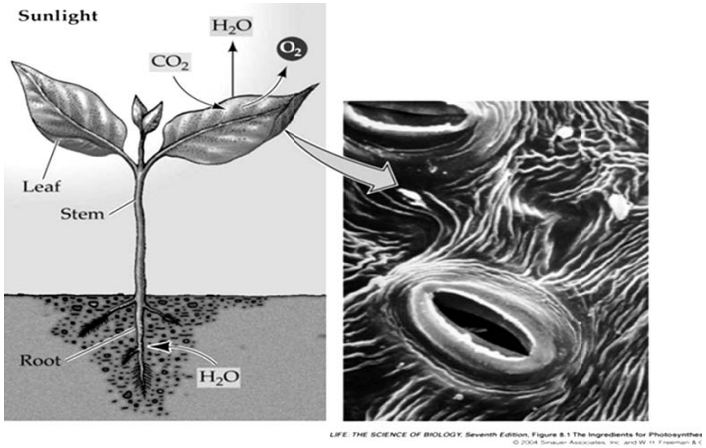


Protection against desiccation:

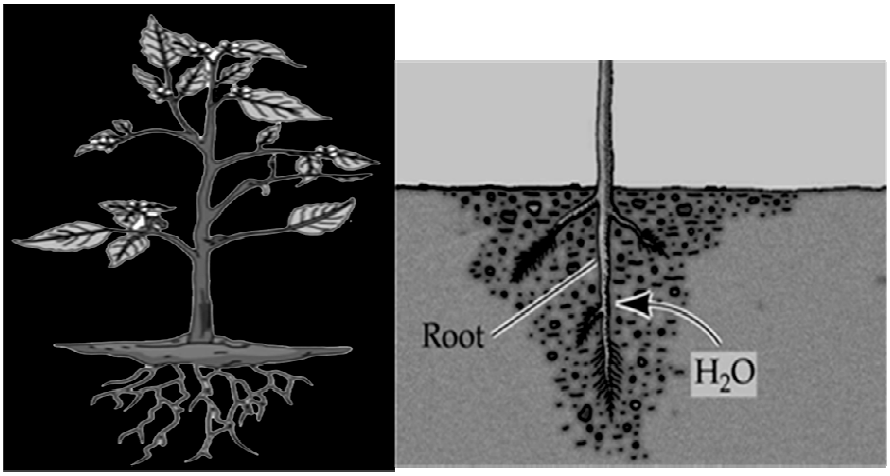


Trilete spore with sporopollenin on outer wall

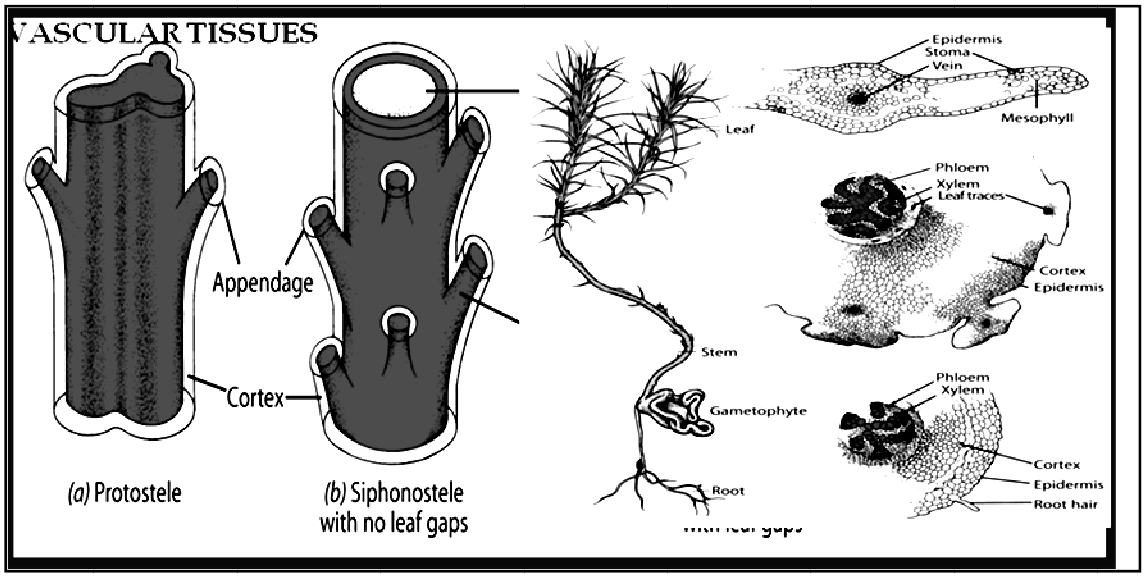
Formation of pore for gaseous exchange:



Anchoring mechanism:



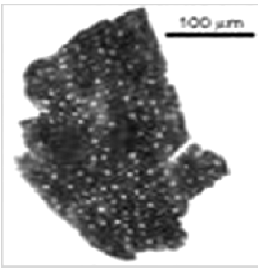
Development of specialized cells for water and nutrient uptake:



Transitional plants (Enigmatic non vascular plants) with land adaptive features:

***Nematothallus* (Early Silurian-Devonian)**

- Plant body thalloid
- Pseudoparnchymatous cells comprised of filament and surrounded by thick cuticle
- Branching tubes with secondary wall thickening that have the appearance of tracheids
- Some produce thick walled spores

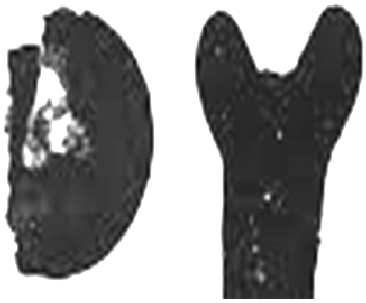


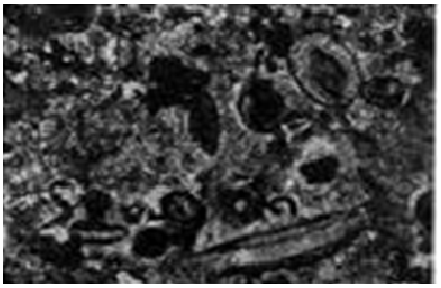
Protosalvinia (Devonian)



- Thalloid plant body
- Plant body developed depression within which thick walled spores present in a tetrahedral manner
- Though biochemical evidences pointed out towards the affinity with brown algae, but authentic botanical affinity unknown; thus it is considered as transitional non-vascular plants

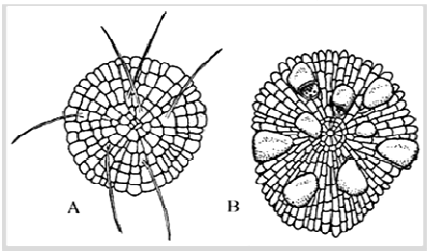
Pachytheca (Late Silurian – Early Devonian)





- Single sphaeroidal body, 1.5-5.0 mm in diameter with outer and inner zone
- Inner zone consists of multicellular filament oriented randomly
- Radially arranged filament present in outer zone
- The plant having affinity with algae

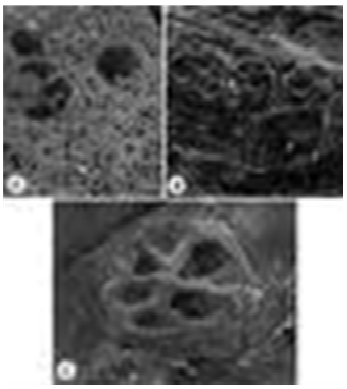
Parka (Early Devonian)

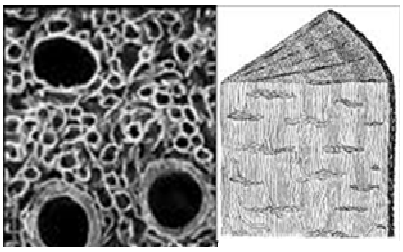


- Dorsiventral thallus like plant circular to ovoid up to 5 cm with clustered spores
- The plant body cellular, upper and lower epidermis parenchymatous attached with a ventral holdfast
- Spores appear cuticularized, 28-34 µm in size, interrupted to be in a sporangium at the surface

Spongiophyton (Middle Devonian)

- Thalloid, branching plant thought to have a habit similar to modern liverworts
- Typically preserved as a cuticular sheath with a porate (200-300 µm raised areas with holes) and non porate surface
- Porate cuticle are three times thicker than non porate one
- There is no evidence of vascularization
- Stein et al. (1993) suggested that thase plants are lichens





Prototaxites (Silurian-Devonian)

- Giant, terrestrial, saprotrophic organism, axes termed as pseudostem, 1.25 m in diameter and more than 8 m long
- Outer surface smooth or mildly ribbed
- Pseudoparenchymatous or plectenchymatous internal organization constructed to longitudinally oriented tubes of three different types
- In addition tubes, several lacunae are scattered throughout the pseudoparenchyma
- Previous chemosystematic data suggested an affinity with algae, where most recent interpretation (Hueber, 2001) hypothesizes that it may belong with the basidiomycetes



Early non vascular land plants (Bryophytes)

Poor record because of the delicate nature of many bryophytes

LIVERWORTS

1. Oldest fossil bryophyte: *Pallavicinites devonicus* (thallus liverwort)
Mode of preservation: Compression fossil
Age: Upper Devonian age (Paleozoic)
Preserved form: Dichotomizing axis with some cellular details
Affinity: Jungermanniales-Anacrogynae (having the female sex organs arising from any cell below the apex of the stem, thereby not terminating its growth): high degree of similarity with extant genus *Pallavicinia*.
2. *Hepaticites kidstoni* (leafy liverwort)
Age: Carboniferous (Paleozoic)
Affinity: Anacrogynous *Treubia*
3. *Thallites willsi*
Age: Carboniferous
Affinity: *Metzgeria*
Preserved from: Dorsiventral dichotomizing thallus
4. *Metzgerites* (anacrogynous liverwort)
Age: Triassic (Mesozoic)
5. *Jungermannites* (leafy liverwort-Jungermanniales-Acrogyne)
Age: Tertiary
Some specimen of leafy liverworts and mosses are found preserved in famous Oligocene (Tertiary) Baltic amber deposits and are assigned to modern genera
6. *Hepaticites cyathodoides*
Age: Middle Triassic
Affinity: Marchantiales
Preserved from: Undeffertiated, dorsiventral dichotomizing thallus, pores with air chamber, small scales on the ventral surface
7. *Ricciopsis*
Age: Jurassic: Triassic rock ((Mesozoic)
Affinity: *Riccia*
8. *Marchantites sezannensis*
Age: Eocene **(**Tertiary)
Affinity: Marchantiales
Preserved from: Antheridial gametophores similar to *Marchantia*

At least eight *Hepaticites* and ten *Thallites* are also found from Mesozoic rocks

Naiadita lanceolata: an enigma

(Discovery by T. M. Harris in 1938)
Age: Triasic

Unquestionable liverwort with unusual combination of characters of both Hepaticae and Musci; as such it is not possible to place in a family of Hepatics and so family Naiaditaceae is suggested for this fossil genus belonging to Sphaerocarpales order of Hepatics.

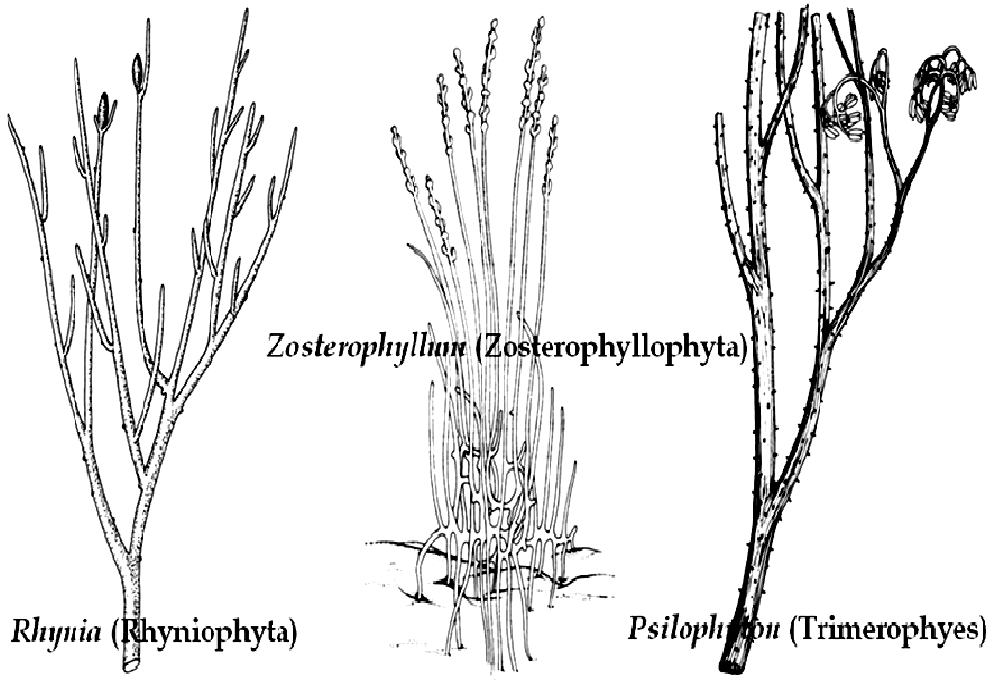
- Unicellular rhizoids
- Sessile archegonia with venter wall one cell layer thick, and
- Leaf cells of a similar shapes;
- Erect gametophores with spirally arranged leaves like most of mosses;
- Leaves lack costa which is a characteristics of mosses;
- Archegonia are enclosed in a perianth of several leaf like lobes;
- Sporophytes are found at the tips of a short lateral branch of gametophore;
- Sporophyte with poorly developed bulbous foot and globose sporangium

❖ MOSSES:

❖ Bogmoss:

❖ Distinct leaf cell pattern like bog moss reported from: Permian of Russia
❖ ***Protosphagnum:***
❖ **Age:** Lower Jurassic
❖ **Preserved from:** Leaves with midribs and occasional lateral vein
❖ **True Moss (Eubrya)**
❖ ***Muscites polytrichaceus*:** Carboniferous
❖ ***Intia:*** Permian; looks like modern *Mnium* and *Bryum*
❖ ***Merceria:*** Permian; permineralized from looks like Bryales
❖ ***Aulacomnium heterostichoides:*** Tertiary (Eocene); *Aulacomnium heterostichum*
❖ ***Ditrichites:*** Palaeocene; assigned to Districhales

Some early land vascular plants:
Early Pteridophytes



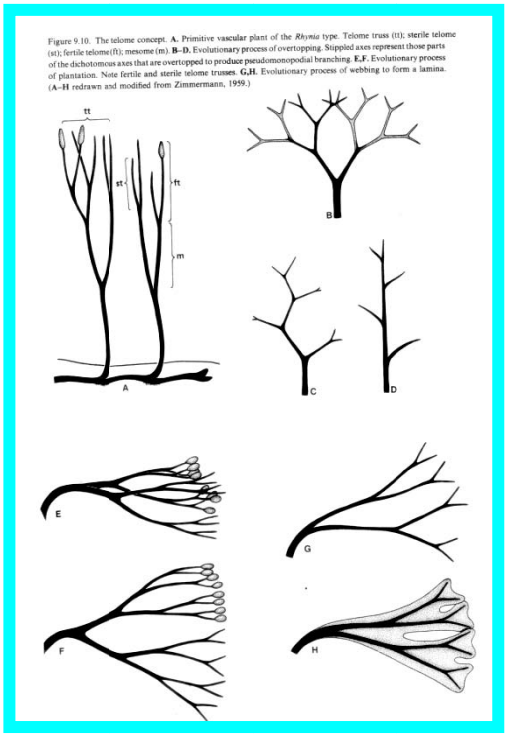


Figure 9.10. The telome concept. **A.** Primitive vascular plant of the *Rhynia* type. Telome truss (tt); sterile telome (st); fertile telome (ft); mesome (m). **B–D.** Evolutionary process of overtopping. Stippled axes represent those parts of the dichotomous axes that are overtopped to produce pseudomonopodial branching. **E,F.** Evolutionary process of plantation. Note fertile and sterile telome trusses. **G,H.** Evolutionary process of webbing to form a lamina. (**A–H** redrawn and modified from Zimmermann, 1959.)

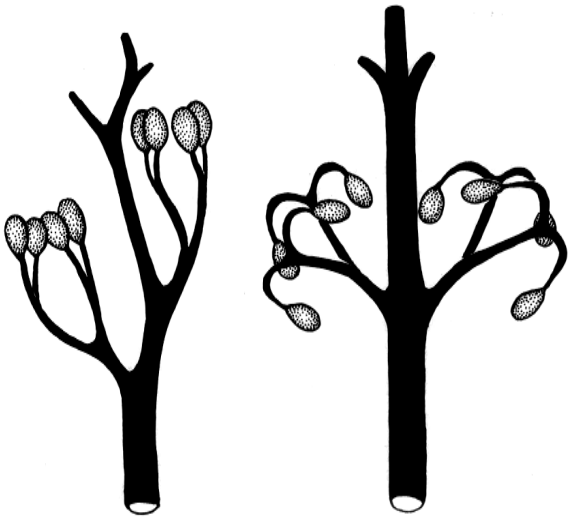


Figure 9.12. Evolutionary process of recurvation of fertile telomes so that the terminal sporangia are directed toward the axis. (Redrawn from Zimmermann, 1959.)

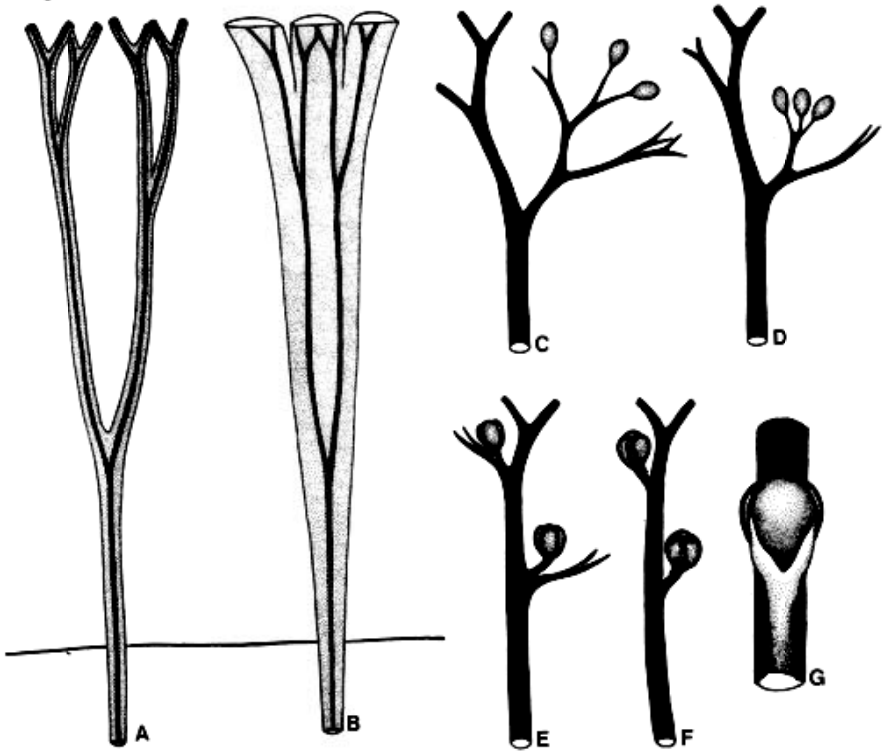


Figure 9.11. The telome concept continued. **A,B.** Evolutionary process of syngenesis, the tangential fusion of telomes and mesomes. **C–G.** Series of steps using the evolutionary process of the telome theory to depict the origin of the *Psilotum* synangium. (**A,B** redrawn and modified from Zimmermann, 1959; **C–G** redrawn and modified from Emberger, 1944.)

Evolution of non vascular land plants

Between the first occurrences of prokaryotic algal like plants (Cyanobacteria) in the Precambrian and the first well documented terrestrial plants there is a gap of about 3 billion years. This delay in colonization of terrestrial habitats has been related to oxygen levels in the palaeo-atmosphere and specifically to the lack of a sufficient ozone shield to protect terrestrial organisms from UV radiation, which acts as a strong mutagen in organisms. In considering the history of early terrestrial plants, it is essential to distinguish between the first authentic vascular plants and the more primitive land plants that undoubtedly preceded them. In the search for the most primitive vascular plant, some palaeobotanists have called attention to certain enigmatic organisms that they believe might represent early land plants or even progenitors of the earliest vascular plants – earlier than late Silurian. Some form represented by sheet of cells, had tubes with annular or helical thickenings that resemble tracheids. Also thick walled resistant spores with tri-radiate ridges have been described, but bryophytes or some extinct algae could have produced this type of spore.

Isolated cuticle fragments, spores and tubes are found in lower to middle Ordovician rocks and extend through the Silurian into the Lower Devonian. Although even less is known about these fragments than the enigmatic fossils, they constitute an important source of palaeobiological data because they occur at a point in geologic time that coincides with the origin of a terrestrial biota. The presence of land plants with a bryophytic level of organisation as early as the Ordovician is supported by both palaeobotanical and molecular evidence. The first evidence of plants on land comes from spores of Mid-Ordovician age (early Llanvirn, ~470 million years ago). These spores, known as cryptospores, were produced either singly (monads), in pairs (dyads) or groups of four (tetrads), and their microstructure resembles that of modern liverwort spores, suggesting they share an equivalent grade of organization. They are composed of sporopollenin – further evidence of an embryophytic affinity. A close examination of algal spores shows that none have trilete spores, either because their walls are not resistant enough, or in those rare cases where it is, the spores disperse before they are squashed enough to develop the mark, or don't fit into a tetrahedral tetrad.

The earliest megafossils of land plants were thalloid organisms, which dwelt in fluvial wetlands and are found to have covered most of an early Silurian flood plain. They could only survive when the land was waterlogged. There were also microbial mats. Once plants had reached the land, there were two approaches to dealing with desiccation. The bryophytes avoid it or give in to it, restricting their

ranges to moist settings, or drying out and putting their metabolism "on hold" until more water arrives. Tracheophytes resist desiccation. They all bear a waterproof outer cuticle layer wherever they are exposed to air (as do some bryophytes), to reduce water loss, but—since a total covering would cut them off from CO_2 in the atmosphere—they rapidly evolved stomata, small openings to allow gas exchange. Tracheophytes also developed vascular tissue to aid in the movement of water within the organisms, and moved away from a gametophyte dominated life cycle. Vascular tissue also facilitated upright growth without the support of water and paved the way for the evolution of larger plants on land. The establishment of a land-based flora caused increased accumulation of oxygen in the atmosphere, as the plants produced oxygen as a waste product. When this concentration rose above 13%, wildfires became possible. This is first recorded in the early Silurian fossil record by charcoalified plant fossils. Apart from a controversial gap in the Late Devonian, charcoal is present ever since.

Some fossils with land adaptive features have been recovered from various strata and it is concluded that the structures do not represents the remains of ancient vascular plants but are parts of other plant forms and known as enigmatic plant fossil. e.g. :

Protosalvinia **(Devonian):** Thalloid plant body. Botanical affinity unknown so it is considered as transitional non-vascular plants. The plant body developed depression within which thick walled spores present in a tetrahedral manner. Biochemical evidences has pointed towards the affinity with brown algae.

Pachytheca **(Late Silurian–Early Devonian):** The plant having affinity with algae consisting of single, sphaerical body, 1.5-5.0 mm in diameter with outer and inner zone. The inner zone consists of multicellular filament oriented randomly whereas in outer zone radially arranged filament present.

Parka **(Early Devonian):** Dorsiventral thallus like plant, circular to ovoid upto 5 cm with clustered spores. The plant body cellular, upper and lower epidermis parenchymatous attached with a ventral holdfast. Spores appear cuticularized, 28-34 um in size, interpreted to be in a sporangium at the surface.

Spongiophyton **(Middle Devonian):** Thalloid, branching plant thought to have a habit similar to modern liverworts that is typically preserved as a cuticular sheath with a porate (200-300 um raised areas with holes) and non porate surface. Porate cuticle are three times thicker than non porate one. There is no evidence of vascularisation. Recently Stein et. al. (1993) has suggested that these plants are lichens.

Nematothallus **(Early Silurian–Devonian):** Plant body thalloid, characterized by pseudoparenchymatous cells comprised of filament and surrounded by thick cuticle. Branching tubes with secondary wall thickenings present in the plant body that have the appearance of tracheids. Some produced thick walled spores.

Prototaxites **(Silurian and Devonian):** Largest representative of Nematophytes known to date. It occurs in the form of compressed or silicified axes, termed as pseudostem, some of which up to 1.25 m in diameter and more than 8m long. Outer surface is smooth or mildly ribbed. Thin section show a pseudoparenchymatous or plectenchumatous internal organisation constructed of longitudinally oriented tubes of three different types:

- Skeletal hyphae, which are thick walled, large, long, straight or flexuous, aseptate or unbranched.
- Generative hyphae, which are large and thin walled, septate with an open and occluded core and profusely branched and
- Binding hyphae, which are small, thin walled with a pore in the septum, and profusely branched. Arrangement of the tissue made up of these hyphae, along with the presence of well defined borders of growth increments marked by increased tissue density, suggests some type of periodicity in growth. In addition to the tubes, numerous lacunae are scattered throughout the pseudoparenchyma.

Nematoplexus **(Lower Devonian):** It consists of a meshwork of intertwined aseptate and septate tubes of two or three different sizes. Most of the larger aseptate tubes have annular or helical thickening; some of the narrow smooth walled tubes display distinct septa with a centrally located elliptical aperture or pore.

8. Early vascular plants to early spore producing trees (arborescent pteridophytes & progymnosperms): Geologic time, environment, advancement in plant adaptive features for land dwelling with fossil evidences.

Stem Genera

Lepidodendron, Lepidophloios and *Sigillaria*

❑Stem genera show raised leaf bases (also known as leaf "cushions") with a small leaf scar at the apex of the elevated cushion: the point of attachment of abscised leaves.

❑Within the leaf scar, there are three other scars: The middle scar representing the leaf's vascular trace, flanked by two parichnos scars.

❑**Parichnos scars mark the position of parichnos tissue** (parenchymatous with lots of air space between cells) **within the leaf and stem which aided photosynthetic gas exchange much like the lenticels of modern trees (e.g., *Betula*).**

❑**Above the leaf scar, the position of the ligule can be noted which is marked by a small pit known as ligule pit.**

Lepidodendron: Diamond shaped, taller than width, spirally arranged

Lepidophloios: Diamond shaped, wider than height, spirally arranged

Paralycopodites: Shape intermediate

Sigillaria: square or rectangular, with hexagonal leaf scars, vertically arranged

Anatomical feature of Stem Genera

❖An exarch siphonostele surrounded by secondary xylem.

❖Primary xylem is composed of metaxylem tracheids (scalariform) and have fimbrils traversing the apertures between adjacent wall thickenings.

❖The small protoxylem tracheids form vertical ridges at the periphery of the primary xylem cylinder. In cross section, the ridges appear as undulations or small radiating teeth of protoxylem, named the structure as corona.

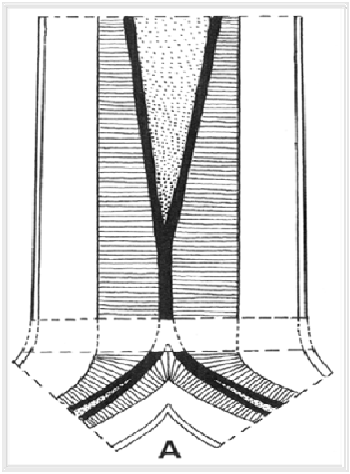
❖The secondary xylem was produced by a unifacial vascular cambium. Unlike the vascular cambium of arborescent seed plants, the vascular cambium of a lepidodendrid did not produce secondary phloem.

❖A continuous band of cortical parenchyma immediately outside the secondary xylem.

❖Departing leaf traces and strands of primary phloem are embeddedin this tissue.

❖Cells of the cortical parenchyma can assume a meristematic function to produce cells of secondary cortex or the so called periderm. One of the distinctive features of this stem is the production of copius amount of this tissue, play major role for mechanical support of the plants.

Epidogenesis



The primary vascular system at the base of a mature tree was protostelic

The primary cylinder became medullated, with the resulting pith increasing in size at higher levels in the trunk.

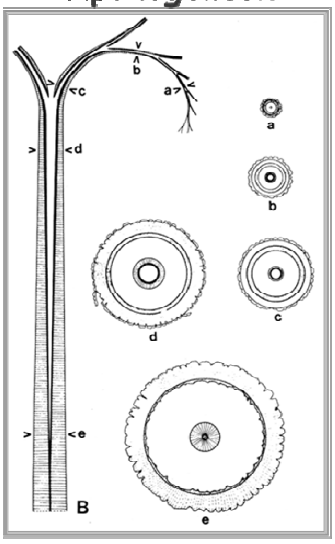
This aspect of development, where there is progressive enlargement and increase in complexity of the primary vascular system, has been termed epidogenesis.

Where dichotomous branching occurred in the aerial axes, it is apparent that the resulting branches were smaller than the axis from which they are formed. Thus, with each successive dichotomy, there was an ever-decreasing size of branches.

This is reflected in a decrease in size of the stele so that in penultimate branches, the vascular cylinder is only slightly medullated while in the ultimate branches only a tiny protostele remains.

Concomitant with the diminution of the primary vascular system was a decrease in the amount of secondary xylem and secondary cortex. This type of development, which involves the production of progressively smaller and simpler anatomy, is called apoxogenesis.

Apoxogenesis



9. Early spore producing trees to early seed producing trees (gymnosperms): From isospores to free sporing heterospores, origin of ovule, hydrasperman reproduction with fossil evidences.

10. Origin and evolution of flowering plants (angiosperms): Geologic time, evolutionary trends -angiosperm derived characteristics, fossil evidences for early flowering plants, place of origin, radiation, phylogeny

Division: Tracheophyta			Age of Occurrence	Significant Characters
Class- Spermatopsida	Subclass- Angiospermae		Early Cretaceous - Recent	Ovules are enclosed in carpels, within a flower, and fertilization is double
	Subclass - Gymnospermae	Gnetales	Triassic - Recent	Leaves opposite each other, and vessels in the wood; male and female cones are flower like
		Coniferales	Mississippian - Recent	Trees with resin canals, and needle or scale like leaves
		Ginkgoales	Late Triassic - Recent	Trees with seed bearing shoots and with fan shaped or more divided leaves
		Cycadales	Mississippian - Recent	Bushy to tree like plants with leaf traces that girdle the stem; frond like leaves; seeds attach to a megasporophyll stalk below a leaf like structure
		Bennettitales	Late Triassic-Late Cretaceous	Bushy to tree like plants with sterile scales between the seeds; frond like leaves; flower like cones with enclosing structures that surrounds ovules and pollen sacs
		Medullosales	Mississippian - Permian	Primitive seed plants with large pollen grains and unusual stem anatomy
Class- Progymnospermopsida			Late Devonian	Plants with gymnosperms like wood but free sporing (fern like) reproduction
Class-Filicopsida			Mid Devonian - Recent	Ferns, flat leaves which uncurl as they develop; sporangia are grouped in clusters usually on the underside of leaves
Class-Equisetopsida			Late Devonian - Recent	Vertical stems with jointed structure and a whorl of fused leaves at the nodes, sporangia grouped in cones
Class-Lycophyta			Late Silurian - Recent	Small to large plants with lateral sporangia and small leaves
Class-Rhyniopsida			Mid Silurian – Early Devonian	Simple vascular plants with dichotomously branching stems and terminal sporangia

Indian Gondwana system: Indian Gondwana sequence, classification and megafloristics assemblages in Gondwana sequence

Indian Gondwana sequence

World's earliest forests appeared during Permo-Carboniferous Periods that is ~345-245 million years ago and those forests of the geological past eventually transformed into the coal deposits of the modern world. Depending on the typical floral assemblages of the earliest forests, four distinct palaeo-phytoprovinces were categorized which are 1. Euramerian phytoprovince, 2. Angara phytoprovince, 3. Cathaysian phytoprovince and 4. Gondwana phytoprovince and the corresponding floral assemblages are Lepidodendron, Angara, Cathaysian and Glossopteris respectively. The first three phytoprovinces (Euramarian, Angara, Cahaysian) were placed at the equatorial belt of the Northern Hemisphere continents whereas the Gondwana province extended at the higher latitudes of the Southern Hemisphere region.

The term 'Gondwana' or 'Gondwanaland' is derived from the ancient territory of the 'Gond' (a tribal community inhabiting the area south of the Narmada River in Central India), from where Medlicott and Oldham, staff of Geological Survey of India, first recognized well exposed distinctive

sedimentary strata during the 1870s (Lele 1964, Maheswari 1992). Some workers applied the term 'Gondwana' only to the region hosting the classical Permian-Cretaceous sequences of India and also employed a broader term 'Gondwanaland' to denote the Southern Hemisphere supercontinent that existed from Palaeozoic to mid-Mesozoic times (Wadia 1957, Metcalfe 1991, Li and Powel 1993). The supercontinent Gondwanaland eventually broke apart by the influence of Plate Tectonics (continental drift) to form the modern continents of Australia, Africa, South America, Madagascar, Indian sub-continent, New Guinea and New Zealand.

Classification and megafloristics assemblages in Indian Gondwana sequence

The earlier studies (Oldham 1893, Cotter 1917, Fox 1931) mostly employed a two-fold subdivision of the Indian Gondwanan strata- the 'Lower' and 'Upper' Gondwanas for Late Palaeozoic (*Glossopteris* bearing) and Mesozoic (*Ptilophyllum* bearing) sequences, respectively. Later on, the *Dicroidium* dominated fossil plant bearing Triassic (a Period under the geological Era Mesozoic) sequence was documented in between *Glossopteris* and *Ptilophyllum* fossil assemblages and accordingly a three-fold subdivision of the Gondwanan sequence was adopted by the workers (Feistmantel 1882, Vredenburg 1910, Wadia 1957, Lele 1964). This three-part scheme remains a useful subdivision of the Indian Upper Carboniferous-Permian to Early Cretaceous succession although geologists and palaeontologists subsequently recognized numerous distinct litho- and bio-stratigraphic units within these sequences (Sukh-Dev 1987, Maheswari 1992).

The dominant member of the Lower Gondwana flora is recognized under a single genus *Glossopteris*, accommodating many species exhibiting wide diversity in their morphological characters which flourished during Upper Carboniferous-Permian for about fifty million years. Origin of the Glossopteris flora occurred during the phases of climatic amelioration in the intermittent interglacial phases. By the end of the Upper Carboniferous glacial phase, the Glossopteris flora emerged as a distinct and dominant vegetation in the Southern Hemisphere continents and Indian subcontinent and gradually started to diversify since Early Permian constituting the earliest forest. In Indian subcontinent, the flora is recorded from the Upper Carboniferous to Permian rocks of extra-Peninsular region whereas from Early Permian to Early Triassic rocks in the Peninsular region.

Studies on the Glossopteris flora started about 200 years ago since the *Glossopteris* leaves were first discovered by Adolph Brongniart (1822, 1828) from the 'Gond' tribe inhabited areas in Central India (now in Madhya Pradesh), India from which the name of the supercontinent Gondwanaland was proposed by Eduard Suess (1855, 1898) although Medlicot (1872) first formally utilized the term Gondwana. Since then, many leaves, stems, roots, fertile organs belonging to glossopterid group are described by many workers from different Lower Gondwana sediments. Besides, different plant remains belonging to bryophytes, pteridophytes, and other gymnosperms are also recovered from the Indian Lower Gondwana sediments in Glossopteris flora. The Triassic aged Middle Gondwana sediments recorded Dicroidium flora comprising with *Lepidopteris, Cladophlebis, Taeniopteris, Cyclopteris, Neomariopteris, Algacites, Hepaticites, Sphagnophyllites, Bosea, Nidpuria* etc. and the Pitlophyllum flora is documented from Upper Gondwana sediments of Late Cretaceous–Jurassic age with the discovery of fossil plants *Williamsonia, Pentoxylon* etc. A comprehensive list of some megafloral assemblages recovered from Indian Gondwana sequences is represented in Table 6.

Table 6: Characteristics megafloral assemblages in Indian Gondwana sequences

Stratigraphic Age	**Megafloral assemblages**		
UPPER GONDWANA			
Early Cretaceous - Jurassic	*Ptilophyllum, Pterophyllum, Pagiophyllum, Elatocladus, Marattiopsis, Brachyphyllum, Araucarioxylon, Hausmannia, Pachypteris, Dictyozamites, Gleichenia, Williamsonia, Weltrichia, Pentoxylon, Carnoconotes, Nipaniophyllum* etc.		
MIDDLE GONDWANA			
Triassic	*Lepidopteris, Cladophlebis, Taeniopteris, Cyclopteris, Neomariopteris, Algacites, Hepaticites, Sphagnophyllites, Bosea, Nidpuria* etc		
LOWER GONDWANA			
Permian - Late Carboniferous	***Bryophytes***	*Dwakea, Buthelezia, Bryothallites, Hepaticites, Talchirophyllites*	
	Pteridophytes	***Lycopsids***	Cyclodendron, Boldihadendron
		Sphenopsids	Phyllotheca, Schizoneura, Raniganjea, Trizygea, Lelstotheca (Stelotheca), Annularia, Notocalamites, Giridia, Gondwanostachys, Tulsidabaria, Rajmahaliyastachys
		Filicopsids	Neomariopteris, Dizeugotheca, Damudopteris, Cuticulatopteris, Searsolia, Botrychiopsis
	Glossopteridales	**Leaf**	*Glossopteris, Gangamopteris, Euryphyllum, Rubidgea, Palaeovittaria, Macrotaeniopteris*
		Root	*Vertebraria*
		Stem	*Araucarioxylon, Dadoxylon, Damudoxylon*
		Fertile organ	*Ottokaria, Dictyopteridium, Scutum, Lidgettonia, Eretmonia, Lanceolatus, Glossotheca, Senotheca, Veekaysinghia, Birbalsahnia*
		Seedling	*Diphyllopteris, Deogharia*
	Cordaitales	*Noeggerathiopsis*	
	Coniferales	*Buriadia, Feruglioclad*us,	
	Ginkgoales	*Ginkgophyton, Psygmophyllum, Rhipidopsis, Polyspermophyllum, Ginkgophyllum*	
	Cycadales	*Pteronilssonia, Pseudoctenis*	
	Incertae sedis	*Sharmastachys, Saharjuria, Chitraphyllum, Santhalitheca, Pachwarophyllum*	

11. Let’s sum up

Carbonate: a salt or ester of carbonic acid (H_2CO_3); carbonates can combine with other elements to form minerals, for example with calcium (calcium carbonate) or with iron (iron carbonate)

Cast: opposite of mold; a preservation type that forms within a mold; casts are usually three-dimensional and therefore preserve form

Chert: a type of rock; chert can form as a primary deposit preserving fossils within it, or replace organic materials in fossils embedded in different rock material

Coal: a general name given to stratified accumulations of carbon-rich material derived from vegetation. The starting point for coal formation is usually peat or some similar accumulation of partially decayed plant matter. By the process of compaction, heating, and chemical alteration, the peat is converted by a series of stages into coal. The type of coalification corresponds to the amount of heating that a peat has undergone:

- Peat
- Lignite (brown coal)
- Bituminous coal
- Anthracite

Coal balls: a type of preservation known from Carboniferous and Permian coal seams, in which plants are preserved by calcium carbonate and other minerals; see petrifaction and permineralization.

Compaction: in sedimentation: the formation of massive rock from loose sediment, normally brought about by the weight of the overlying sediments; in preservation: organic preservation in three dimensions, for example in peat or clay

Compression: a fossil preservation type in which a thin film of organic matter is preserved; compare to impression

Cuticle : an impermeable layer of cutin on the outer walls of epidermal cells

Cutin: the waxy substance of which a cuticle is composed

Fossil: any evidence of prehistoric life; any remains of any once living organism preserved in the Earth's rocks

Lignite: see coal

Midden, packrat midden: midden: garbage or refuse heap; packrats (*Neotoma* sp.) collect and store plant and animal parts in middens; plant material is preserved and cemented together by urine and feces from the packrats; packrat middens are only found from the later part of the Pleistocene through the present, but their fossils have been instrumental in reconstructing Pleistocene and Holocene vegetational changes in the North American southwest and have also been important in archeological studies

Mold: a three-dimensional preservation type that represents a negative image of the plant; compare to cast

Silicification: a type of fossilization in which silica (SiO_2) infills intercellular spaces (permineralization) or replaces the cell walls (petrifaction)

Silica: SiO_2; occurring in crystalline (quartz), cryptocrystalline (very finely crystalline; crystals are very, very small) (opal) and non-crystalline (chert) forms; one of the most common minerals in the crust of the earth; an important mineral in the process of silification; also see permineralization

12. Suggested Reading

1. Gifford Ernest M. & Foster Adriance S. (1989). Morphology and Evolution of vascular plants. 3rd edn. New York: Freeman Publ.
2. Golenberg, E. M., D. E. Giannasi, M. T. Clegg; C. J. Smiley, M. Durbin; D. Henderson and G. Zurawski, 1990. Chloroplast DNA sequence from a Miocene Magnolia species. Nature 344: 656-658.
3. Kumar, R. 1985 Fundamentals of Historic al geology and stratigraphy, Wiley Eastern Ltd.
4. Meyen, Sergei, V. 1987 Fundamentals of Palaeobotany. Chapman and Hall
5. P. Mcl D. Duff 1994 Holmes' Principles of Physical Geology Ed. volume 4th edition Chapman and Hall
6. Stewart W. N. & Rothwell G. W. (1993). Palaeobotany and the evolution of plants. 2nd edn. Cambridge: Cambridge University Press.
7. Surange, K. R., Lakhanpal, R.N., Bharadwaj, D. C. 1974 Aspects and appraisals of Palaeobotany Edited Volume, Birbal Sahni Institute of Palaeobotany, Lucknow
8. Taylor Thomas N., Taylor Edith L. & Krings Michael. (2009). Palaeobotany: The biology and Evolution of fossil plants. 2nd edn. Elsevier Publication.
9. Willis K. J. & McElwain J. C. (2002). The evolution of plants. 1st edn. New York: Oxford University Press.

13. Assignment

1. What is Palaeobotany?
2. Define Fossil.
3. What is Taphonomy?
4. Enumerate the types of fossils.
5. What is index fossil? Give example.
6. Briefly describe different modes for preservation of fossils.
7. Mention the differences between coal and coal ball.
8. What are the external and internal factors responsible for fossilization?

9. Mention the difference between amber and amberat.
10. What is the difference between ‘plant fossils’ and ‘fossil plant’?
11. How does the naming of fossils occur?
12. Describe any one technique for studying anatomical details of plant fossils.
13. What is peat? How does it analyze for extracting fossil?
14. Mention the time of first appearance of Prokaryotes.
15. Name commercially important organic deposits.
16. Name the dominant plant of Indian Lower Gondwana coal forest.
17. Mention the forces responsible for movement of tectonic plates.
18. State the differences between relative and absolute dating method.
19. Define geological time scale.
20. Which mode of evolution (gradualism or punctuation & stasis) is predominates in the fossil record?
21. How does the fossil plant record fit into either of these two evolutionary theories?
22. Enumerate the pattern of evolutionary change in plant fossil record.
23. What is meant by Turnover-pulse hypothesis?
24. Describe the mechanisms driving the evolutionary change.
25. Describe the causes behind mass extinction events in evolutionary history.

COURSE – BOHCT 2.4
PALAEOBOTANY, PALYNOLOGY AND ANATOMY

Hard Core Theory Paper **Credit: (Groups A+B+C) = 3**

Group – B (PALYNOLOGY)

Content Structure

1. Introduction
2. Course Objective
3. Spore-pollen morphology: units, polarity, symmetry, shape, size, aperture; NPC system for numerical expression of apertural details; harmomegathic mechanism; evolution of aperture types.
4. Sporoderm: stratification & sculptures; LO- analysis; pollen wall evolution.
5. Pollen Wall: Development; Ubisch body; sporopollenin; adaptive significance of pollen wall architecture.
6. Extraexinous wall materials: pollen connecting threads, perine, pollen-kit.
7. Natural spore/pollen traps: types, their implications in floristic & environment reconstruction.
8. Branches of Palynology & their Application: Branches of palynology; palynology in taxonomic & phylogenetic deductions; palynology in academic & applied aspects including melissopalynology, medical palynology, forensic palynology, entomopalynology & copropalynology.
9. Let's sum up
10. Suggested Reading
11. Assignment

1. Introduction

Palynology is the study of plant pollen, spores and certain microscopic plankton organisms (collectively termed palynomorphs) in both living and fossil form. Botanists use living pollen and spores (actuopalynology) in the study of plant relationships and evolution, while geologists (palynologists) may use fossil pollen and spores (paleopalynology) to study past environments, stratigraphy (the analysis of strata or layered rock) historical geology and paleontology. Palynology is a useful tool in many applications, including a survey of atmospheric pollen and spore production and dispersal (aerobiology), in the study of human allergies, the archaeological excavation of shipwrecks, and detailed analysis of animal diets. Entomopalynology is the study of pollen found on the body or in the gut of insects. It is useful for determining insect feeding and migratory habits, especially as it involves economically important insects (as the boll weevil, or earwigs). Forensic palynology, or the use of pollen analysis in the solving of crimes, is used by Crime Scene Investigators around the world.

2. Objectives

It is the branch of biology which has important contribution in both classical and applied aspects which encompassing wide spectrum of life, earth and biomedical science. Due to immense contribution in immerging field of both advanced as well as classical studies it may represent itself as sphere of knowledge of science and technology. Being an interdisciplinary science, it has diverse range of application. By studying this branch of science student get knowledge of -

- Palynotaxonomy and evolutionary studies
- Aerobiology
- Melissopalynology
- Forensic palynology
- Biostratigraphy and geochronology
- Paleopalynology
- Archaeopalynology

3. Spore-pollen morphology: *units, polarity, symmetry, shape, size, aperture*; NPC system for numerical expression of apertural details; Harmomegathic mechanism; Evolution of aperture types

Palynology is the branch of science concerned with the study of pollen grains, spores, and similar palynomorphs, both living and fossil. H. A. Hyde & D. A. Williams (1944) coined the term Palynology but the study of pollen analysis was started in 1916 by E. J. Lennart Von Post (Fig.1). **Pollen grains** are male reproductive unit of the flowering plants and **Spores are** various types of reproductive bodies in non-flowering plants formed by sexual and asexual methods.

Fig. 1: Renowned Palynologists



Spore-pollen morphology: units, polarity, symmetry, shape, size, aperture

UNIT

Pollen unit is the product of microsporogenesis (Fig. 2) through which a single pollen mother cell forms pollen units (Fig. 3A-K) by meiotic division either singly (monad- Fig. 3a) or aggregation of two (dyad- Fig.3B), four (tetrad- Fig. 3C-I), and more (polyad- Fig.3J).

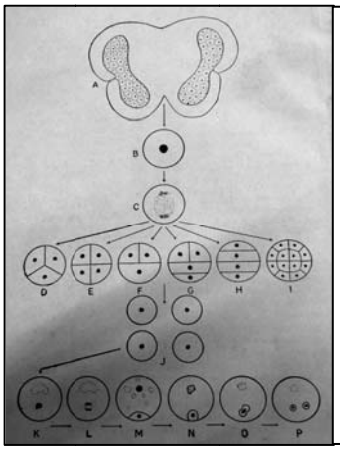


Fig. 2: Microsporogenesis

A. Cross Section of anther showing the pollen sacs with the sporogenous tissue

B. Pollen mother cell

C. Meiosis stage

D. – H. Tetrad type

I. Polvad

Dyads:

Pollen grains which are united in pairs and shed from the anthers as doubles are called dyads (Fig. 3B). Dyads are present in *Scheuchzeria palustris* and other members of Podostemonaceae. The dyads are formed due to the incomplete break up of individual grain or monad.

Tetrads:

Four pollen grains are united to form tetrad. Tetrads are product of non-separation of meiotic cell. Tetrads maybe categorized into different types based on their arrangement (Fig. 3C-I).

Tetrahedral tetrad:

Pollen grains are arranged in two different planes (Fig. 3C). Three grains are in one plane and one lies centrally over the other three. In some cases, the pollen grains are released from the anther in the tetrad condition. These types of tetrads are called obligate or permanent tetrads, namely, *Drymis* (Winteraceae), *Drosera* (Droseraceae), *Rhododendron* Ericaceae).

Tetragonal tetrad:

All the four pollen grains are arranged in one plane (Fig. 3D), as for example, *Typha latifolia* (Typhaceae), *Hedycaria arborea* (Monimiaceae).

Rhomboidal tetrad:

All pollen grains are arranged in one plane forming rhomboidal shape (Fig. 3E) as for example, *Annona muricata* (Annonaceae).

Decussate tetrad:

Pair-wise the pollen grains are at right angle to each other (Fig. 3F), as for example, *Magnolia grandiflora* (Magnoliaceae).

T-Shaped tetrad:

The first division of pollen mother cell is transverse to form a dyad. The upper or lower cell of dyad undergoes a vertical or longitudinal division instead of transverse, yielding either straight or inverted T-shaped configuration (Fig. 3G), as for example, *Aristolochia* sp. (Aristolochiaceae), *Polyanthes* sp; (Amaryllidaceae).

Linear tetrad:

The first division of pollen mother cell is transverse, and a dyad is formed. Each cell of the dyad again divides transversely to form a linear tetrad (Fig. 3H), as for example, *Mimosa pudica*.

Cryptotetrad or Pseudomonad:

Here tetrads are formed without partition walls between the four compartments. One out of the four nuclei develop normally and the rest three obliterate. Thus, an apparent monad but homologous to the tetrad is formed (Fig. 3I), as for example in Cyperaceae.

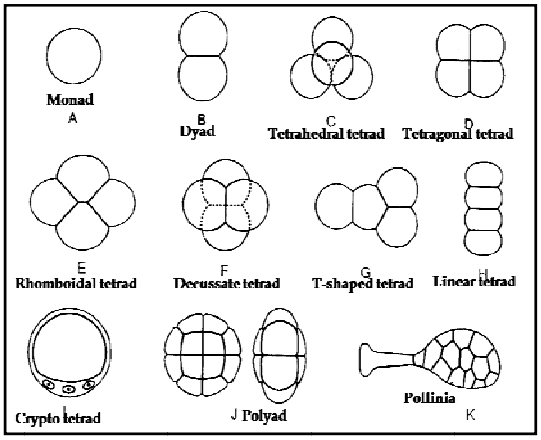
Polyads:

In most of the Mimosaceae members each of the tetrad cells divides once or twice or more, yielding a group of 8 to 64 cells which remain together after maturity. These compound grains are usually held together in small units and are called polyads (Fig. 3J) as for example, *Acacia auriculiformis*, *Adenanthera pavonina*, *Calliandra hematocephalla*, *Samania saman*, *Albizzia lebbeck*.

Pollinia:

In Orchidaceae and Asclepiadaceae the whole contents of an anther or anther locule which shed as one united mass of pollen are called Pollinia (Fig.

Fig. 3: Different types of pollen unit



3K). The pollinium (singular) apparatus is the functional unit of a “corpusculum” with its two attached arms (translator) and Pollinia. as for example, *Calotropis* sp., *Daemia* sp., etc., of the Asclepiadaceae and majority of the family Orchidaceae

POLARITY

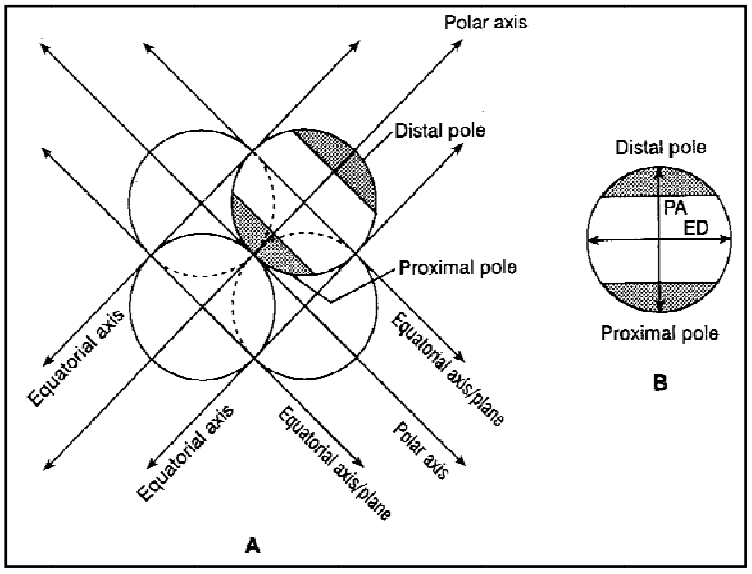
Polarity of individual pollen grains is expressed in terms of their arrangement in the tetrad; end of the grains directed towards the centre of the tetrad is proximal pole & towards the outside is the distal pole. The orientation of polarity is an important criterion in identification and description of pollen grains, as apertural position is of primary phylogenetic and functional significance. All pollen grains are in tetrad stage during development and the polarity is determined in this stage, prior to their separation.

The part of the pollen grains which is nearest to the centre of the tetrad is the proximal pole and that towards the opposite side is the distal pole (Fig. 4). The imaginary line between the proximal and distal pole of the grain is called the Polar Axis (PA) which passes through the centre of the spore to the centre of the tetrad.

The plane perpendicular to the polar axis through the middle of the grain is the equatorial plane (equatorial diameter). Positions on the surface of the grain may be determined by their latitude comparing to the latitude on a regular sphere. Similarly, surface features in a pole to pole direction at right angles to the equatorial plane are called meridional.

Fig. 4: Polarity in tetrad and monad

A= Polarity in tetrad stage; B= Length of polar axis (PA): *hypothetical line connecting two poles; perpendicular to it is the equatorial axis;* ED: Breadth of equatorial diameter in a monad



The pollen grains maybe either apolar or polar. In apolar spores, poles or Polar Regions cannot be distinguished in individual spore (monad) after separation from tetrad. Among the polar types the pollen grains are either isopolar or heteropolar depending upon the demarcation between two equal or unequal polar faces, respectively (Fig. 5)

Apolar spore: In individual spore, poles or Polar Regions cannot distinguished after separation from tetrad stage

Isopolar grain: If equatorial axis demarcates two equal polar faces

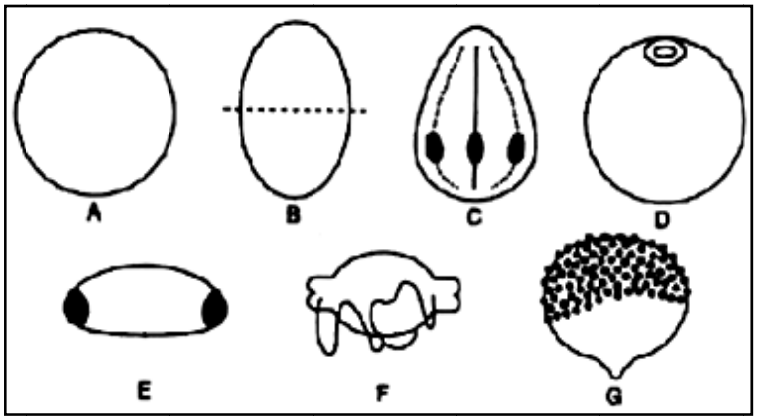
Paraisopolar or subisopolar: slight differences exist between the distal and proximal faces for example *Carya, Ulmus*, etc.

Heteropolar grain: If equatorial axis demarcates two unequal polar faces so that two faces are distinctly different either in shape, ornamentation of apertural system

Cryptopolar: Distal and proximal faces have dissimilar sculpturing and lack tetrad mark for example *Calobryum dentatum, Haplomitrium hookeri*

Fig. 5: Different types of Polarity

A. Apolar; B. Isopolar; C & D. Heteropolar; E & F. Paraisopolar; G. Cryptopolar



SYMMETRY

Pollen grains or spores may be of symmetric or asymmetric (Fig. 6).

Asymmetric grain: It has no plane of symmetry and it is rare in occurrence.

Nonfixiform: not having fixed shape

Fixiform: having fixed shape

Symmetric grain:

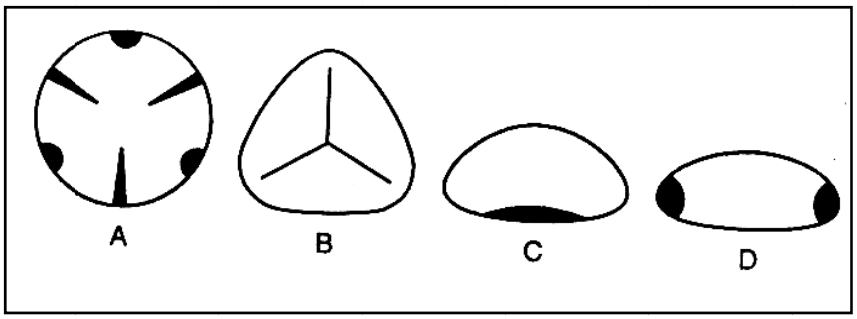
Radiosymmetric- having a radial symmetry

Bilateral symmetry-having a single plane of symmetry

In radiosymmetric grain the shape is such that any plane including the polar axis that passes through will produce identical halves. So the radiosymmetric grains have more than two vertical planes of symmetry. Radial symmetric isopolar grains have one horizontal and two or more vertical planes of symmetry. Radial symmetric heteropolar grains have no horizontal plane of symmetry. Bilateral heteropolar pollen grains have two vertical planes of symmetry. Bilateral isopolar grains have three planes of symmetry, one horizontal and two vertical. In some bean- shaped or boat-shaped spore/pollen there is only one vertical plane of symmetry with an opening towards the end of the grain.

Fig. 6: Pollen Symmetry

A. & B. Radial symmetry; C. & D. Bilateral symmetry

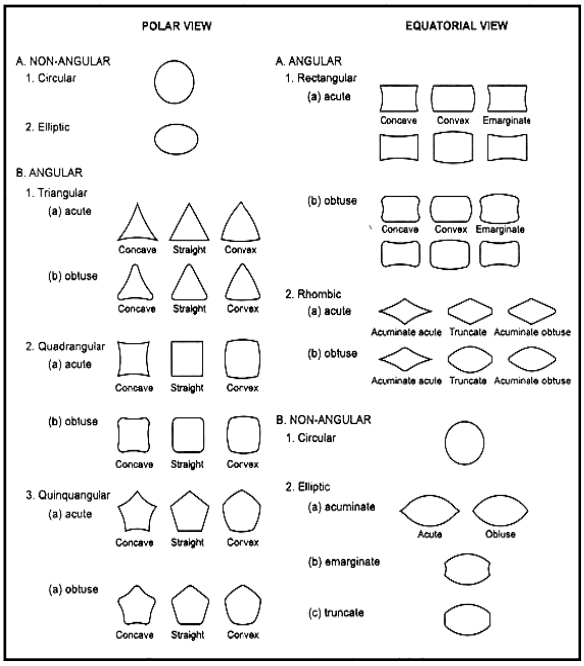


SHAPE

The shape of the pollen grains varies from species to species. Shape of the grains is found to be useful in spore/pollen identification. However, the shape may vary considerably within one grain type or even within one species.

Pollen grains and spores are often described by the shape (non-angular and angular) of their outline both in polar and equatorial views. The shape of the pollen/spores may be circular, elliptical, triangular, rectangular, and quadrangular or in other geometrical shapes (Fig. 7).

Fig. 7: Shape of grains in polar and equatorial views

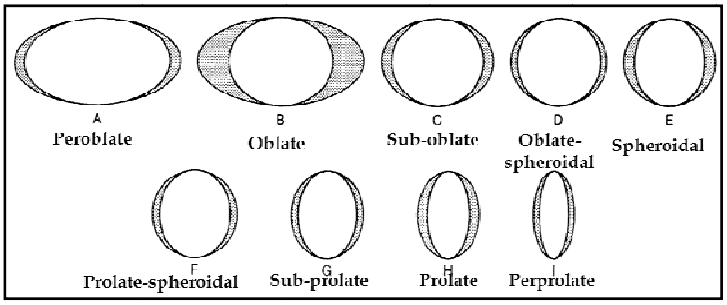


Determination of Shape: Various shape classes (Table 1; Fig. 8) can be determined by calculating the ratio between the length of polar axis (PA) and equatorial diameter (ED) multiplied by 100. In bilateral grains, pollen grains are plano-convex, concavo-convex or biconvex in lateral view.

Table 1: Shape class

Shape Class	**(PA/ED) X 100**
Per-oblate	<50
Oblate	50-75
Sub-oblate	75-88
Oblate-Spheroidal	88-99
Spheroidal	100
Prolate-Spheroidal	101-114
Sub-prolate	115-133
Prolate	134-200
Per-prolate	>200

Fig. 8: Different shape classed as categorized by PA/ED ratio



SIZE

Pollen grains show a great variety in their sizes. Smallest pollen grains of about 5 x 2.4 µm is noted in *Myosotis palustris* and some members of Boraginaceae, while the largest pollen grains (> 200 µm in diameter) are observed in Curcurbitaceae, Nyctaginaceae and *Orectanthe ptaritepuiane* (Abolbodaceae). In taking measurements of size the length of polar axis (PA), equatorial diameter (ED) and sometimes equatorial breadth (EB) are considered in bilateral grains.

In radial symmetric pollen grains the PA and the greatest ED can be measured in equatorial view, while the EB can be measured in polar view only. It is also necessary to measure exine elements, taking into consideration the thickness of exine, sexine/nexine thickness ratio and the thickness of the exine projections greater than 0.5 µm if any.

Erdtman (1945) categorized the different pollen size classes based on the size expressed as length of the longest axis (Table 2).

Table 2: Pollen size class

Pollen size class	**Length of longest axis (in um)**
Very small grain	<10
Small grain	10-25
Medium sized grain	26-50
Large grain	51-100
Very large grain	101-200
Gigantic grain	>200

APERTURE

Aperture is a specialized region of the sporoderm that is thinner than the remainder of the sporoderm and generally differs in ornamentation and/or in structure. Morphologically aperture is an opening or thinning of the exine where the in tine is usually thick; physiologically it is a germination zone or a harmomegathus (A mechanism accommodating changes in volume of the semi-rigid pollen exine) or both.

Regarding their position, the apertures are polar, global or equatorial. The polar apertures are either monopolar (either in proximal or in distal pole) or bipolar (both in proximal and distal face). Global apertures are uniformly distributed over the pollen/spore surface. Equatorial apertures are meridionally arranged.

Some taxa have ‘atreme’ (trema, a Greek word means aperture) pollen/spore, i.e., they seem to have no special aperture, are termed as ‘inaperturate’ or non-aperturate.

Majority of the pollen grains described as ‘inaperturate’ seem to be ‘omniaperturate’, that is, the entire pollen wall is made up of a thin exine and a thick intine or at least thick as the exine, for example *Canna* sp. of Cannaceae. There are two types of apertures known as Pores (Porus, in plural Pori) and furrows (Colpus, in plural Colpi. or Sulcus, in plural Sulci). In most cases the furrows act as harmomegathi.

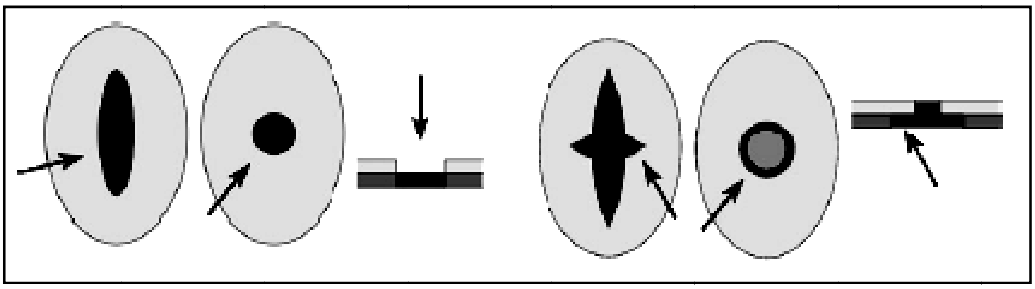
In living pollen grains or spores the apertures usually function as

- sites of germination;
- they may also provide routes for transfer of water and other substances;
- play a part in harmomegathy

Aperture types

The term is often used in conjunction with a prefix or suffix, as for example in, ectoaperture, endoaperture, inaperturate, omniaperturate, pseudoaperture, triaperturate. Depending upon the position of the aperture on the pollen wall it is categorized as simple and compound aperture (Fig. 9)

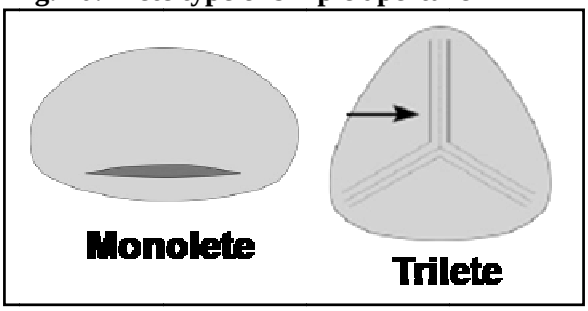
Fig. 9: Position of aperture on pollen wall



Simple Aperture: Apertures are described as simple if they are present in only one wall layer. Different types of simple apertures (Fig. 10, 11) are presented below-

Lete: Slit like; present on proximal end; depending on the number of lete it is either monolete (1 lete) or trilete (3 letes). These types of apertures are found in spores of lower group of plants like pteridophyte.

Fig. 10: Lete type of simple aperture



Other various types of simple apertures are present on pollen grains of plants belong to gymnosperms and angiosperms.

Porus: circular or elliptic aperture with a length/breadth ratio less than 2

Ulcus: A rounded ectoaperture situated at the distal or proximal pole of a pollen grain. Examples: *Sparganium (Sparganiaceae), Typha* (Typhaceae). Pollen grains are called ulcerate.

Ulculus: A rounded ectoaperture which is not situated at a pole. Example: Poaceae.

Colpus: An elongated, aperture with a length/breadth ratio greater than 2.

Sulcus: An elongated latitudinal ectoaperture situated at the distal or proximal pole of a pollen grain. A sulcus has the same shape as a colpus, but differs in orientation. Sulci are essentially latitudinal apertures whereas colpi are essentially longitudinal apertures.

Trichotomosulcate: pollen grain with a three-armed sulcus. Example: some Palmae

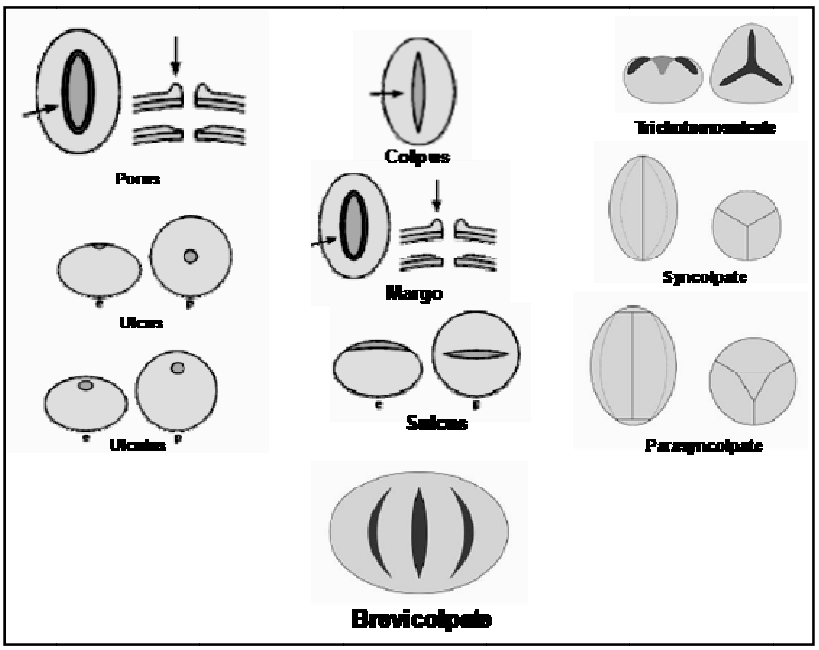
Syncolpate: pollen grain with two or more simple colpi the ends of which anastomose at the pole. Example: *Primula farinosa*

Parasyncolpate: syncolpate pollen grains in which the apices of the colpi divide into two branches and anastomose towards the poles, delimiting an isolated area known as the apocolpial field. Examples: *Nymphoides peltata*

Brevicolpate: brevi is a prefix for short; it is pollen grain with shorter length of colpi.

Breviaxial: pollen grains with a polar axis that is shorter than their equatorial diameter.

Fig. 11: Different types of simple apertures present in pollen grains

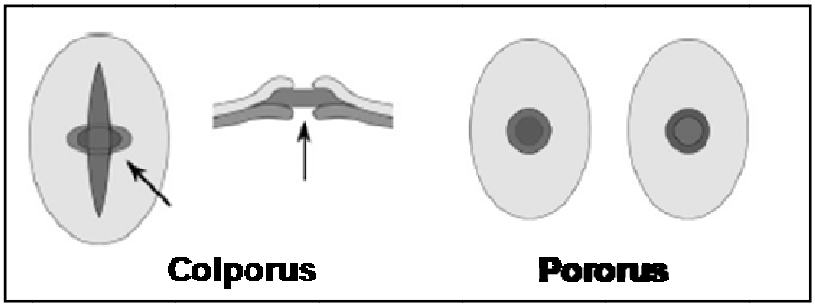


Compound Aperture: Compound aperture is combination of two simple apertures present both at ektexine- (ektoaperture-Colpi or pore) and endexine (endoaperture-ora) so that they affect more than one layer of the wall. In compound apertures the shape of apertures may differ between layers. Ectoapertures occur in the sexine/ectexine, endoapertures in the nexine/endexine, and mesoapertures are sometimes found in an intermediate position between an ecto- and endoaperture. Below the types of compound apertures (Fig. 12)-

Colporus: A compound aperture consisting of an ectocolpus with one or more endoapertures; aperture type colporate; example Asteraceae, Fabaceae etc.

Pororus: pollen grain with compound apertures in which both the ectoaperture and the endoaperture are pores and the two are not congruent; aperture type pororate; *Example: Myrica gale* (Myricaceae) etc.

Fig. 12: Different types of compound apertures



Besides simple and compound apertures sometime other conditions are also found. **Pseudoaperture:** A thinning of the exine which, although superficially resembling an aperture, is not associated with a thickening of the intine and is presumed not to function as an exitus *(the site of exit of the pollen tube from the sporoderm)*

pseudoacolpus- colpus like pseudoaperture;
pseudopore- pore like pseudoaperture

Spiraperturate: It is 'anomotreme' aperture, referred to as N_8 in the NPC classification. Here the spiral apertures are irregularly arranged over the surface of the pollen, irrespective of their number, for example *Thunbergia* (Acanthaceae), *Eriocaulon* (Eriocaulaceae).

Pollen grains with other intermediate types are also noted, and they include:

Synorate or Svnclinorate: Pollen with lalongate ora anastomose latitudinally, for example, *Polygala*, *Solanum*.

Multi orate: Pollen with two or more ora found latitudinally along the length of the colpus, for example, *Congea*, *Viticipremna*.

Colporoidate: Pollen have well developed colpi with weakly developed ora (= Oroids) for example, *Rex canariensis*, *Salix myrsinites*, *Phalline lucida*.

Colpoidorate: Pollen have weakly developed colpi (= Colpoids) with well developed ora, for example, *Alangium villosum*.

Pororoidate: Pollen have well developed pores with weakly developed ora (=Oroids).

Poroidorate: Pollen have weakly developed pores (= Poroids) with well developed ora.

Colpororate: A compound aperture characterized by an ectoaperture, a shorter lolongate mesoaperture and a lalongate endoaperture for example, *Sonchus* (Compositae).

Heterocolpate: Pollen grains have both simple and compound colpi for example, *Lythrum*, *Peplis* (Lythraceae), *Myosotis* (Boraginaceae).

Porocolpate: Pollen grains with an arrangement of apertures in which colpi alternate with pores round the equator, for example, *Pardoglossum* (Boraginaceae).

Edges of apertures

Annulus: A distinct ring-like thickening or thinning of the ektexine bordering the pore is called annulus (Fig. 13A). A halonated area around a pore is found in Poaceae.

Costa: A distinct rib-like thickening of the endexine bordering the aperture is called Costa (Fig. 13B). Costae colpi maybe transverse i.e. Costae – transversales, or, if they are continuous around the equator in uninterrupted, equatorial rings, they are called Costae-equatorials.

Margo (margin): An area of exine around an ectocolpus that is differentiated from the remainder of the sexine, either in ornamentation or by difference in thickness (Fig. 13C).

Operculum: The thick membrane, either of ektexinous, endexinous or both, covering the aperture is called Operculum (Fig. 13D). This may be circular, elliptical, annular or bridge-like. The panto-opercular nature of the pollen may be ascertained by studying the intine thickening under the panto-operculum.

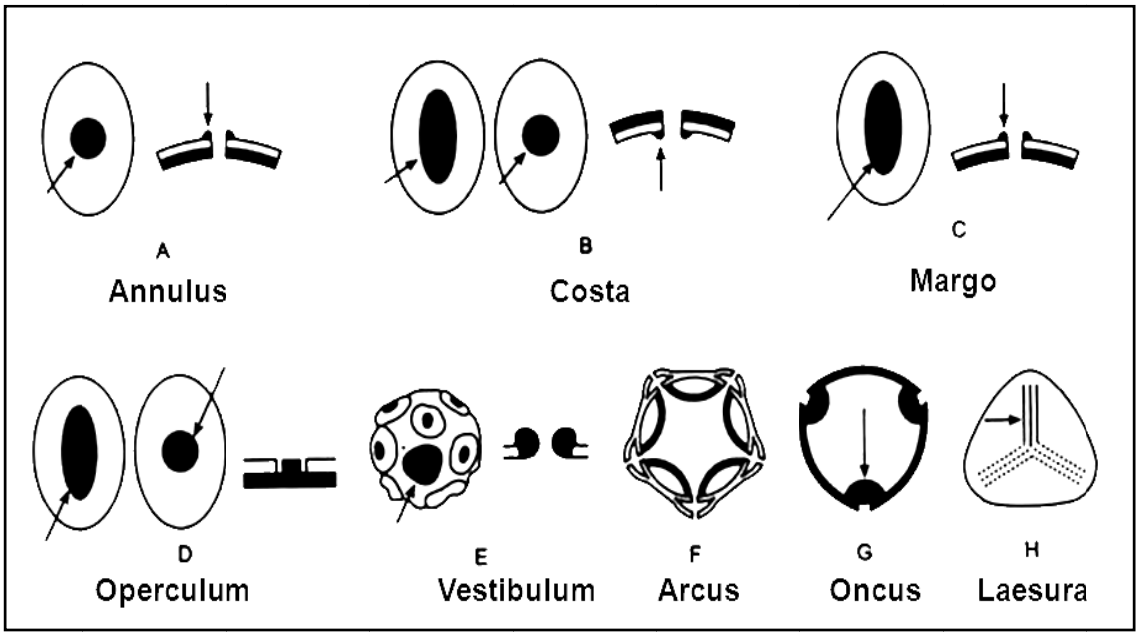
Vestibulum or Aspidote: The apertures or at least their outermost parts are borne on small, more or less circular, shield-shaped areas (aspidote), protruding as round domes from the general surface of the grains for example *Betula*, Dorstenia (Fig. 13E). If the chamber (space) is formed between an outer (ectopore) and inner (endpore) aperture opening, it is called an atrium. Here endopore is much larger than ectopore. In this condition apertures are also referred to as oriferous colpi for example, *Myrica*.

Arcus: In *Alnus*, *Rhoiptelea* pollen grains, band-like locally thickened parts of ektexine extending in a sweeping curve (arcus) from one aperture to another. Pollen having such arcus is referred to as arcuate type (Fig. 13F).

Oncus: Oncus is a lens-shaped structure that is not resistant to acetolysis and occurs beneath the aperture of many kinds of pollen grains (Fig. 13G), for example *Corylus* (Betulaceae).

Laesura (in plural Laesurae): Laesurae (Fig. 13H) are the tetrad scars of moss and fern spores which show the contact of spores with their neighbours in the original tetrad from which they were separated. Laesura may be trilete or monolete. A laesura has a centre suture or commissure which serves the purpose of providing a zone of weakness for rapture upon germination. The separated arms of trilete laesura are called radii, and the terminal ends of radii are forked.

Fig. 14: Edges of apertures

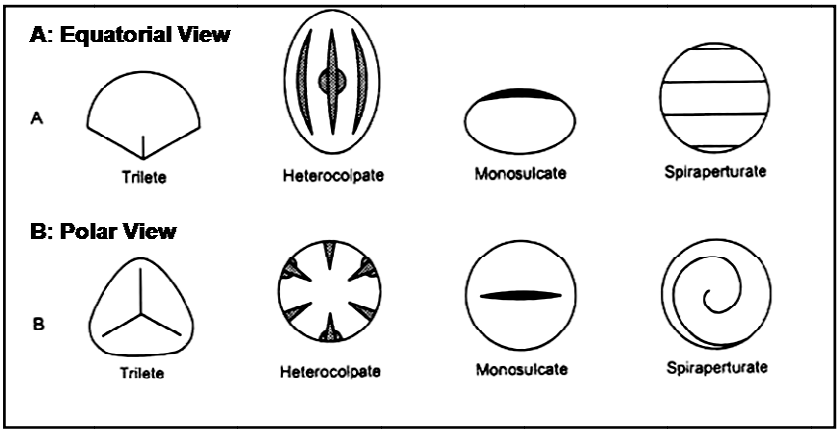


Views of grains with reference to apertures:

Polar view: In this view the pollen/ spores are viewed with one of the poles exactly uppermost i.e. with the polar axis directed straight towards the observer (Fig. 15). In polar view lete/sulcus/ulcus type of apertures will be observed centrally as they are situated at polar region. The polar axis will not be viewed here. The zonal apertures like colpi, pore, etc. will be viewed in the circumference of the pollen grains.

Equatorial view: In this view the apertures are arranged meridionally-pole to pole at right angles to the equator (Fig. 15).

Fig. 15: Views of grains with reference to apertures



Both polar axis and equatorial diameter will be viewed, and they can be measured in this view.

Amb: Outline i.e. contour or circumference of the grain in polar view is called amb (ambit, L. ambitus) which is viewed with one of the poles exactly uppermost directed towards the observer. In isopolar, equatorially non-constricted grain amb is as same as equator, while in equatorially constricted grain amb does not coincide with equator. Amb of radiosymmetric pollen grains are of different types based on the nature of circumference and arrangement of apertures (Fig. 16):

Peritreme: Apertures are more or less uniformly distributed along a circular amb for example, Solarium.

Goniotreme: Amb is angular and the apertures lie at the angles of the grain, for example, Myrtaceae, Proteaceae, etc.

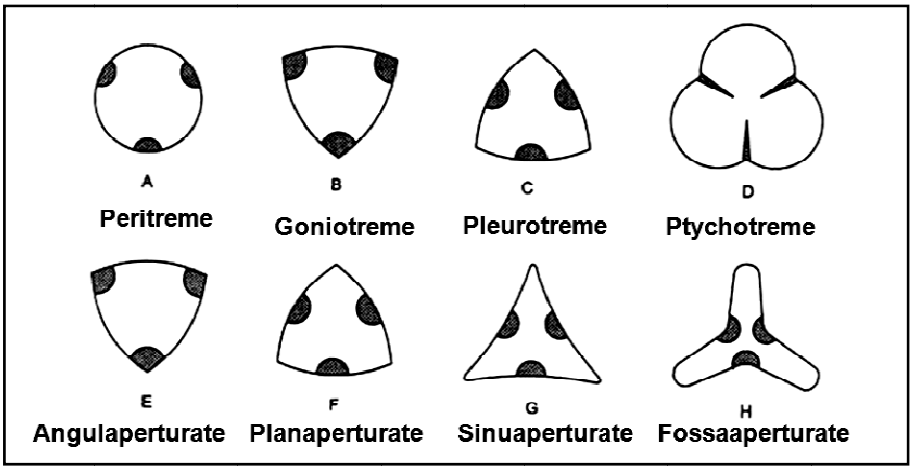
Pleurotreme: Amb is angular like the goniotreme, but the apertures are situated at the mid-points of the sides and the sides are more or less straight, for example, *Bombax*, *Ceiba* of Bombacaceae.

Ptychotreme: The sides of the amb are concave or lobate and the apertures are situated half way between the angles, for example, *Gunnera*.

Pollen grains with equatorial apertures and angular amb may be categorized (Fig. 16) into following types:

i. Angulaperturate: Apertures are situated at the angles of the amb (sides of amb convex, straight or concave) for example, Proteaceae, Olacaceae.

Fig. 16: Different Ambs (Outline that is contour or circumference of the grain in polar view)



Planaperturate: Apertures are situated at the mid-points of the sides of the amb (sides of amb straight), for example, *Bombax*.

iii. Sinuaperturate: Apertures are situated equally halfway between the angles (side of the amb concave).

iv. Fossaperturate: Apertures are situated at the ditch-like indentations between the lobes of lobate amb, for example, *Anomopanax* (Araliaceae).

NPC SYSTEM FOR NUMERICAL EXPRESSION OF APERTURAL DETAILS

Gunner Erdtman (1969) proposed NPC-System pollen/spore classification based on the apertures, their Number (N-whether single or two or many), Position (P- polar: distal or proximal; global; meridional) and Characters (C – circular or elongated) regarding microspore tetrad (Fig. 17). Under this system the term 'treme' (aperture) has been used for preparing keys for the classification of the pollen grains/spores.

The pollen number (N) groups are of nine types. The grain without aperture is named "Atreme" and is designated as No. Depending upon the number of apertures, the types of pollen are Monotreme (N_1) with one aperture, Ditreme (N_2) with two apertures; Tritreme (N_3) with three apertures, Tetratreme

(N_4) with four apertures, Pentatreme (N_5) with five apertures, Hexatreme (N_6) with six apertures and Polytreme (N_7) having more than six apertures. Irregularly arranged spiral apertures over the surface of the pollen irrespective of their number are designated as 'Anomotreme' (N_8).

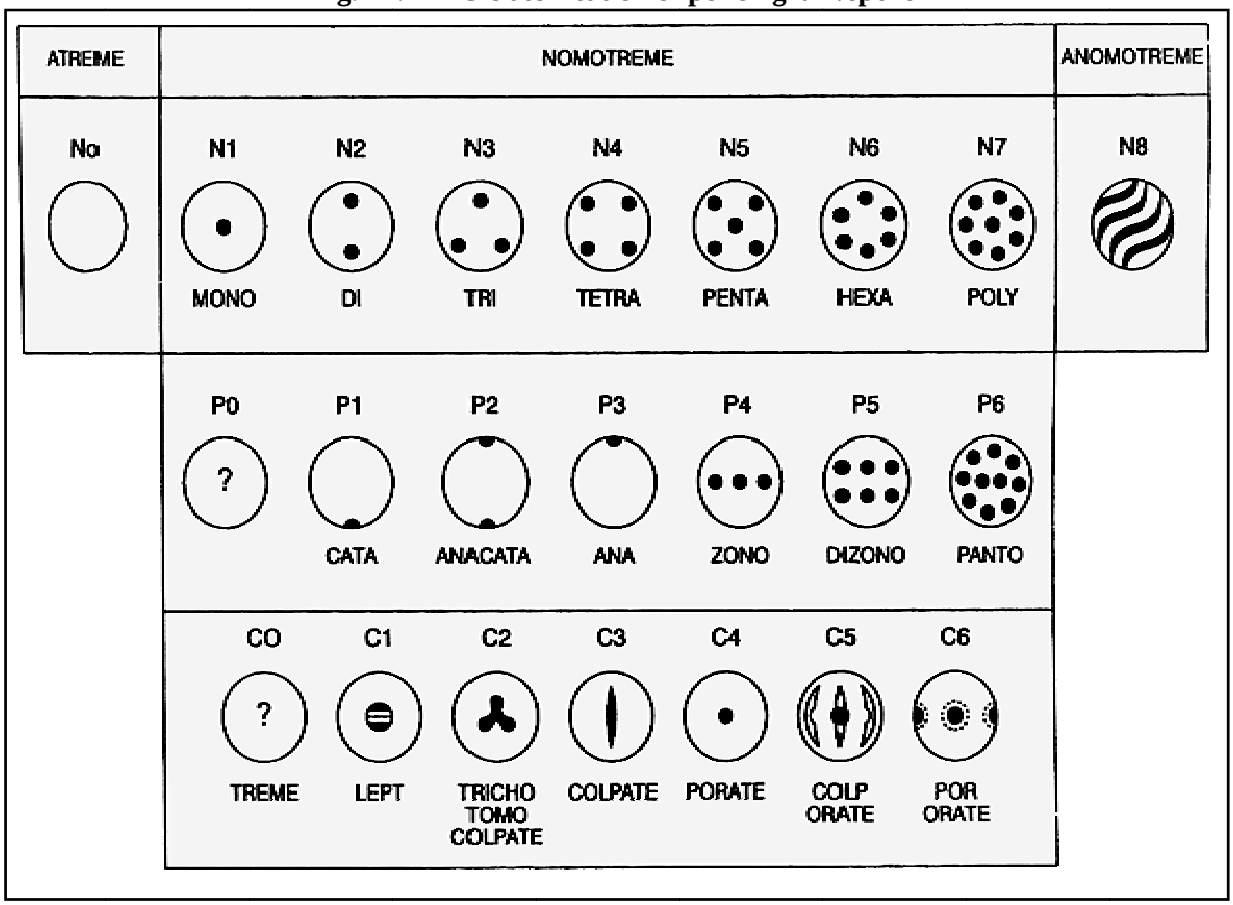
On the basis of the position (P) of apertures, pollen grains are categorized into seven groups (P_0 to P_6). In 'Catatreme' (P_1) pollen aperture is in proximal face, while in 'Anatreme' (P_3) it is in distal face. The pollen grains are designated as Anacatatreme' (P_2) where apertures are both in proximal and distal faces.

The pollen grains are referred to as 'Zonotreme' (P_4), when the apertures are located on the equatorial zone. 'Dizonotreme' (P_5) are like zonotreme, but with two rows of apertures on the equatorial region. In 'Pantotreme' (P_6), apertures are globally distributed all over the pollen surface.

Like position groups the character (C) groups are of seven types ($C_0$to C_6). If the character of the aperture is not known, it is designated as C_0. Pollen having an aperture like thin area or Leptoma is designated as C_1. Pollen with one leptoma is called Monolept, it may be called Cataleft if present in the proximal face, or Analeft if in the distal face.

Pollen with three- slit like colpus are called Trichotomocolpate which belongs to C_2 category. The remaining character classes i.e., C_3, C_4, $C_5$and C_6 include Colpate (with colpa i.e. furrow), Porate (with pore i.e. circular aperture), Colporate (both with colpa and pore/ora apertures), Pororate (aperture with pore and ora) respectively.

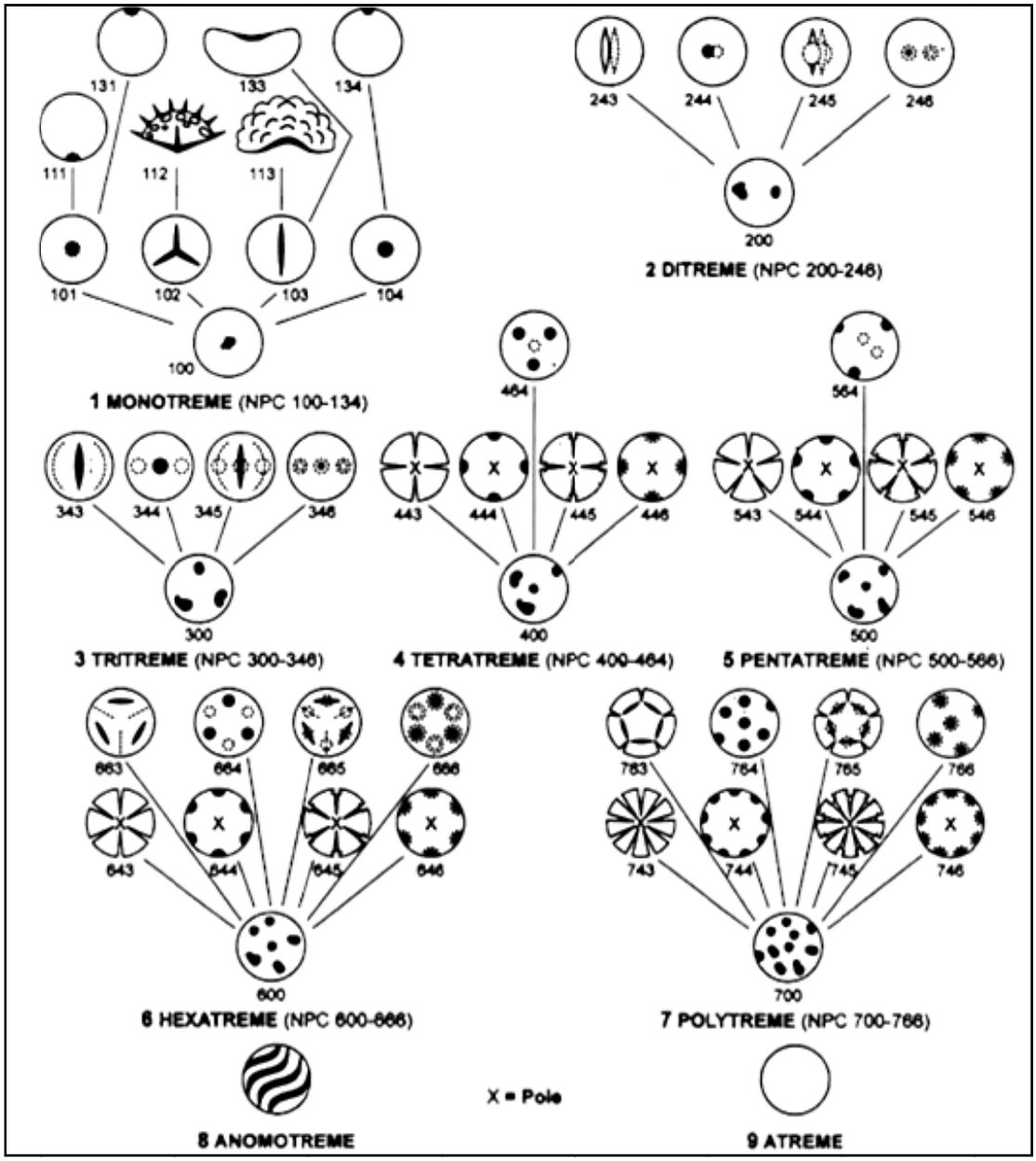
Fig. 17: NPC classification of pollen grain/spore



Based on NPC classification, each pollen type is designated by using a three digit number (Fig. 18). The first digit denotes the number of aperture, for example, 100 is assigned to monotreme, 200 to ditreme, 300 for tritreme, 400 for tetratreme, 500 for pentatreme, 600 for hexatreme, 700 for polytreme, and 8 for anomotreme and 9 for atreme.

The second digit denotes the position of the aperture, e.g. 010 to proximal aperture, 030 for distal aperture, 040 for equatorial aperture, and 060 for global aperture. The third digit denotes the characters of the aperture, e.g., 002 for trilete, 003 for colpate, 004 for porate, 005 for colporate. Therefore, the number 112 is assigned to trilete grains, similarly 133 to monosulcate grains, 343 to tricolpate and 345 to tricolporate grains, etc. (Fig. 18).

Fig. 18: Pollen grain/spore types designated by three-digit numbers



HARMOMEGATHIC MECHANISM

Harmomegathy is one kind of adaptive mechanism associated with the phases of desiccation and rehydration and the contrivances needed for this mechanism help to avoid plasmolysis of the cytoplasm. The term is first described by Wodehouse (1935).

Pollen grains can close off their apertures to avoid losing water in arid environments by a mechanism. It prevents water loss until it arrives in a wetter environment.

Harmomegathus: It is an organ or mechanism which accommodates a semi-rigid exine to change in volume; **Harmomegathy:** it is volume change accommodation.

Different Harmomegathic Mechanisms

- **Invagination of the aperture membrane in colpate, porate and colporate pollen grains**, comparable to meridional folding
- **Extensive invagination in synorate endoapertures**, comparable to equatorial folding
- **Invagination of the Polar Regions** which becomes prominent by the thinning of exine at the polar region or by the **fusion of the apertures at the poles** (syncolpy)
- **Development of striate ornamentation, linear operculum, conduplicate margin and periapertural thinning** to control the direction of folding
- Enhancement of the flexibility of pollen grains by the discontinuity of the sexine layers and by absence or reduction of the nexine to a lamellar condition
- Band like sexine elements that spreads over the aperture membrane (*e.g. Crocus)*
- Development of protruding aperture with oncoid plugs
- Thinning or development of perforations in the proximal part of the dyads, tetrads, polyads to function as a single harmomegathic unit (Asclepiadaceae, Mimosaceae)

Harmomegathic Mechanisms of the Halophytes **(Table 3)**

Genera	Adaptation
Aegialitis	Wide colpi; thin non apertural exine
Avicennia	Wide endo and ecto apertures
Bruguiera, Ceriops, Kandelia, Excoecaria	Equatorially extended large endoaperute
Rhizophora	Endoaperture often fused
Nypa	Meridionosulcus
Heritiera	Thin non apertural nexine
Lumnitzera	Pseudocolpi fused at poles
Sonneratia	Thin non apertural nexine; pseudocolpi sometime present

EVOLUTION OF APERTURE TYPES

Bryophytes, Pteridophytes: Aperture *(either trilete or monolete)* is proximal in position

Primitive extinct gymnosperm like pteridosperm *(*Lyginopterids-*trilete,* Medullosans-*monolete),* Cordaitales *(trilete),* voltziales *(trilete)*: Pre-pollen which is characterized by proximal aperture presuming proximal germination rather than distal as in case of other seed plants.

Extant gymnosperm: either inaperturate or if aperture present, it is in distal position

Angiosperm: along with distal and zonal position, global distribution of apertures is also found which increase the probability of pollen germination in any part of the pollen surface and its adherence to stigmatic surface may accelerate pollen germination

Positional change of aperture from proximal to distal: required to meet up the physiological and ecological needs that occurred due to diversification of new plant taxa.

APERTURE TYPES IN EXTINCT PRE-ANGIOEPERMIC PLANTS

Most primitive trilete spores: Mid. Silurian to Early Devonian vascular plants- *Rhyniales, Zosterophyllales, Trimerophytales, Lepidodendrales, Sphenophyllales, Calamitales, Primofilicales, some Cycadeoids*

Pre-pollen: *Pteridosperms, Cordaitales, Voltziales*

Monosulcate pollen: Bennettitales

APERTURE TYPES IN EXTANT PRE-ANGIOEPERMIC PLANTS

Monolete spore: *Psilotales and some members of Filicales*

Inaperturate spore: *Equisetales*

Monosulcate pollen grains: *Cycadales, Coniferales, Ginkgoales*

As such, pre-angiospermic plants have following morpho-forms:

a. Basic form-trilete, mon-perinous, non-saccate

b. Derived form-i. Trilete, perinuous non-saccate

ii. Trilete, saccate

iii. Monolete, non perinous, non-saccate

iv. Monolete, perinous

v. Monosulcate, saccate

vi. Inaperturate

Perine *(occurs especially in certain pteridophytes. It seems to develop after the exine proper and forms a usual wrinkle, loosely attached covering of the mature spore)* **and saccus** *(occurs in most of the gymnospermous pollen grains; winged like expansion made as a windborne mechanism)* **developed later during the morphological evolution of spore and pollen which indicates**

Origin of monosulcate and inaperturate forms **-** *reduction of the basic structural organization i.e. trilete*

Origin of perine and sacci **-** *modification in form of expansion of the basic structural organization*

Angiospermic Plants: Earliest record is from Lower Cretaceous

Most primitive angiosperms: monosulcate aperture *(pollen grains were named as* ***Clavatipollenites, Retimonocolpites, Liliacidites*** *– these may have originated from a common Mesozoic ancestor Benettitales)*

This monosulcate aperturate condition is distributed among followings:

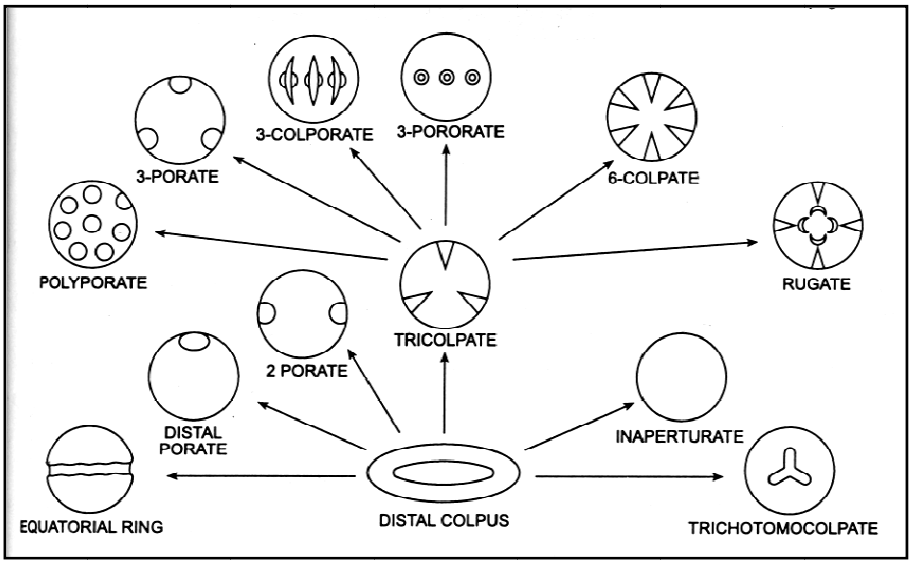
- **Most gymnosperms:** *Cycas* (Cycadales); *Pinus, Abies, Cedrus* (Coniferlales); *Ginkgo* (Gikgoales)
- **Primitive dicotyledoous angiosperms:** *Magnolia, Degeneria* etc.
- **Many monocotyledonous angiosperms:** members of Arecaceae

Distal sulcate aperture then modified to a few other types –

- distal porate (some monocots-poaceae);
- distal trichotomosulcate (both in dicots & monocots);
- equatorial ring like aperture (Nymphaeaceae);
- Inaperturate (by having reduced thin exine and thick intine)

As such, the main trend of evolution of pollen aperture is the transformation of the distal monosulcate (monocolpate) into tricolpate form. Takhtajan (1980) hypothesized (Fig. 19) that tricolpate condition developed because of evolutionary deviation of the primitive stage of sporoderm development and this is derived form of monocolpate condition. Tricolpate type independently given rise to other types namely polycolpate, triporate, pantoporate. Highest stage of evolution of pollen aperture: compound apertures like tricolporate or tripororate etc. are form simple tricolpate type.

Fig. 19: Evolution of apertures in pollen (Takhtajan 1980)



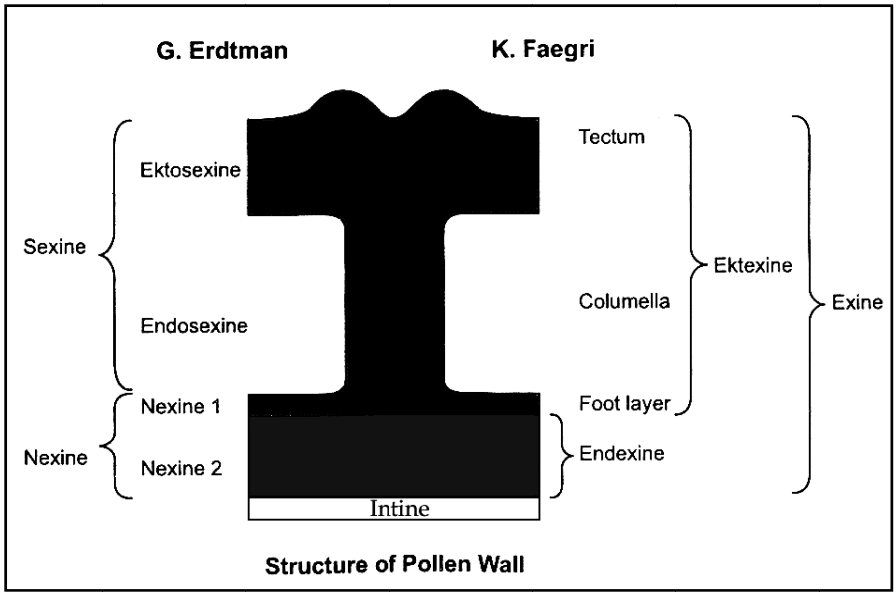
4. Sporoderm: stratification & sculptures; LO- analysis; pollen wall evolution

Sporoderm Stratification:

The pollen wall, the sporoderm is generally stratified i.e. layered (Fig. 20). The walls of the mature pollen, at least in angiosperms, consist of two fundamentally different layers, intine and an outer acetolysis resistant layer exine composed of sporopollenin.

The exine covers the entire pollen surface except germinal apertures where it is absent or greatly reduced. The exine of pollen grains can be divided into an outer sculptured sexine and an inner unsculptured nexine (Fig. 20).

Fig. 20: Sporoderm stratification



Sexine again consists of two layers: the outer, ectosexine and inner, endosexine. The sexine is generally constituted of a set of radially-directed rods supporting a roof-like structure (tectum or tegillum), which may be partially perforated or completely absent.

Rods supporting the tectum are known as columella, and rods not supporting anything but standing vertically on the nexine are called bacula. Columella are usually simple but may be branched. In Compositae the columellae are either distally branched (digitate) or proximally branched (conjunctate) or sometimes the columella hangs down from the tectum, e.g., Caryophyllaceae (Fig. 21).

The nexine has been divided into two layers namely nexine I and nexine II. Knut Faegri (1964) proposed an alternative terminology for exine stratification (Fig. 20). He recognized two layers of exine, the outer ektexine (including sexine and nexine I) and endexine (nexine II). He designated nexine I as foot layer and considered it to be the basal part of ektexine for its identical chemical composition and staining property as that of sexine. Faegri's ektexine is quite different from the endexine because the former contains denser sporopollenin and stains more deeply. The ektexine may be regarded as a three-layered structure in which the granules form small columns, columella, thus dividing an outer tectum and inner foot layer strata. The endexine is often well developed in dicots but is virtually absent or have it only in the apertural region in monocots.

In some pteridophyte spores (e.g. Polypodiaceae) and a few gymnosperms pollen (e.g., *Taxodium*) there is a hyaline loosely organized sporopolleninous envelope called Perine covering the exine (Fig. 22). The perine maybe continuous or sometimes folded in various ways. In some gymnosperms, especially among conifers, the ektexine enlarges to form bladdery wings (Saccus, in plural Sacci) generally two (*Pinus, Cedrus, Abies, Picea* etc.) or one (*Tsuga*) in number.

Types of Pollen in respect to Sporoderm

The pollen grain with a tectum which covers most of the surface of the grain is called tectate. Here the structural elements fused distally forming a continuous roof (Tectum). **Faegri and Iversen (1964) divided tectate grains into three categories (Fig. 21):**

(a) Tectum solidum (unbroken tectum with or without supratectal processes viz., *Betula, Zea*).

(b) Tectum perforatum (perforated tectum, viz., *Stellaria*).

(c) Tectum perfossulatum (fossulated tectum viz., *Saxifraga oppositifolia*)

Pollen grains without an apparent tectum are called intectate (Fig. 21). Here the structural elements are free or absent. Intectate grains may be either (a) without granules (e.g., *Juncus*) or (b) with granules (e.g., *Ilex*). The granules maybe more or less crowded (e.g., *Populus trimula*), or somewhat scattered (e.g., *Callitriche*).

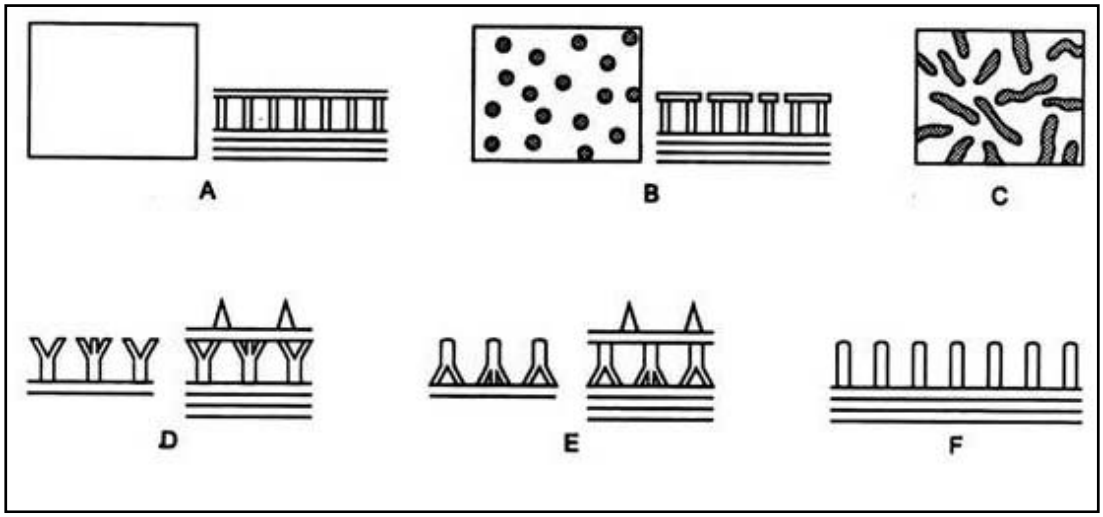
They may be evenly dispersed or form patterns (e.g., *Croton*) with a great variation in their shape, club-shaped type (e.g., *Ilex, Croton* etc.) being the predominant. The graules are generally simple, but occasionally branched and anastomose (e.g., *Geranium*).

Some of the pollen grains are an intermediate between tectate and intectate grains. They are called semitectate or sub- tectate (Fig. 21) in which tectum is not continuous i.e., tectum is partially absent. Here columellae are generally found underneath the fragmentary tectum, but they may also exist as free granules that do not support a tectum.

Semitectate grains maybe (a) Per-reticulate (where structural elements fused distally forming an open reticulum, e.g., *Salix),* (b) Frustillate (synonym of areolate) (where structural elements fused distally forming isolated frustillae, e.g. *Populus balsamifera*).

Fig. 21: Nature of tectum

A: Tectum imperforatum/solidum (Tectate/Eutectate); B: Tectum perforatum (Semi-tectate); C: Tectum perfossulatum; D: Digitate columella; E: Conjunctate; F: Intectate



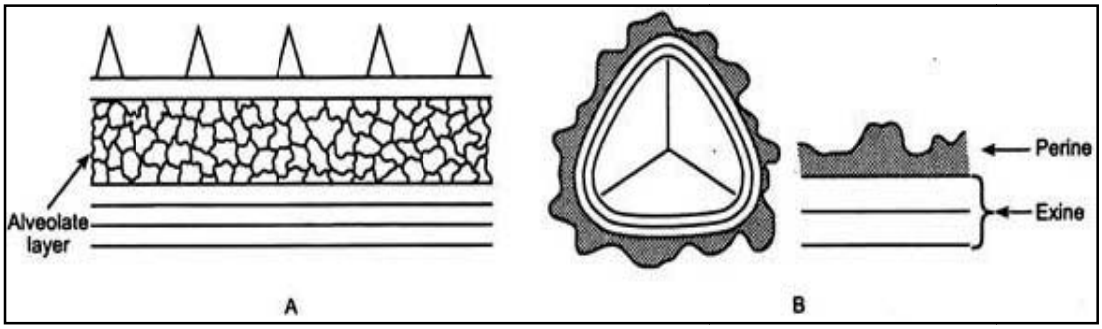
Angiosperm exines show a middle layer of distinct, well-defined columella. Gymnosperm pollen can be distinguished from the angiosperm pollen due to the presence of an irregular spongy, alveolate middle layer instead of columella (Fig. 22). In addition, the endexine of angiosperm pollen is relatively homogenous, whereas in gymnosperm it is typically laminate.

Morphologically the spores of bryophytes and pteridophytes can easily be distinguished from the pollen of gymnosperms and angiosperms. The walls of bryophyte and pteridophyte spores often appear to be laminated throughout their thickness and without having wall stratification (there is no ektexine/endexine division in spore).

The spores have either one long slit-shaped aperture (monolete) or with a trifurcated slit (trilete) forming 'Y' shape. In some pteridophytes a loose outer layer called perine or perispore surrounding the exine is often noticed (Fig. 22).

Fig. 22: Gymnosperm and Pteridophytic sporoderm

A: Alveolate layer of Gymnosperm pollen grain; B: Perine and exine of Pteridophyte spore



Sporoderm Sculpture- Exine Ornamentation

There are two different types of exine ornamentation, the structure or texture and the sculpturing. The structure comprises of all the internal (infratectal) baculae of various form and arrangements. All the ektexine characters belong to the structural features, while the sculpturing comprises external (supratectal) geometric features without reference to their internal construction.

1. Supratectal sculpturing:

There are several external textural modifications of the exine of pollen grains and spores (Fig. 23). The sculpture is a usual feature of the ektexine but may be a perine character. Tectum may be smooth i.e., psilate or with processes or excrescence of various kind like spinules, spines, pila, verruca, gemma, clava, granules, etc. Tectum with the processes is referred to their respective terminology (Fig. 23a, b).

2. Sculpturing on subtectate sexine:

In subtectate or semitectate grains the tectum may be provided with minute perforations having a diameter of 1.0 µm called puncta and the tectum is referred to as punctate (Fig. 23). If the width of such perforation is more and the sexine displays a net-like pattern usually forming a honey-comb like hexagonal meshes, it is called a reticulum (Fig. 23).

Fig. 23a: Supratectal sculpturing *(Gemmate, Clavate, Verrucate, Baculate, Echinate, Spinulose)* and **sculpturing on subtectate sexine** *(Punctate, Reticulate, Striate)*

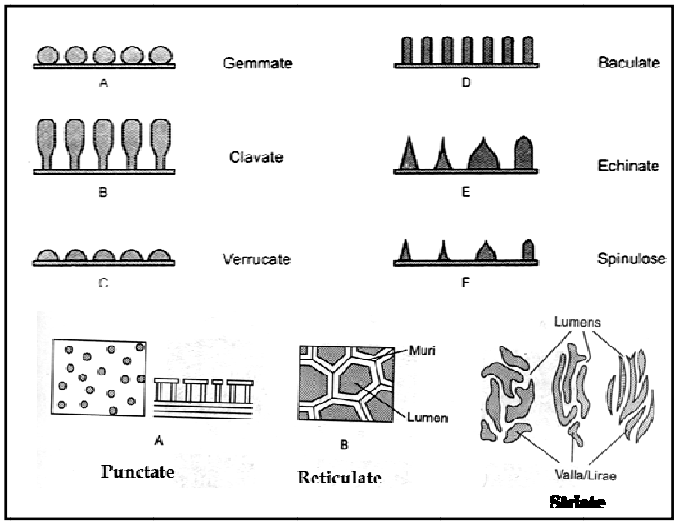
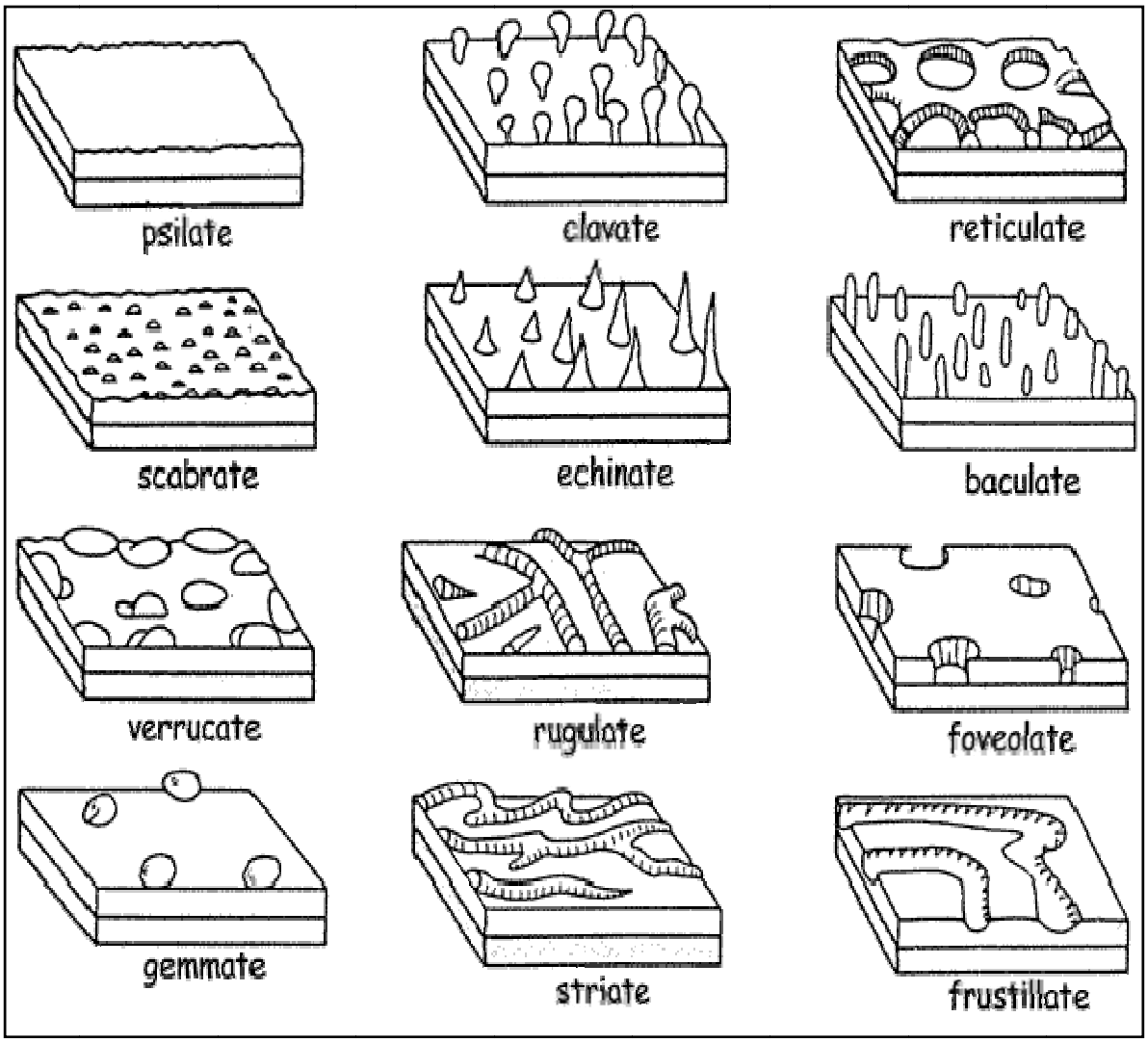


Fig. 23b: Supratectal sculpturing



Faegri and Iverson (1964) proposed a key for identification of various sculpturing types (Table 4). In tectate grains the sculpturing types are given the prefix supra-. So the supra-reticulate grain means a reticulation on the outside of the tectum, while in infra-reticulate grain columellae form a reticulation beneath the tectum.

A typical reticulum comprises of a system of ridges called muri (singular murus), separated by empty roofless interspaces called lumina (Fig. 23). The lumina have the nexine as floor and muri as walls. The muri have an upper tectal part and a lower part consisting of baculae (infratectal) standing between the outer surface of nexine and the lower surface of tectum.

A mesh consists of a lumen and the adjoining half of the muri which separate that lumen from other lumina. A mesh is referred to as brochus (plural brochi). A reticulum may be homobrochate (Fig. 24) (with brochi of the same size) or heterobrochate (Fig. 24) (with brochi of distinctly different sizes).

The muri in which the upper tectal part is supported by a single row of baculae is called simplibaculate (Fig. 24) and the muri supported by two rows of baculae is called duplibaculate (Fig.

24) (e.g., *Avicennia*). In some cases, a negative reticulum is observed where sexine areas are separated by narrow reticulately arranged grooves (Fig. 24).

Table 4: Key to the sculpturing types of pollen grains

A. Sculpturing elements absent	
B. Surface even or diameter of pits <1µm	**Psilate**
BB. Surface pitted, diameter of pits >=1µm	**Foveolate**
BBB. Surface with grooves	**Fossulate**
AA. Sculpturing element present	
B. Radial portion of sculpturing elements ± isodiametric *(sphaerical or polyhedral)*	
C. No dimensions >=1µm	**Scabrate**
CC. At least one dimension >=1µm	
D. Sculpturing elements not pointed	
E. Lower part of element constricted	
F. greatest dia of radial projection is more of equal to height of element	**Gemmate**
FF. height of element is more than greatest dia of projection, club shaped	**Clavate**
EE. Lower part of element not constricted	
F. greatest dia of radial projection >=height of element; wart like	**Verrucate**
FF. height of element >greatest dia meter of projection, cylindrical	**Baculate**
DD. Sculpturing element pointed	
E. Spines long, > 3µm	**Echinate**
EE. Small spines, <3µm in length	**Spinulate**
BB. Radial projection of sculpturing elements elongated	
C. Elements regularly distributed	**Rugulate**
CC. Elements parallel	**Straite**
BBB. Sculpturing elements forming a reticulate pattern	**Reticulate**

Based on the nature of lumina, the surface ornamentations are of different types (Fig. 25) namely, scrobiculate (very small circular, distantly placed lumina separated by sexinous streaks, scrobiculate

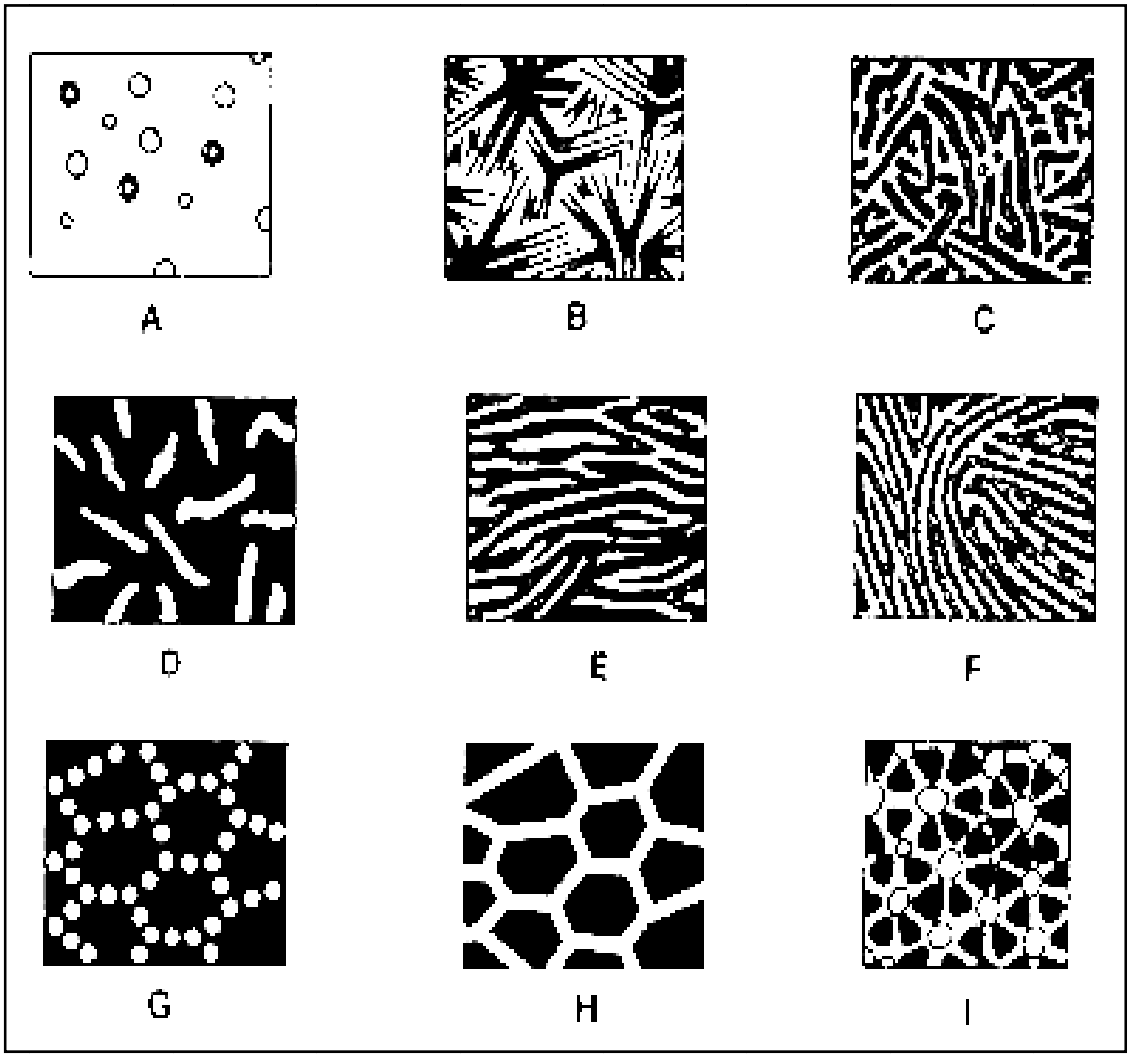
is a synonym of punctate), foveolate (circular, closely placed lumina, diam. of pits 1.0 µm), rugulate (ridges run irregularly, lumina parallel and anastomosing), fossulate (elongated lumina, surface with irregular grooves).

In striate ornamentation, the ridges run parallel instead of forming a network. In this case the ridges are often called lirae (singular lira) or valla (singular vallum) and the streak-like furrows between them are called striae (sing, stria) (Fig. 25). If the lira forms a definite reticulation, the pattern is called striato- reticulate. Elements are very irregularly distributed. If pila instead of muri form a reticulum, then the sexine pattern is called retipilate (Fig. 25).

Crotonoid pattern is a characteristic type of ornamentation comprising of five or six (sometimes more) raised, often triangular, sexine elements arranged around a circular area, usually formed by pila (Fig. 25). For example, *Croton*, *Jatropha* (Euphorbiaceae), *Callitriche antarctica* (Callitrichaceae), *Pimelea arenaria* (Thymelaceae).

Fig. 25: Sculpturing types

A: Foveolate; B & C: Rugulate; D: Fossulate; E & F: Striato-Reticulate; G: Retipilate; H: Negative reticulum; I: Crotonoid pattern



It is observed that two or more grain types show same surface ornamentations (Fig. 26). Say for example, regulate, striate or reticulate can have very different fine structure. One grain type is tectate

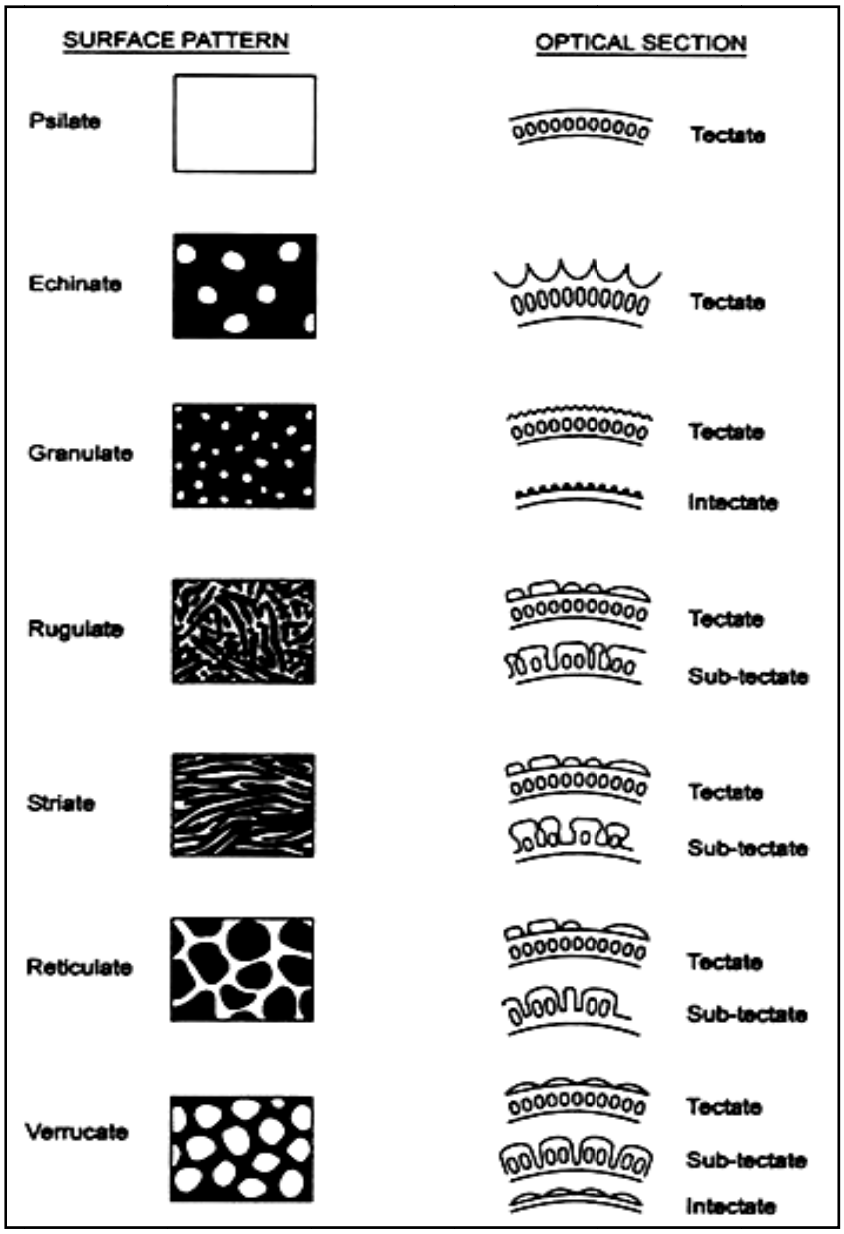
with ornamentation on the top of the tectum, and the other is sub-tectate where surface ornamentations are formed of columellae.

The verrucae sculpturing may be produced by three different exine structures (tectate, semitectate and intectate) (Fig. 26). The optical section would determine the type of the grain (whether tectate or semitectate). The other sculpturing types namely, psilate or echinate are produced only by tectate grains, while granulate type is formed of by two different exine types (tectate and intectate) (Fig 26).

3. Other exine patterns (Fig. 27):

Areolate: An ornamentation feature where the ektexine is composed of circular or polygonal areas separated by grooves which form a negative reticulum. For example, *Phyllanthus*

Fig. 26: Sculpturing pattern visible in surface view and optical section
(all raised areas are shown light and lower areas or holes are shown dark)



(Euphorbiaceae), *Apama* (Aristolochiaceae).

Caveate: In some Compositae like Ambrosia, a cavity is formed between two layers (ektexine and foot layer) of the exine extending to the colpus margin where the layers meet. The cavity is called cavea or cavum and the pollen of such type is termed as caveate or cavate. When cavea is highly developed the pollen grain is said to be vesiculate or saccate.

Lophate: Describing a pollen grain in which the outer exine is raised in a pattern of ridges (lophae) surrounding depression (lacunae). For example, *Taraxacum*, *Hieracium* (Compositae). A lophate pollen grain with echinate (spines) ridges is called echinolophate, while a lophate pollen grain which lacks spines is known as psilolophate.

Fenestrate: Describing a class of pollen grains characterized by large, window-like spaces lacking a tectum. The term is accepted as a category in the classification of Iversen and Troels-Smith (1950) which includes lophate pollen grains. This term is not recommended in descriptions and such pollen grains can be described as lophate.

Halo: A clear zone around a well-defined feature such as spine or an aperture. For example, *Ranunculus* (Ranunculaceae), *Valeriana* (Valerianaceae).

Hamulate: Describing a form of rugulate ornamentation consisting of irregularly arranged, winding, or angular rounded muri of varying thickness, which do not form a distinct reticulum, but rather a maze-like pattern. For example, *Lycopodiella inundata* (Lycopodiaceae). This term is mostly used in spore terminology.

Haploxylonoid (= Haploxylon-type): Describing a bisaccate pollen grain in which the outline of the sacci in polar view is more or less continuous with the outline of the corpus, so that the grains appear a more or less smooth ellipsoidal form. For example, *Pinus*, *Picea* (Pinaceae).

Diploxylonoid (= Sylvestris-type): Describing a bisaccate pollen grains in which the outline of the sacci in polar view is discontinuous with the outline of the corpus, so that the grains seem to consist of three distinct, oval parts.

Metareticulate: A reticulum which is characterised by the consistent presence of one porate aperture in each lumen. For example, *Kallstroemia mexicana* (Zygophyllaceae), *Froelichia floridana* (Amaranthaceae).

Urceolate: Describing a type of ornamentation consisting of urn-shaped elements situated on the foot layer. For example, *Pinanga aristata* (Arecaceae).

Polumbra: A darkened triangular or subcircular area centred on the proximal pole. This feature appears to be most commonly observed in specimens that have lost a perisporal outer exoexinal layer. For example, *Retusotriletes distinctus*.

Bireticulate: A two layered reticulum consisting of a suprareticulum supported by a microreticulate tectum. For example, *Entelea arborescens* (Tiliaceae), *Phyllanthus oppositifolius* (Euphorbiaceae), *Salvia azurea* (Lamiaceae).

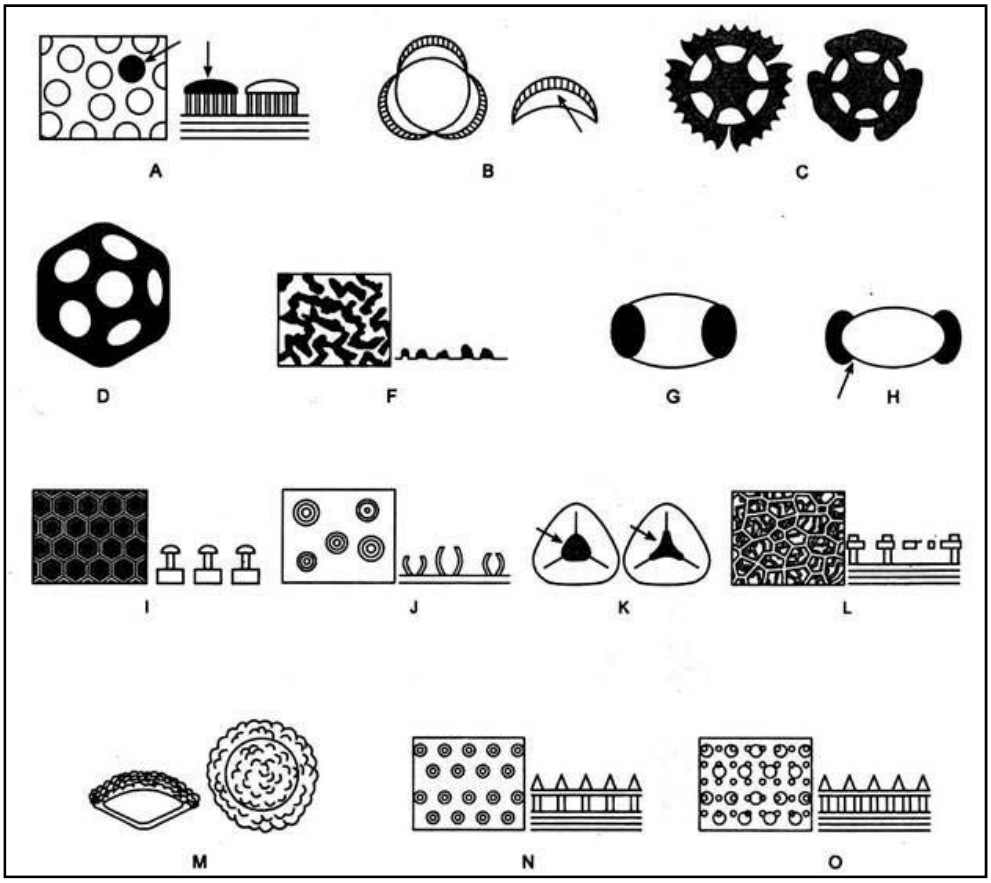
Velum: A feature of a monosaccate pollen grain in which the saccus is convoluted. For example, *Tsuga* (Pinaceae). The pollen having this feature is called velate.

Concordant pattern: A pattern in a tectate pollen grain in which the arrangement of the columellae is the same as that of the elements upon the tectum. For example, *Lilium* (Liliaceae).

Discordant pattern: A pattern in a tectate pollen grain in which the arrangement of the columellae is different from that of the elements on the tectum. For example, *Geranium* (Geraniaceae).

Fig. 27: Other exine pattern

(A: Areolate; B: Caveate; C: Lophate; D: Fenestrate; F: Hamulate; G: Haploxylonoid; H: Diploxylonoid; I: Metareticulate; J: Urceolate; K: Polumbra; L: Bireticulate; M: Velum; N: Concordant pattern; O: Discordant pattern



'LO' Analysis:

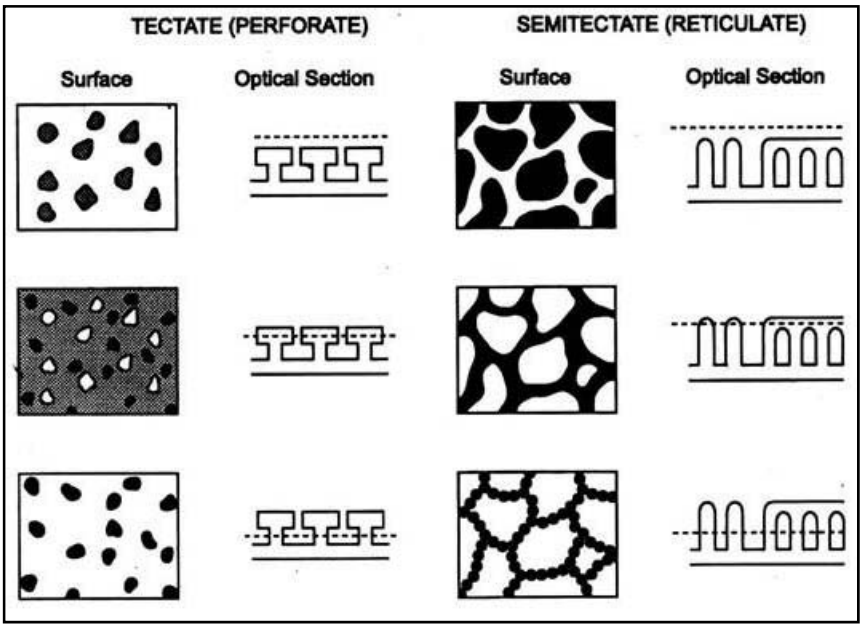
An optical section does not always make the fine structure of the sexine as clear as one might expect. A careful focusing through the sculpturing and patterning presented in a surface view of the grain provide a good deal of information.

Erdtman (1952) proposed the term LO-analysis (derived from two Latin words: lux means light and obscuritas means darkness) which is a method for analysing patterns of sexine organisation by means of light microscopy. This method is valuable for elucidating exine patterns. The surface types show the holes or lower areas to be dark and any raised areas or projecting elements to be light (Fig. 28).

On focusing carefully down through the exine their appearance would change due to a changing that diffraction images produced. For example, when focused at high level, raised sexine elements appear bright, whereas holes in the tectum are relatively dark (Fig. 28).

At lower focus holes become lighter and the sexine elements become darken (Fig. 28). If a reverse sequence occurred i.e. a pattern of ornamentation that appears to show "dark islands" at high focus and that become bright at low focus, it is given the term "OL- pattern". This system works very well if the pollen grains are embedded in such a medium having lower refractive index.

Fig. 28: 'LO' Analysis



Pollen wall evolution

The colonization of land by plants in the Palaeozoic was a highly significant event in Earth's history, both from an evolutionary point of view and because it fundamentally changed the ecology and environment of the planet. Land plants evolved to form crucial components of all modern terrestrial ecosystems through evolutionary adaptations involving changes in anatomy, physiology and life cycle. Key adaptations include rooting structures, conducting tissues, cuticle, stomata, and sex organs such as gametangia and spores/pollen. **Development of a durable spore wall is essential for terrestrialization as it enables the spore to withstand physical abrasion, desiccation and UV-B radiation. As part of their life cycle, sexually reproducing embryophytes manufacture either spores, or their more derived homologues pollen. The major component of the spore/pollen wall proposed to be of primary importance in enabling resistance to the conditions described above is the highly resistant biopolymer sporopollenin.**

It seems reasonable to hypothesize that colonization of the land by plants was not possible prior to the evolution of the sporopollenin spore wall, and this adaptation is considered to be a synapomorphy of the embryophytes. While the basic components associated with spore/ pollen wall development and structure can be localized in different wall regions and influence wall development at different stages across the principal land plant groups, their presence in the majority or all of these groups suggests that their involvement in wall development and sporogenesis as a whole is a signature of embryophytes. Additionally, spore walls are not present in the hypothesized embryophyte antecedents, the green algae. However, the production of sporopollenin is highly likely to be pre-adaptive as it is present in a number of different algal groups such as the charophyceans, which have been proposed as the sister group to the embryophytes. In certain charophyceans, sporopollenin occurs, but is located in an inner layer of the zygote wall.

The main differences among different groups of embryophytes concern the absence, presence and role of callose, and also the mode of sporopollenin deposition. Callose would appear to play a major role in pollen wall development in angiosperms, where a callose wall surrounds the tetrad and serves as a template for exine development. The role of callose in wall development in other groups is less well defined. In some groups such as pteridophytes, with the exception of *Selaginella*, callose has yet to be identified during sporogenesis and is currently thought to be absent. In some bryophytes

(Hepaticopsida: *Anthoceros*, *Geothallus*, *Riccia*; Bryopsida: *Mnium*) callose has been identified around the spore mother cell but its link to wall development, if any, is not well understood. In this instance, it could be the case that callose is relictual as it is involved in the reproductive systems of some algal groups.

Certain modes of sporopollenin deposition are not confined to individual plant groups and different modes have been observed on numerous occasions in a single species. For example, white-line-centred lamellae (WLCL), while not a constant feature, have been observed in species belonging to all phylogenetic levels of land plants. However, the accumulation of sporopollenin within a pre-patterned microspore surface coat, or primexine, is seemingly confined to gymnosperms and angiosperms.

In the spore/pollen walls of embryophytes have multiple layers and components that are laid down in a regulated manner during spore/pollen development. Layers containing the macromolecule sporopollenin are the component enabling the resistance of the spore/pollen wall to numerous environmental factors that make life on land challenging.

Modes of sporopollenin deposition in spore and pollen walls

The basic mechanisms involved in the formation of the spore wall, and the deposition of sporopollenin in the exospore/exine, have been illuminated by numerous ultrastructural studies performed on extant and fossil species across the plant kingdom. A number of sporopollenin deposition processes apparent in the spore wall were proposed by the scientists. Firstly, the accumulation of sporopollenin on an array of white-line-centred lamellae (WLCL) is regarded as being the most primitive method of sporopollenin deposition and has been identified in a number of algal groups and most, if not all, embryophytes. These lamellae materialize at the plasma membrane with sporopollenin polymerizing out onto either side of the white line. They accumulate in a variety of ways to form the spore/pollen wall. Another mode of exospore/exine formation involves the deposition of sporopollenin from the surrounding cells of the tapetum. Transmission electron microscopy has shown that the tapetal cells possess a highly active secretory system containing lipophilic globules, which are thought to contain the precursors of sporopollenin and are deposited onto the surface either directly contributing to the exospore/exine or forming extraexosporal layers. Blackmore et al. (2000) suggested that a tapetal contribution to the spore wall can take place in a variety of ways, including the addition to the layers formed by the WLCL or directly onto WLCL. Studies of pollen wall formation in angiosperms highlight the role that tapetal cells play in supplying nutrients and lipid components to developing microsporocytes and microspores.

Interestingly, the most basal extant land plants (liverworts) lack a tapetum, which is acquired in mosses and vascular plants. An alternative deposition process involves centripetal accumulation of sporopollenin onto previously formed layers. Blackmore et al. (2000) noted that exospore formation may be achieved by sporopollenin accumulation below a pre-existing layer, either by WLCL accumulation or by the deposition of granular or unstructured sporopollenin. A further mode of deposition is observed in seed plants where sporopollenin accumulates within a pre-patterned cell surface glycocalyx referred to as the primexine, which is essentially an exine precursor.

Spore wall development in bryophytes

Spore wall development has been studied in all three of the traditional bryophyte groups. In the majority of liverworts, immediately after meiosis, a polysaccharide wall (the spore special wall) is laid down outside the plasma membrane. In many liverworts, this spore special wall seems to function as a primexine in which the pattern of exospore ornamentation is established. However, in some liverworts exospore ornamentation appears to be determined by exospore precursors produced by the diploid sporocyte prior to meiosis and formation of the haploid spores. The exospore develops centripetally based on WLCL formed outside the spore cytoplasm. At completion, the entire exospore comprises sporopollenin deposited on WLCL.

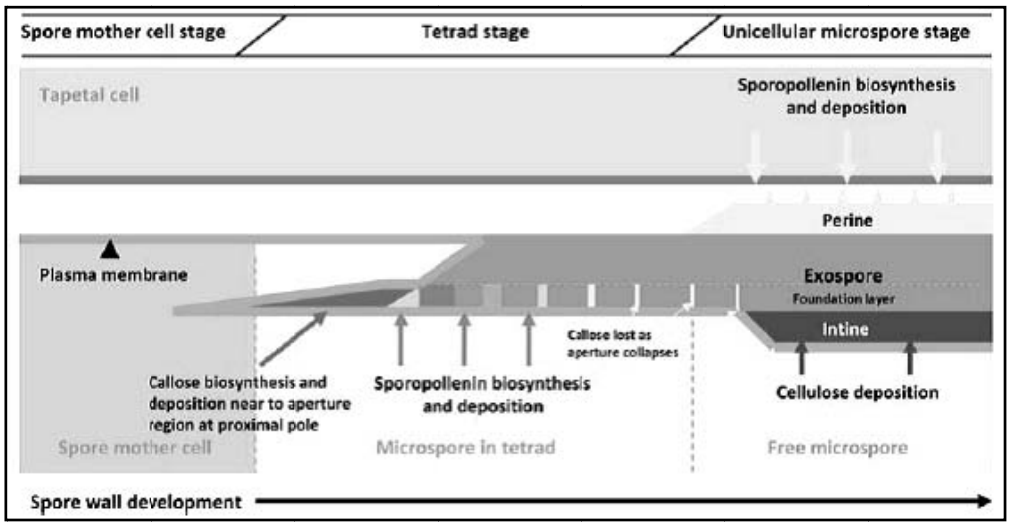
At maturity, the lamellate structure thus formed is clearly discernible and is highly characteristic of the liverwort exospore. Liverworts lack a tapetum and there is therefore no input from this source. The innermost layer of fibrillar intine is the final wall layer to be formed. Studies of spore wall development in hornworts are limited. As with liverworts, a spore special wall is formed after meiosis and functions as a primexine in which the exospore is set down. It was initially thought that the exospore formed in the absence of WLCL, but Taylor and Renzaglia have recently demonstrated their presence.

Recent analyses of *Phaeomegaceros fimbriatus* have shown that the mature spore wall has a thin perine-like outer layer, but this represents the remnants of the spore mother cell wall rather than extra-exosporal material derived from a tapetum. Three types of spore wall have been recognized in mosses:

- Bryopsida type
- Andreaeidae type
- Sphagnidae type

All three of these types appear to form in the absence of a spore special wall. Bryopsida-type spore walls are homogeneous except for an inconspicuous foundation layer (Fig. 29). This foundation layer forms first via sporopollenin accumulation on WLCL. Subsequently, the homogeneous exospore layer is laid down outside the foundation layer in a centrifugal manner. This layer is probably mainly extrasporal in origin. Sometimes additional homogeneous material is also deposited inside the foundation layer. This layer is almost certainly derived from the spore. Following the accumulation of homogeneous material, the spores are coated by an additional extraexosporal layer, referred to as the perine or perispore, which is derived from the tapetum. Finally, the intine forms.

Fig. 29: Proposed model for spore wall development in *Physcomitrella*



Spore wall development in the Andreaeidae type is unique among mosses in that they have a spongy exospore that appears to form in the absence of WLCL. By studying *Andreaea rothii*, it is demonstrated that the exospore is instead initiated as discrete homogeneous globules within the coarsely fibrillar network of the spore mother cell. These globules accumulate and form an irregular layer with numerous interstitial spaces. The sequence of spore wall layer development is essentially the same as that of other mosses and the mature wall consists of an inner intine, a spongy exospore and an outer perine.

Sphagnidae-type moss spore walls are more complex than those of the other mosses and consist of five layers. Unlike other mosses, the exospore of Sphagnidae type comprises two layers: an inner

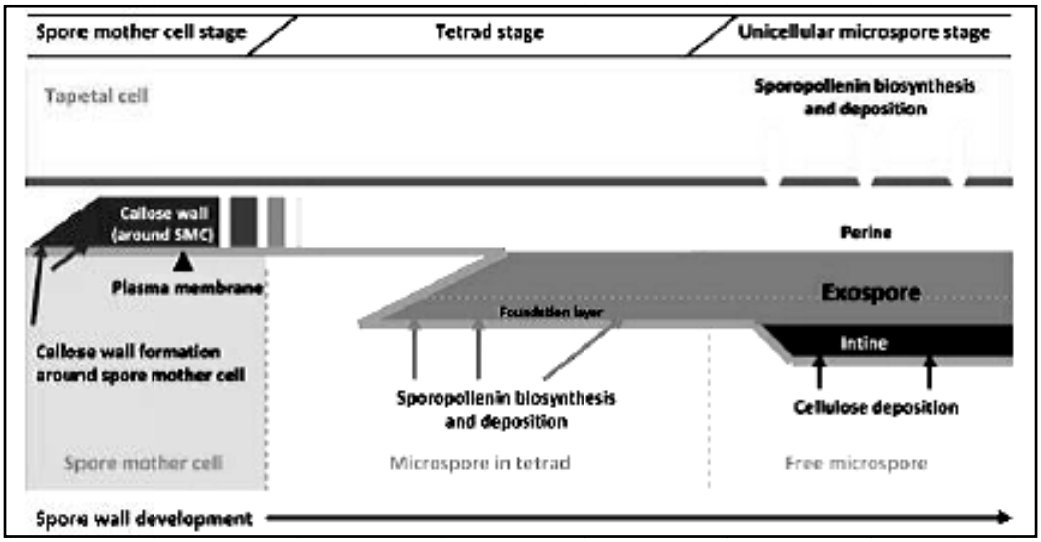
lamellate layer (A-layer) and a thick homogeneous outer layer (B-layer). In addition to the exospore, there is an intine, a unique translucent layer and the outermost perine. The A-layer is the first to form and does so by sporopollenin accumulation on WLCL, and develops evenly around the young spore immediately after meiosis. The homogeneous B-layer is deposited outside the A-layer. Overlying the exospore is a translucent layer that consists of unconsolidated exospore lamellae in a medium of unknown composition. The tapetally derived perine is deposited on top of this unique layer. The study of spore wall development in *Sphagnum lescurii* suggests that the ontogeny of the wall layers is not strictly centripetal.

Spore wall development in pteridophytes

Spore walls have been investigated in a number of pteridophyte species representing all of the major pteridophyte groups. Spore wall development is well understood in the homosporous lycopsid *Lycopodium clavatum*. Shortly after meiosis, the plasma membrane of the sporogenous cell folds into a pattern that later becomes the reticulate spore sculpture. Small WLCL form on the plasma membrane and accumulate in a centripetal fashion, forming the greater part of the exospore. After the main lamellate part of the exospores is formed, an inner granular layer, possibly derived from the spore cytoplasm, is deposited. In some *Lycopodium* there are no extra-exosporal layers whereas in others a thin extra-exosporal layer is deposited after the completion of the exospore. Spore structure and development in heterosporous lycopsids differ between microspores and megaspores.

In the clubmoss *Selaginella*, microspores possess an exospore consisting of two layers (Fig. 30).

Fig. 30: Proposed model of spore wall development in *Selaginella* microspore



The thin inner layer is the first to develop and comprises imbricate lamellae that are formed on WLCL in a centripetal direction. The outer layer starts to form only once the inner layer is complete. Some *Selaginella* species may also develop a thin perispore or a paraexospore. In the microspores of the heterosporous lycopsid *Isoetes japonica*, a large gap is developed between the two exospore layers. The outer exospore layer is regarded as a paraexospore as it begins to form before the inner exospore, consists of similar sporopollenin, and is completed at the same time as the inner exospore. *Selaginella* megaspore walls contain two layers of similar thickness. The inner and outer layers consist of lamellae and poorly segregated components, respectively. The inner layer does not thicken during exospore development and a dense basal layer is formed by the lamellae. In contrast, the outer layer

increases significantly in thickness due to self-assembly. During the final stages of sporogenesis, the endospore forms between the plasma membrane and the exospore. In *Isoetes*, the megaspore wall is similar to that of *Selaginella* in terms of development and structure, consisting of two layers, with the formation of the outer layer commencing prior to that of the inner layer. Substantial quantities of silica are deposited within and on top of the outer layer before the exospore are completed. Finally, the endospore is laid down between the plasma membrane and the exospore.

The exospore in homosporous ferns develops centrifugally and is once again bilayered. The inner layer acts as a substructure and consists of varying numbers of fused sheets (extensive interconnected laminae) that form by sporopollenin accumulation on WLCL. The homogeneous outer layer is considerably thicker and contains thin radial fissures and small cavities. An extra-exosporal layer (perispore) forms once the exospore is complete and is deposited from the decaying tapetum. Spore wall development in heterosporous ferns is similar to that observed in homosporous ferns, and is also similar in both microspores and megaspores. In sphenopsids the spore walls appear to be highly derived, and observations of *Equisetum arvense* have shown that four layers are present in the form of an exospore, an endospore, a middle layer and pseudoelators. The exospores comprise inner and outer exospores. The broad and homogeneous inner exospore forms first by way of plate like structures accumulating on the plasma membrane. The outer exospore is then formed by the deposition of granular material on the inner exospore and is similarly wide and homogeneous. Once exospore formation is complete, the middle layer forms in the gap between the exospore and the plasma membrane. The pseudoelators are the next structure to form and consist of two layers. The inner layer comprises longitudinal microfibrils during the early stages of development but eventually becomes homogeneous. The outer layer is also homogeneous and is formed by granules that are released from vesicles in the plasmodial cytoplasm. The pseudoelators are connected to the spore, by way of the middle layer, at the aperture. The endospore is the final component of the wall to form on the inside of the exospores

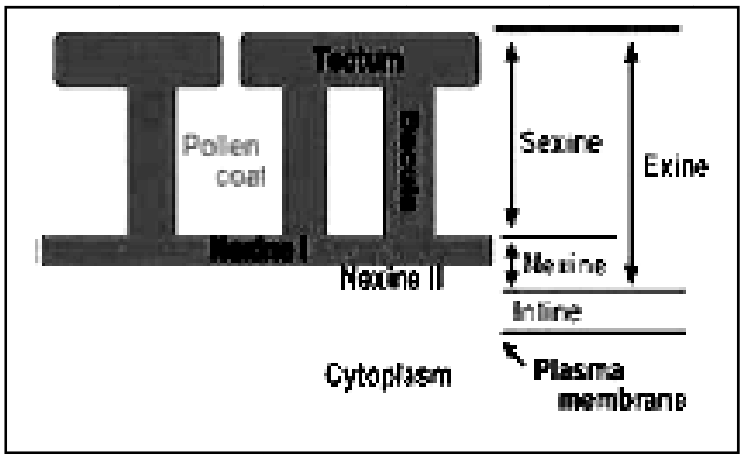
Pollen wall development in gymnosperms

Although differences in pollen wall structure and development are evident in different extant and extinct gymnosperm groups, the main ontogenetic elements appear to be homologous. The pollen mother cell undergoes meiosis to form four haploid microspores. Subsequent development of the exine consists of a number of stages. Firstly, a callose wall forms around the pollen mother cell and subsequently extends around each of the microspores. Next, a matrix develops around each microspore upon which the fibrillar microspore surface coat and later the sexine (consisting of tectum and infratectum components) pattern is established. The microspore surface coat is deposited between the surface of the microspore and the surrounding tetrad wall prior to the formation of the wall components. This layer is regarded as being equivalent to the primexine in angiosperms. The sexine then begins to form on and within the microspore surface coat. The nexine (inner pollen exine wall) lamina is then formed below this coat; therefore, the sexine is partly developed when the nexine begins to develop. The exine as a whole appears to form in a centripetal direction from the outside inwards. Finally, an intine is deposited on the inside of the pollen exine.

Pollen wall development in angiosperm

Pollen walls in angiosperms typically consist of an outer exine composed of sporopollenin and an inner intine composed of cellulose and pectin (Fig. 31). Models of development have been proposed based on observations on numerous species, including *Lilium* and *Arabidopsis*. Similar processes have been described in both these species.

Fig. 31: Diagram of *Arabidopsis* pollen wall structure



Once again, prior to meiosis, the pollen mother cell is surrounded by a callose special cell wall. Immediately after meiosis, four microspores derived from the pollen mother cell form a tetrad. A callose special wall surrounds the microspores. A cellulose primexine then forms between the plasma membrane and callose wall of each microspore. Both the callose wall and primexine are deposited at the surface of the microspore through processes mediated by the plasma membrane. A section of the primexine is then adapted to form column-like structures called the probaculae upon which sporopollenin, secreted by the microspore, will eventually accumulate and polymerize. Sporopollenin deposition and accumulation extend the probaculae, which form the baculae and the tectum. The callose wall then degrades and the developing baculae and tectum are exposed to the fluid of the locule and receive sporopollenin secreted by the tapetum. Wall formation is complete when the nexine and intine layers are formed and the primexine recedes and disappears. The mature pollen grain is then coated by tryphine and pollenkitt, which are synthesized by the tapetum.

5. Pollen Wall: Development; Ubisch body; sporopollenin; adaptive significance of pollen wall architecture.

Pollen wall development

Pollen wall development may be considered under exine and intine growth phases.

1. Exine Growth Phase:

After meiosis of microsporocyte (microspore mother cells) the tetrads of haploid microspores are enveloped by a callosic wall. The callose wall can be detected around the microspore mother cells during initiation of meiosis (Figs. 32a and 33a). It forms a layer between the cytoplasm and pollen mother cell wall.

Additional callose is formed after the second meiotic division which isolates the young microspores from each other. The callose special cell envelope, persists until it is enzymetically digested at the end of the tetrad stage. The pollen wall formation is discussed under two models, viz., the primexine model and the undulation model.

a) Primexine Model:

Heslop-Harrison (1971) considers the primexine as the blue print for the exine. The primexine has a matrix presumably made of cellulose microfibrils, and is deposited in between the spore and the callose wall. Scott (1994) believes that **"the primexine acts as a loose scaffold on to which sporopollenin monomers (fatty acids and phenols) are covalently attached by the localized action of super oxide radicals generated at the plasmalemma".**

Beside its function as a controlled deposition of sporopollenin it also functions as a pathway for the diffusion of other substances such as enzymes, including those responsible for digestion of the callose wall.

In the beginning the primexine is discontinuous at certain specific regions of the plasmalemma (Figs. 32b and 33b) which marks the position of the future germ pore. During the later period of primexine deposition additional gaps appear and these are occupied by columns of electron dense intricate tubular lamellae of 70 nm diameter, called probaculae that rests on the plasmalemma (Figs. 32c and 33c).

The probaculae appear to condense round the plasmalemma and become more evident by the deposition and polymerization of the sporopollenin precursors. Later during the late tetrad stages, the probaculae become disjointedly differentiated into electron dense baculae.

The baculae become laterally expanded at the base to form the foot layer (Figs. 32d and 33d).

Fig. 32: Ubisch bodies formation and the development of pollen wall

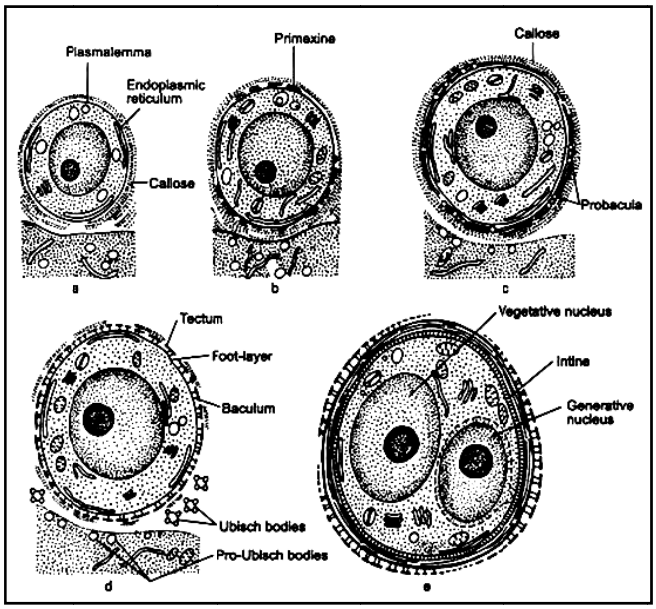
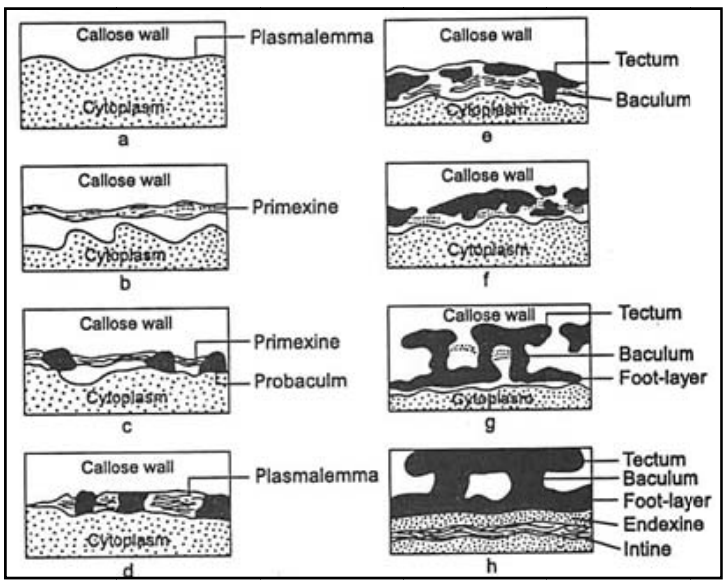


Fig. 33: Diagrammatic development of the pollen wall.



Depending upon the taxon they may remain free above or increase in electron density due to rapid deposition of sporopollenin, and their heads expand laterally to form the tectum (roof), over the primexine matrix.

The foot layer represents the future nexine 1, while the baculae and the roof layer, the future sexine. Further deposition of sporopollenin continues and the whole pollen grain expands laterally and radially as the pollen grain enlarges.

The chemical constituent of the probaculae and the foot layer is not clear and their electron opacity is different from that of the sporopollenin of mature pollen, for which it has been described as protosporopollenin. In the initial stages these layers are not resistant to acetolysis, however, they become resistant with the development of the tetrad stage.

In many marine angiosperms primexine is absent and consequently do not develop normal exine. The nexine2 is deposited below the sexine. In the initial process a number of very thin electron transparent lamellae appear to arise from the cytoplasm and provide a locus around which sporopollenin is deposited. With the progress of deposition the lamellae thicken and merge with each other to from the nexine2.

The presumptive germinal apertures of pollen grain are already demarcated during the microspore tetrad stage. The sites of pre-aperture are distinguished by the absence of the primexine matrix and is associated with an underlying plate of endoplasmic reticulum oriented parallely to the plasma membrane.

This endoplasmic reticulum may physically prevent the movement of membranous structures (coated with primexine material) to the cell surface. Sheldon and Dickinson (1986) believed that the meiotic spindle plays a role in aperture positioning.

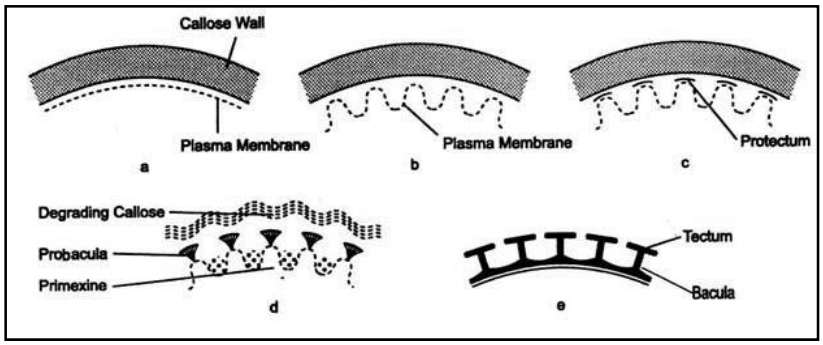
Later the callose wall dissolves, thus releasing the tetrads. The spores now expand rapidly and the primexine matrix is largely disrupted, and only the residue can be observed between the baculae of the mature exine. A rapid conversion of the protosporopollenin occurs, and the primexine acquires the staining properties of the exine.

b) Undulation Model:

Studies on exine development by Takahashi, (1989, 1993, 1995) in *Caesalpinia* and *Lilium* gives little support to the primexine model. It explains that the exine formation commences with the tetrad stage by the invagination or undulation of the plasma membrane which is possibly under the control of cytoskeleton elements. These invaginated localized regions match to the regions of future lumina and distensions that correspond to the muri of the mature exine.

Takahashi (1995) observed that in Lilium the plasma membrane assumes a reticulate pattern which matches the pattern of the mature exine. Fibrous threads (10- 20nm diameter) together with granules (10 nm diameter) aggregate at the regions of protuberances of the plasma membrane and slowly these aggregates develop into a smooth protectum of 0.5 to 0.7 µm diameter (Fig. 34).

Fig. 34: Diagrammatic representation of pollen wall development based on Undulation model



During the later part of the tetrad stage the probaculae and the protectum are more distinguishable beneath the callose wall and the plasma membrane assumes a smooth outline. Probaculae are later formed in between the plasma membrane and the protectum.

At this time the cellulosic fibrous primexine is distinguishable in spaces between the probaculae and the callose wall dissolves, thus releasing the microspores and further differentiation of the exine continues. Thus the undulated plasmamembrane plays an important role in pollen wall development and in fact the protectum is the first exine layer that is deposited on this membrane.

Christensen (1972) outlines the following steps in the development of the pollen wall:

a) In the tetrad stage a new wall called primexine, is deposited around the microspore protoplast within the wall of callose. The primexine appears to contain cellulose microfibrils.

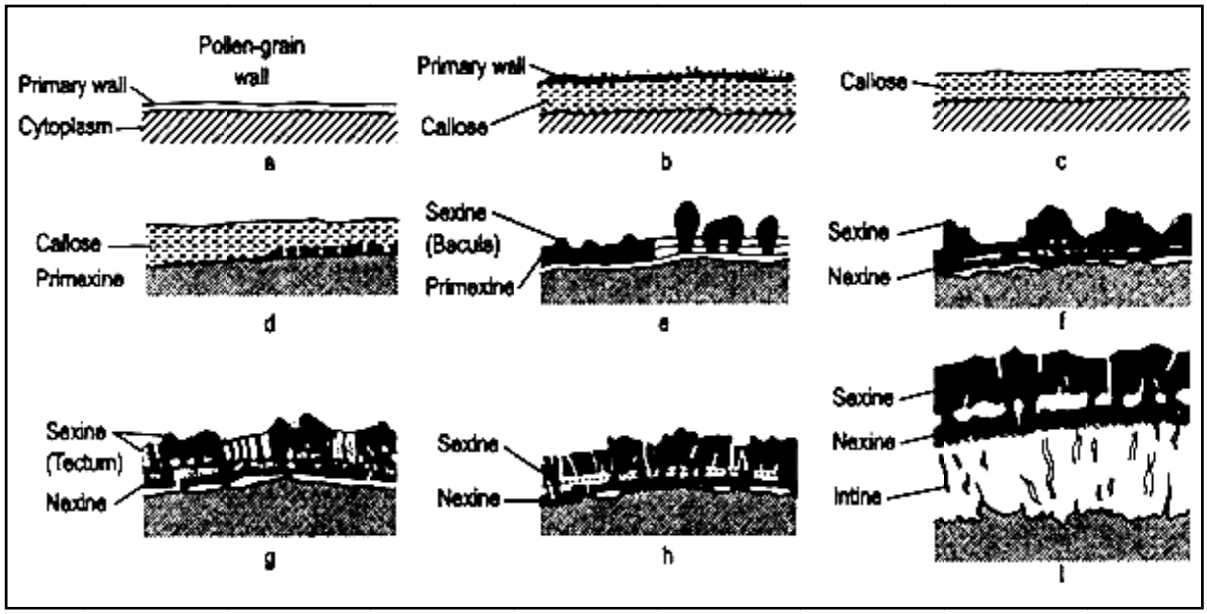
b) In the transition of primexine to exine elements of primexine produces precursors of rod like bacula, which form the sexine. As a result of rapid deposition of sporopollenin the baculae enlarge in electron density and their heads expand laterally to form the tectum.

This is simultaneously followed by the lateral expansion of the base of the bacula to form the foot-layer. Deposition of sporopollenin continues and the whole wall expands laterally and radially. It also witnesses the dissolution of the cellulose wall as a consequence the pollen grain lie free in the pollen sac.

c) Nexine is deposited below the sexine. This is initiated by the deposition of number of electron transparent lamellae originating from the cytoplasm, around which sporopollenin is deposited. Deposition is followed by the thickening of the lamellae which unite with each other to form the nexine (Fig. 35).

Fig. 35: Diagrammatic representation of the pollen wall development

d) In the aperturate region the sexine has very short bacula, while the nexine becomes much thicker and discontinuous in this region than the rest of the pollen wall.



e) At later stage cellulosic intine is formed inside the nexine.

2. Intine Growth Phase:

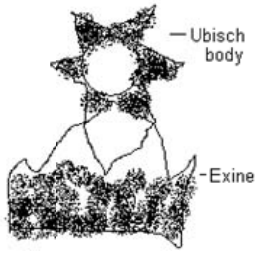
The intine usually starts to develop at the vacuolated stage, beneath the apertures. It increases in thickness under the pores and later on starts to develop under the interapertural parts as a thin layer.

Golgi bodies are frequent during intine synthesis e.g., Ranunculaceae while in others, E.R. and polyribosomes are abundant e.g. *Cosmos*. The intine beneath the pore become comparatively very thick and is provided with fibrillar material and radially arranged membranous units.

In the final period of wall development, additional lipoidal and pigmented substances may accumulate on or/and within the outer exine. This material called the pollenkitt, imparts colour or odour to the pollen and may cause the pollen grains to adhere together during dehiscence.

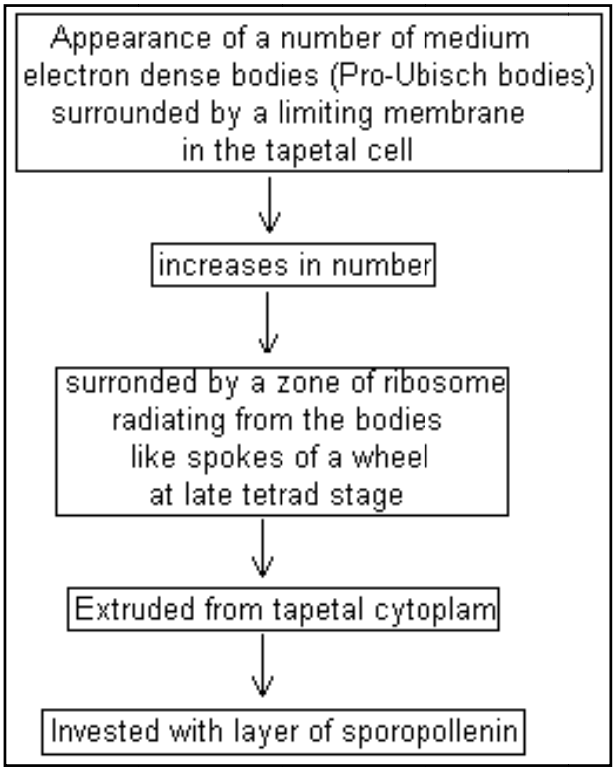
Ubisch bodies

These are spherical structure (2-5 µm) found in the anthers of many genera of angiosperms, both monocotyledons, e.g. *Triticum aestivum* and dicotyledons, e.g. *Betula pendula* and many gymnosperms, e.g. *Ephedra foliata*. They may also oval, rounded, triangular, rod like etc. They frequently fuse into large compound aggregates as, in *Euphorbia caputmedusae*. They generally have a central core and thick wall with microchannels. They are a distinctive feature of secretary or glandular tapetum. Their walls apparently consist of sporopollenin.



It was first discovered by Rosanoff (1865) in close association with the tapetum. Erdtman et al (1961) proposed the term 'orbicules' for these bodies.

Development of Ubisch bodies



Functions:

- Orbicules have no specific function; they are a byproduct of tapetal cell metabolism.
- They might actively partcipate in lysis and degradation of tapetal cell.
- They prevent osmosis and collapses of the developing microspores.

Sporopollenin

Sporopollenin forms the basic structure of the resistant wall of most palynomorphs, like spores, pollens, dianoflagellates and acritarchs. It has also been recorded from spores of *Aspergillus niger*, sexual spores of *Mucor mucedo*, asexual spores of *Pithophora oedogonia*, cell wall of *Phycopeltis epiphyton*, *Chara corallina*, cyst of *Presinocladus marinus*, and spores of Bryophytes, Pteridophytes,

pollen of Gymnosperms and Angiosperms. Besides exine, it is found in perispore, viscin threads, elaters, Ubisch bodies.

Its natural colour is pale yellow, but with thermal maturation it loses O and H, increases percentage of C, the colour deepens through dark yellow, orange, reddish brown, finally to black. The specific gravity is about 1.4 and the index of refraction is 1.48. It is also sensitive to high pH over prolonged period of time.

It is not affected by enzymes so pollen and spore exine pass through most animal guts unchanged.

Functions

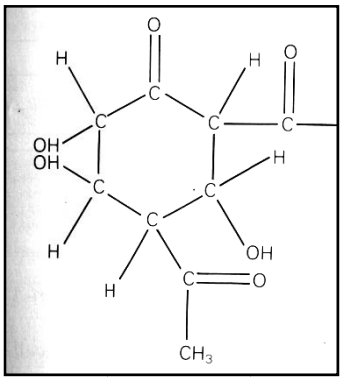
- It played the role of protector of protoplasm against UV radiation and also acts as a sheild against oxidation and desiccation.
- Due to its resistance property it remains intact in the various palynomorph and thus helps in reconstruction of past vegetation, predicting climate changes, understanding the evolution of plant life and in the exploration of hydrocarbons.

Chemical nature

Sporopollenin is highly resistant to physical, chemical and biological degradation procedures. Consequently, its precise chemical composition, structure and biosynthetic route have not yet been ascertained.

It is highly inert compound having empirical formula $C_{90}H_{144}O_{27}$ in spores of *Lycopodium*. It resists acetolysis, but degrades in strong oxidants like H_2O_2 or CrO_3 and exhibits secondary fluorescence when stained with primuline. Zetzsche et al. (1937) determined that the sporopollenin is an oxygeneted hydrocarbon and contains hydroxyl and C-methyl groups and substantial level of unsaturation. Shaw and Yeadon (1966) proposed that the sporopollenin is composed of a lipid fraction of 55-65%, consisting of molecules with a chain length of up to C_{16} and a lignin fraction representing 10-15% of the total mass. Heslop-Harrison (1968) and Brooks et al. (1969) suggested that sporopollenin is a copolymer of β-carotene and xanthophylls such as antheraxanthin and fatty acids. By applying ^{13}C NMR spectroscopy it is seen that sporopollenin contains a series of related biopolymers derived from largely saturated precursors such as long chain fatty acids and oxygenated aromatic rings.

An approximate empirical formula: $C_{90}H_{142}O_{27}$ for *Lycopodium, Picea, Pinus, Corylus* sporopollenin



As such, traditional convention asserts that sporopollenin is a polymer of carotenoid esters. However, modern purification, degradation and analytical techniques have shown that it is comprised of polyhydroxylated unbranched aliphatic units with small quantities of oxygenated aromatic rings and phenylpropanoids.

Adaptive significance of pollen wall architecture

Presence of a complex mixture of lipid substances (pollenkitt) on the surface of mature pollen grains

- which provide the necessary shield against the harmful effects of the environment especially radiation hazards (as because ultra violet ray absorbing pigments are present in pollenkitt)
- which protect the pollen grains from desiccation due to its impermeability to water and water vapour

Presence of a mucilage coat around the pollen of some aquatic taxa

- which protects the cytoplasm from the chances of desiccation and from fungal and microbial attacks

Stratified structure of pollen exine

- which is most difficult to bend. During hydration and dehydration the radial and tangential differentiation of exine layers absorb the bending stresses.

Early delimitation of apertures

- which is considered to be the most justified first direct pressure sensitive control. For example-

  Triaperturate: an expression of mechanical stress mode

  Periaperturate: an equal spacing mode

  Multiaperturate: law of equidistance causes proportional alteration of apertural and non apertural regions

Stratification of apertural intine

- which has special functional significance

Enzymatic inclusion in this layer is sealed above and below by a continuous polysaccharide layer until hydration. Following hydration the outer layer is loosened and disrupted releasing the underlying enzymes. The inner layer then becomes the precursor of the pollen tube. Since the enzymes of the apertural intine are lytic they are associated with the digestion of stigmatic substrates, thus providing the initial nutrition to the pollen tube. The compatibility reactions are also determined by the enzymes of the apertural intine. This explains the reasons for the absence of an enzymatic layer in the intine of the spores of pteridophytes and bryophytes.

Omniaperturate pollen grains (exine thin or absent and intine thick, so that no specific apertural region is distinguished and thus the whole surface can be considered apertural in nature)

- where the entire sporoderm represents the potential site for pollen tube emergence and clearly the entire intine of the grain is loaded with enzymes

Increase in exine thickness at the non apertural areas in case of multiaperturate pollen grains

- multiaperturate pollen grains have the selective value of rapid germination and early fertilization but increase in aperture logically reduces the mechanical efficiency of the wall and this lacuna is compensated by increase in exine thickness at the non apertural areas e.g. *Hibiscus*, *Ipomoea* etc.

Presence of tapetal proteins in the chambered exine

- which acts as a recognition substance that is responsible for the acceptance or rejection of the pollen by the stigma

- This functional outcome means that the pollen lacking a chambered exine would be incapable of sporophytic incompatibility

6. Extraexinous wall materials: pollen connecting threads, perine, pollen-kit

Pollen connecting threads

The selective factors in pollination biology include both structural and functional efficiency. It is observed that anemophilous angiosperms produce mostly small, single and dry pollen grains whereas, in zoophilous angiosperms, flower visitors transfer large clusters of pollen grains that are certainly a positive selective factor in pollination biology. There are three different types of pollen clusters occur in angiosperms: a. sticky pollen grains b. polyads and c. pollen connected by threads.

Types and Function of pollen connecting threads

Previously most of the known pollen connecting threads were grouped under the term "viscin threads". But recent knowledge interprets that principally there are two main types of adhesion threads, either containing sporopollenin or not. The adhesion strings containing sporopollenin is named as 'viscin threads' whereas threads lacking sporopollenin cannot be called as viscin threads.

In modern day, 5 different types of pollen connecting threads are categorized on the basis of nature and ultrastructure, which are as follows:

- Filiform sporopollenin threads
- Non Sporopollenin threads of uncertain origin
- Cellular threads
- Elastoviscin threads of tapetal origin
- Threads from degenerated materials

Sporopollenin Threads: It occurs only in three zoophilous unrelated families of angiosperm viz., Onagraceae, in many Ericaceae and in a single genus *Jacqueshuberia* of Caesalpiniaceae but the nature and function of the threads is same.

Patel et. al. (1985) described the ultrastructure of sporopollenin threads and added some interesting characters:

Often four grains are found connected by threads – this indicates that four microspores of post-meiotic tetrads remain connected.

- Before acetolysis the *Jacqueshuberia* threads and pollen grains are widely covered and sculptured by some granular tapetal debris. After acetolysis the pollen grains and threads are found free of debris with smooth surfaces.
- The sporopollenin threads are very flexible but apparently not elastic, their average diameter is similar to that of columella.
- The columellae are pilar like and distally branched, with tangled ribs forming a network.Some enormously elongated ribs of the columellae form the pollen connecting threads, which are extension of ektexine.
- The sporopollenin threads of Ericaceae and Onagraceae pollen also represent distal elongations of sculpture elements i.e. branched columellae. The ektexine characters are strikingly similar to that of viscin threads.
- In Ericaceae and Onagraceae, like *Jacqueshuberia*, the ektexine of viscin thread pollen consists of distally branched and vaulted columellae forming a peculiar type of tectum, while

some extremely elongated, not vaulted and not thickened columellar branches form sporopollenin threads.

- In onagraceae and Mimosaceae, in addition to long sporopollenin threads, small filiform bridge like threads and fragile, short non-sporopollenin fibres are present.

Function:

i. Strictly ektexinous viscin threads connect the neighboring pollen grains or polyads and help in easy dispersal of large quantity of pollen grains by flower visitors.

ii. Extremely short bridge like threads act as additional cohesion elements in polyads and does not connect neighboring polyads. These are formed from endexinous material.

Non Sporopollenin threads: These types of threads are formed by:

- Cells – in only one zoophilous angiosperm family
- Exclusively by 'dead cell' secretions – in most of the zoophilous angiosperm families

Cellular threads: This type of threads is rough and massive and formed by modified anther cells (e.g. *Strelitzia* of Musaceae). The threads are covered by thin, lipid coating and stick on the pollen grains. These large, massive and rough threads connected the pollen for carrying by heavy pollinators while flexible threads seem to be associated with small insect pollinators.

Non-Celllular threads: It is found in several zoophilous angiosperm taxa.

In Orchids, there are at least two different types of threads are present:

- **Elastoviscin threads** – These are produced by specialized tapetum cell cytoplasm and not by plastids. Threads are elastic, non sticky, often non homogenous, mostly lipid materials in and outside the pollinia connecting the individual pollen grains. When the pollen grains are physically separated, the elastoviscin forms highly elastic threads.
- **Lipid threads** – This type of threads are sticky, highly viscous but inelastic substances originate from some immature, degenerating cells in the transition region between the caudicle *(a slender, elastic appendage, to which the masses of pollen in orchidaceous plants are attached)* and the massulae *(=polyad)* (e.g. *Habenaria*).
- **Slimy, viscous fibres** (containing protein and lipid) connect individual pollen grains in some zoophilous angiosperm families. Very little is known about their nature and origin. Slimy threads connecting pollen are either coated with small droplets or resemble a rather rope like fluid (e.g. Aristolochiaceae, Annonaceae). These slime threads may act as special pollen cement or may play some role in pollen germination.

Perine

It is a hyaline loosely organised sporopollenin envelope covering exine and occurs in some pteridophytes (Polypodiaceae) and a few gymnosperms (e.g. *Taxodium*). It is secondary in origin and developed after differentiation of exine. It may be continuous or sometimes folded in various ways. Well-developed, complex and highly ornamented perine is found in advanced groups of ferns. The presence of fold and well developed excrescences is possibly a special advancement of retention of moisture and adherence to the substrate to enhance healthy germination. Sometime it is difficult to decide whether a layer is perinous or exinous. In such case, Erdtman suggested the term 'Sclerine' (Greek *Skleros*, hard) can be used as a comprehensive name for perine and exine. Generally, perine is used for pollen, and perispore for spore.

Pollen-kit

In the final period of pollen wall development, additional lipoidal and pigmented substance may accumulate on or/and within the outer exine. This material is called pollen kitt.

Originally the term was used to describe the whole content of the mature anther. However, the term is now referred as an oily often pigmented layer found coating the exine. In entomophious flower, it is electron-dense homogenous films on the pollen surface; in anemophilous flower it is present as small, heterogenous lumps in the cavity of exine.

Chemical nature- It is fluid, viscous, sticky and partially with crystalline inclusions. It contains carotenoid and other lipid; its principal pigment is carotene 5, 6-epoxide.

Ontogeny- It is synthesized from plastids of anther tapetum. Within the tapetal plastid, osmiophilic globuli appear as small droplets; they coalesces upon contact and attain certain size; finally they occupy the whole plastid and interact with plastid membrane; after breaking the membrane they release in cytoplasm; degeneration of cytoplasm results in discharge of globules which is deposited as pollen kitt on surface of pollen grain.

Functions-

- Act as insect attractant;
- Sticky nature would serve as adherent to insect's body and because of its hydrophilic nature might even be associated with dispersal of the pollen grain;
- Its carotenoid can protect the genetic content of the pollen against radiation damage during dispersal;
- Imparts colour and odour to the pollen.

7. Natural spore/pollen traps: types, their implications in floristic & environment reconstruction

The study of natural spore/pollen traps demonstrates that the trapped palyno-assemblage reliably reflects the modern flora of the studied areas. Different types of natural traps used in studies of modern spore/pollen rain are lake sediments, soil surface, moss and lichen cushions, bark and leaves, spider webs, seed hairs of some plants e.g. *Populus* etc. Much of the pollen found in these natural traps was produced by anemophilous plants in the surrounding area. It was carried by air currents and fell like rain onto the earth's surface. The composition of this pollen rain should reflect the composition of the vegetation that produced it. Assuming the fossil pollen assemblage to be indicative of the vegetation that produced it, a study of modern pollen rain in relation to present vegetation should aid in interpreting the environments responsible for the pollen recorded in pollen diagrams.

LAKES: Lakes are ideal natural traps as their surface samples provide an exact analogue of the depositional environment of any fossil pollen cores taken from a lake. Palynologists would attempt to correlate present vegetation with the pollen assemblages found in the uppermost sediments of the lakes being sampled. However, the spatial distribution of lakes rarely matches that of the ecosystems under study and the extent to which bioturbation has occurred is usually unknown. Samples that have been contaminated through stream action or erosion processes should be avoided. Furthermore, variations in lake size and basin morphometry can be major complicating factors.

SOIL: Soil samples have been more widely used than other types of natural traps in the tropics because of the ease of sampling and their ubiquitous occurrence. Samples may be taken from the surface of materials not generally incorporated in deposits analyzed for pollen; chiefly these include forest litter and mineral soils. The main problems with the use of soil samples are that in most environments oxidation, microbial degradation occur resulting in poor pollen preservation and rates of sedimentation are not ordinarily known. The compactness of the soil or litter will probably also influence differential vertical migration of the spores.

BRYOPHYTIC POLSTERS: Analysis of polsters of mosses, liverworts and lichens has been frequently employed to sample modern pollen rains. It has been found that the more compact growth

forms are superior to the loose, many-branched forms as pollen traps. The polster collections should be representative of the pollen rain over the last few seasons. Moss polsters are not an appropriate method of sampling in much of the tropics as many tropical ecosystems, such as savannas and deciduous dry forests, contain few mosses. However, Weng *et al*. (2003) and Grabandt (1980) have shown that pollen from moss polsters provide a very effective means of distinguishing plant communities along the eastern flank of the tropical Andes above 500 m elevation.

BARKS: Bark may be used as an easily available pollen trap in the reconstruction of the vegetation. Samples, a few millimetres in diameter, taken from bark of beech, birch, hophornbeam, oak, pine, sycamore and willow contained enough pollen grains for reliable counts (between 200 and 600 pollen grains per slide). Pollen deposition at various levels in the vegetation and at different times of the year provides useful information on vegetation composition, and the possible effect of wind direction. Analysis of archaeological bark samples forms another promising application besides pollen data from other samples.

LEAVES: Leaf surfaces are differential spore/pollen traps. Their efficiency as traps depends upon whether they are horizontal or vertical, wet or dry, hairy or glabrous, glossy or mat, waxy or non-waxy and so on. Not all spores that land become securely attached. Some are washed off by rain, blown off by wind or redistributed by dew. Virtually any spore which may become air-borne can be found on leaves.

SPIDER WEBS: Spider webs are used to trap insects and provide useful environmental information on caves. Bera et al. (2002) and Song et al. (2007) have shown that spider webs have also proved to be an efficient natural trap of airborne spores and pollen grains. Pollens collected from these samples have been also used to interpret about the vegetation and environment of the area.

SEED HAIRS OF POPLAR: The seed hairs and pericarp of poplar (*Populus* spp.) is efficient natural trap of airborne spores and pollen grains. In a study, these were collected from the trees and on the ground in Beijing Botanical Garden of Chinese Academy of Sciences and around Miyun Reservoir during May 2005 for pollen analysis. Different pollen spectra are recorded from different samples and are characterized by dominant occurrence of pollen grains of arboreal and anemophilous plants. In addition, pollen grains of non-arboreal plants including grasses are also found trapped. Among the 46 trapped pollen grains, 26 are known to be allergenic. This study also suggested that poplar seed hairs possibly make people feel uncomfortable due to the presence of allergenic pollen trapped in the hairs.

8. Branches of Palynology & their Application: Branches of palynology; palynology in taxonomic & phylogenetic deductions; palynology in academic & applied aspects including melissopalynology, medical palynology, forensic palynology, entomopalynology & copropalynology

Palynology is the science that studies contemporary and fossil palynomorphs, including pollen, spores, orbicules, dinoflagellate cysts, acritarchs, chitinozoans and scolecodonts, together with particulate organic matter (POM) and kerogen found in sedimentary rocks and sediments. Palynology does not include diatoms, foraminiferans or other organisms with silicaceous or calcareous exoskeletons. Palynomorphs are broadly defined as organic-walled microfossils between 5 and 500 micrometers in size.

The term *palynology* was introduced by Hyde and Williams in 1944, following correspondence with the Swedish geologist Antevs, in the pages of the Pollen Analysis Circular (one of the first journals devoted to pollen analysis, produced by Paul Sears in North America). Hyde and Williams chose *palynology* on the basis of the Greek words *paluno* meaning 'to sprinkle' and *pale* meaning 'dust' (and thus similar to the Latin word *pollen*).

Palynology is an interdisciplinary science and is a branch of earth science (geology) and biological science (biology), particularly plant science (botany). Palynology can broadly categorize into paleopalynology and neopalynology. Paleopalynology deals mainly with study of fossilized spores and pollen grains and other palynomorphs. Neopalynology is the study of concerned spores and pollen grains of extant plants. Palynological investigations are mainly confined to the exomorphic characters of spores and pollen grains as well as the structural details of exine and their implications in plant systematics and evolution. Sophisticated approaches like use of SEM and TEM provided palynologists with valuable insight into the study of pollen grains with increased accuracy. Palynology is used for a diverse range of applications, related to many scientific disciplines:

APPLICATION OF PALAEOPALYNOLOGY

Origin and evolution of plant through ages:

Earth originated 4.5 billion year ago. At the time, the earth was full of volcanoes and the temperature was so high that life could not exist. This unfavourable condition continued for 1.5 billion years. Thereafter, some macromolecules were formed by reacting different compounds. This chemical evolutionary phase continued until the protenoids were produced. One of the oldest known rocks from where earliest life forms (e.g. *Eobacterium, Eosphaera, Palaeolyngbya, Kakabekia*) were recorded is Fig Tree formation in South Africa. The palaeontological records of spores began in the Cambrian rocks, but fossil spores were not abundant until Devonian time. The most primitive trilete sporoforms (e.g. *Retusotriletes*) were observed in mid Silurian–Early Devonian vascular plants. In the extinct groups like Rhyniales, Zosterophyllales, Trimerophytales, Lepidodendrales, Sphenophyllales, Calamitales and Primofilicales, the spores are always trilete. Pre-pollen in early gymnosperms like Pteridosperms, Cordaitales were nearly always trilete and found in the Devonian rock. Monolete (monosulcate) pollen grains were identified in Bennettitales during late Triassic period. Monosaccate pollen of *Felixipollenites* (Cordaitales) was recorded in Carboniferous sediments and disaccate pollens of *Straitopodocarpites*, *Vesicaspora* were found in Permian rock. The earliest record of angiosperm pollen was from lower Cretaceous. The most primitive angiosperm pollen grain (e.g. *Clavatipollenites*) was boat-shaped monosulcate type with clavate ornamentation. Other types of pollen were derived from this primitive sporoform. Observation on sudden appearance of new types of spores and pollen grains, gradual modification of the existing types and the extinction of such types in sedimentary matrix are found very useful to reach conclusion on the evolutionary aspects of present day's plants, when such information are correlated with geological times.

Reconstruction of past vegetation

The exine of the spore and pollen are highly resistant to decay due to presence of sporopollenin. Variation in exine structure and sculpture together with the number and distributional pattern of apertures provides the morphological diversity of the grains. These phenomena enable palynologists to reconstruct the past vegetation and to interpret the depositional environment in terms of the present day known ecological status. Since pollen grains are indicators of species components in vegetation, dominance or absence of pollen grains can be correlated to deduce a general picture of dominant habitats prevailed during a particular period. Pollen analysis may also throw light on the afforestation or deforestation phenomena in relation to prehistoric life form reflected by agricultural activities.

Reconstruction of palaeoclimate and palaeoenvironment

Vegetation reacts sharply to changes in temperature and other related ecological factors. Vegetation is a definite indicator of climate. Palynomorphs are also sensitive to any minor fluctuation in their surroundings, and they are highly indicative of the environment in which they were deposited. Presence of bisaccate and monosaccate pollen grains in a region confirmed its upland nature. Geothermal alteration studies examine the colour of palynomorphs extracted from rocks to give the thermal alteration and maturation of sedimentary sequences, which provides estimates of maximum palaeotemperatures. Information gathered from fossil pollen grains have made possible to reconstruct the relationship of vegetation to climate changes and finally reconstruction of paleoenvironment for many localities, countries and phytogeographical zones.

Indication of geological age

Geologists use palynological studies to determine the relative age of rock. Palynomorphs are great indicators of narrow time ranges because of the rapid evolution of the samples. The earliest known angiosperm pollen, *Clavatipollenites* was found only in lower Cretaceous rock.

Biostratigraphy through correlation

The fundamental principle in stratigraphy is that the sedimentary rock in the earth's surface accumulated in layers, with the oldest at the bottom and youngest on the top. Biostratigraphy is the differentiation of rock units based on the fossils contained in them. Various fossil groups can be found in different sedimentary environments, namely, terrestrial and marine. Spore/pollen has big advantage for biostratigraphy over marine fossils, since they occur in all sedimentary environments. Biostratigraphy plays a critical role in the building of geological models for hydrocarbon exploration.

Determination of ancient shore lines

Most fossil spore/pollens in sediments have been transported sometimes very long distances, by streams and by ocean currents. It is possible to determine distance and direction of ancient shorelines by the kinds and quantities of microfossils, as pollen and spores will decrease in density in a seaward direction, with a corresponding increase in marine forms. According to Hoffmeister (1954), proximity of an ancient shoreline is indicated where the concentration of spores/pollens is about 7500 per gram of sediment.

Petroleum exploration

The rich presence of fossil pollen grains and spores has been detected in oil shales. Since they are >50 μm in diameter, they are not destroyed or damaged by drilling action. Pollen analysis has yielded good results in petroleum geology, especially in areas of brackish water deposits where correlation by means of even other microfossils and megafossil is practically difficult.

Demarcation of coal layer

Most bituminous coal contains abundant plant spores and majority of the spores are believed to be autochthonous in origin. The spore/ pollens in coals occur in a characteristic association, implying the occurrence of different plant communities associated with the deposition of the peat. Spores/pollens in finer sediments of coal deposit bear a variety of shapes, sizes, organization and ornamentation. These characteristics together with their quantitative data have helped in demarcating coal layers and seams in coalfield. The oldest coal deposits have been dated by palynology to 200-180 million years.

Archaeological palynology

Archaeologists examine human uses of plants in the past by using palynological data. This can help determine seasonality of site occupation, presence or absence of agricultural practices or products and plant-related activity areas within an archaeological context. Archaeological objects are mostly dated by pollen analysis. Intensive paleopalynological investigations have yielded much information about different aspects of human civilization like domestication of plants, nature of prehistoric agriculture, the cereals used by pre-neolithic settlement, the plant economy of prehistoric and historic time.

APPLICATION OF NEOPALYNOLOGY

Palynotaxonomy

The study of pollen morphology is of great significance to the disputed problems of higher plant taxonomy and phylogeny, since the pollen grains posses distinctly unique characters which are genetically lebelled. Palynologically plant families can be segregated into two groups:

Stenopalynous, where taxa of the family display more or less same type of pollen grains; e.g. *Poaceae, Casuarinaceae, Chenopodiaceae, Amaranthaceae* etc.

Eurypalynous, where taxa are characterised by an obvious difference in pollen types; e.g. *Rubiaceae, Acanthaceae, Verbenaceae, Solanaceae, Convolvulaceae, Fabaceae* etc.

Based on stenopalyny or eurypalyny the inter- or intra- family affinity and phylogeny of taxa may be determined. The knowledge of pollen morphology has been to substantive many taxonomic revisions sometimes even up to the formation of new taxa. Segregation of Bombacaceae from Malvaceae*;* Trapaceae from Onagraceae*;* amalgamation of Cannaceae, Musaceae and Zingiberaceae into Scitamineae*;* Moraceae and Cannabinaceae into Urticaceae, are a few examples of the contribution of the pollen morphology to taxonomy. With evidences from other areas like anatomy, embryology, cytology, chemotaxonomy, the conclusions on the basis of palynology would become more reliable and convincing.

Pollen biotechnology

This is the branch of biology which deals with the management or manipulation of pollen grains for the production and improvement of crops and related economic products. The first successful induction of haploids (pollen embryo) in cultured anthers of *Datura innoxia* was made by Guha and Maheswari (1964, 1966). Since then this technique has widely been used for induction of haploids of several crop plants and is found to be an effective measure of plant breeding programme.

Aeropalynology

Studies on atmospheric pollen (aerospora) are of great interest because of their application in paleobotany, medicine and forestry. 'Pollen rain' studies of today furnish a key to those of the past, especially the quaternary deposits. Many air-borne pollen and spores cause allergic reaction in human being especially when they enter into respiratory tracts. As the pollen output and its dispersal in the atmosphere are closely related to seed production in trees, investigations on the pollen content of the atmosphere are providing a new approach in the practice of foresty. The bulk of the atmospheric pollen flora is supplied by anemophilous plants which are prolific producers of pollen which is discharged into the air in enormous quantities. Most of the wind borne pollen grains range from 20 to 50 µm in diameter and some may possess special devices such as air sacs as in *Pinus*.

Iatropalynology

This branch of applied palynology deals with the medical aspects specially the allergies (Greek *Iatro* = healing). The allergic response to pollen is called 'Pollinosis' and may involve any part of the body, the most frequently in nose, eye and chest with resultant symptoms of **hay fever, rhinitis or asthma**. Hay fever is characterized by intense sneezing, watery eyes, nasal obstruction, itchy eyes and nose, and often coughing. If the lower respiratory tract, lungs or bronchi are affected, the clinical reaction will be that of asthma. Other reacting organs may be the skin, the gastrointestinal tract, the central or peripheral nervous system or the cardiovascular system. Pollinosis may be caused by pollen grains produced by one or more plant (e.g. *Ambrosia* sp.). Pollen grains causing allergy are quite variable in different ceozones and also in a particular place from season to season, year to year depending on changes in ceological and climatic conditions. Pollen calender may be prepared to correlate the seasonal occurrence of the pollen types to the patients' allergic symptoms.

Pharmacopalynology

The medicinal properties of spore and pollens have been recognised since a long time. Pollen grains contain proteins, carbohydrates, vitamins, enzymes, trace elements etc. The pollen grains of *Cycas circinalis* are narcotic and those of *Typha laxmanii* are used as a stringent and styptic. Spores of *Lycopodium selago* are employed as a powerful cathartic and that of *L. clavatum* are supposed to be diuretic, demulcent and antispasmodic. The spores of *Adiantum philippense* are effective in the treatment of leprosy and other skin disease. Now-a-days pollen grains are sold in tablets or liquid form as a nutritional supplement. Pollens that form a critical component of the honey have antibiotic qualities, intestinal functions, combat respiratory problems, balance endocrine system etc.

Melissopalynology

This is the study pollen and spore found in honey. While collecting nectar from flowers in the field, pollen grains are occasionally swallowed by bees and carried into the hive. Analysis of pollen grains in honey provides information about the geographical origin and period of honey production as pollen

grain in honey indicate the plants visited by the bees. Many of the identified honey plants are important ecological indicator types in the area of honey production. Based on the quantity of pollen grains in a given honey sample, the degree of its purity in commercial honey can be tested. Palynology plays an important role in bee-keeping industry. It is calculated that about 50-70 lbs of pollen grains are required by a fair sized colony of bees for a single year.

Entomopalynology

It is the study of pollen found on the body or in the gut of insects. It is useful for determining insect feeding and migratory habits especially as it involves economically important insects. Adults of numerous insect species feed on nectar, pollen and other plant exudates that are frequently associated with flowers. As a result of this feeding activity, these adults become contaminated with pollen. From the pollen found on several species of Lepidoptera, Mikkola (1971) determined that some of the insects examined migrated to Finland. Hendrix & Showers (1992) found that black cutworm [*Agrotis ipsilon* (Hufmagel)] and armyworm [*Pseudaletia unipuncta* (Haworth)] adults captured in Iowa and Missouri contained pollen from plants that only grew in South and Southwest Texas. The identification of this pollen indicated that these insects migrated over 1300 km. The most sophisticated pollination mechanisms have been evolved in the orchid family (Orchidaceae). Some bee orchids are pollinated by male wasps because the orchid flowers evolved to mimic female wasps.

Forensic palynology

This is the science of applying modern and fossil pollen and spores to help solve legal problems. In its broader application, the field of forensic palynology also includes legal information derived from the analysis of a broad range of organic walled microscopic organisms such as pollen grains, dinaflagellates, acritarchs and chitinozoans that can be found in both fresh and marine environments. Pollen grains and spores from plants with restricted distribution may, if found in dirt on shoes, clothes etc. indicate more or less clearly where the contamination occurred. The analysis of soil and mud samples containing the pollen grains of the vegetation of that area can provide sufficient information for determination of their source and led to their use in forensic science.

Erdtman (1969) first reported two criminal cases, one in Sweden and other in Austria. In the Swedish case the pollen from the body of a murdered woman did not correspond with the area in Sweden, where the body was found. Thus the murder was committed elsewhere, far from where the corpse was found. In the Austrian case, a man disappeared during a trip near Vienna on a journey down to Danube. A pair of muddy shoes belonging to his suspected killer was examined and the soil revealed much pine and alder pollen along with some early tertiary (oligocene) pollen grains. Fortunately, only one area along the Danube velley, 20 km north of Vienna, contained this precise mixture of pollen. When the suspect was confronted with this fact, he confessed and showed authorities where he buried the body as pinpointed by pollen.

Copropalynology

Analysis of pollen grains contained in faecal materials provides interesting insights into the feeding habits of many herbivorous animals. Pollen analysis of coprolites (fossilized faeces) ranging in age from the middle Paleolithic period to late prehistoric times is providing remarkably specific evidences on diets and seasonal activities of early hunters and farmers and their domesticated animals. The sheep excrements from a Swiss lake dwelling that were found to contain pollen grains of a large number of species indicated the composition of the meadows grazed by sheep several thousand years ago. Guano deposits in caves inhabited by bats may – particularly in tropical and subtropical countries – contain a record of entomophilous plants visited, century after century by moths that were subsequently devoured by bats. Reptile coprolites (fossilized feces) recovered from Jurassic sediments indicate that these animals browsed on the pollen-bearing plant structures (Harris 1945, 1956). It has been suggested that reptiles, not beetles, were the original pollinators of angiosperms (Hughes 1976).

9. Let's sum up

- Palynology is the branch of science concerned with the study of pollen grains, spores, and similar palynomorphs, both living and fossil. H. A. Hyde & D. A. Williams (1944) coined the term Palynology but the study of pollen analysis was started in 1916 by E. J. Lennart Von Post.
- Pollen unit is the product of microsporogenesis through which a single pollen mother cell forms pollen units by meiotic division either singly or aggregation of two, four, and more.
- Gunner Erdtman proposed NPC-System pollen/spore classification based on the apertures, their Number, Position and Characters regarding microspore tetrad.
- Harmomegathy is one kind of adaptive mechanism associated with the phases of desiccation and rehydration and the contrivances needed for this mechanism help to avoid plasmolysis of the cytoplasm.
- The pollen wall, the sporoderm is generally stratified i.e. layered. The walls of the mature pollen, at least in angiosperms, consist of two fundamentally different layers, intine and an outer acetolysis resistant layer exine composed of sporopollenin.
- LO-analysis is a method for analysing patterns of sexine organisation by means of light microscopy. Development of a durable spore wall is essential for terrestrialization as it enables the spore to withstand physical abrasion, desiccation and UV-B radiation.
- Pollen wall development may be considered under exine and intine growth phases. Sporopollenin forms the basic structure of the resistant wall of most palynomorphs, like spores, pollens, dianoflagellates and acritarchs. It is highly inert compound having empirical formula $C_{90}H_{144}O_{27.}$
- In modern day, 5 different types of pollen connecting threads are categorized on the basis of nature and ultrastructure. Perine is a hyaline loosely organised sporopollenin envelope covering exine.
- Different types of natural traps used in studies of modern spore/pollen rain are lake sediments, soil surface, moss and lichen cushions, bark and leaves, spider webs, seed hairs of some plants e.g. *Populus* etc.
- Palynology is an interdisciplinary science and is a branch of earth science (geology) and biological science (biology), particularly plant science (botany). Palynology can broadly categorize into paleopalynology and neopalynology.

10. Suggested Reading

1. Bhattacharya, K., Majumdar, M.R. & Gupta Bhattacharya, S. (2006). A Text Book of Palynology, New Central Book Agency.
2. Bryant, V.M. Jr. and S.A. Hall. 1993. Archaeological palynology in the United States: a critique. American Antiquity, volume 58, pages 416-421.
3. Jarzen, D.M. and McAllister, D.E. 1994. Eco-services: l. Pollination - love in the plant world benefits the whole world. Wildflower, North America's Magazine of Wild Flora, volume 10, number 3, pages 24-27.

4. Jarzen, D.M. & Nichols, D.J. 1996. Chapter 9 Pollen. In: Jansonius, J. & McGregor, D.C. (eds.) Palynology: principles and applications. American Association of Stratigraphic Palynologists Foundation, volume 1, pages 261-291.
5. Erdtman, G. (1986). Pollen Morphology and Plant Taxonomy, Latest Ed., Lelden, E.G. Brill
6. Faegri, K. and Iversen, J. (1964). Text Book of Pollen Analysis, Munksgor, Copenhagen
7. Heslop- Harisson (1971). Pollen: Development and Physiology, Butterworth
8. Nair, P.K. K. (1970). Pollen Morphology of Angiosperms, Latest Ed., Scholar Publications

11. Assignment

1. Distinguish between microspore and pollen.
2. Pollen has equatorial distance 125 µm and polar axis 150 µm. Categorize the pollen based on its size and shape.
3. Name two extraexinous wall materials. Mention their functions.
4. Mention the chemical nature and functions of sporopollenin.
5. Describe different types of pollen connecting threads with examples.
6. Distinguish between 'Lalongate' and 'Lolongate' types of ora.
7. Draw and describe the pollen grain having NPC number 345.
8. Distinguish between exospore and perispore.
9. State the physical and chemical properties of sporopollenin.
10. Mention one adaptive character of pollen grains for anemophily.
11. What is pollen kitt? Mention its functions.
12. Describe different types of pollen connecting threads with examples.
13. What is meant by viability of pollen? What are the factors affecting pollen viability.
14. Enumerate the role of primexine in pollen wall development.
15. What are natural spore/pollen traps? Mention their implications?
16. Mention the chemical nature and functions of sporopollenin.
17. Enumerate different types of pollen units with examples.
18. Illustrate different types of sporoderm sculpturing with examples.
19. Discuss the morphological changes of pollen wall during its development.
20. Describe different types of harmomegathic mechanisms in pollen grains with examples.
21. Enumerate the application of palynology in human welfare.
22. Explain the significance of the following studies: Melissopalynology, Medical palynology, Forensic palynology, Entomopalynology and Copropalynology.

COURSE – BOHCT2.4
PALAEOBOTANY, PALYNOLOGY AND ANATOMY

Hard Core Theory Paper **Credit: (Groups A+B+C) = 3**

Group C (Anatomy)

Content Structure

1. Introduction
2. Course Objectives
3. Meristems and differentiation: meristems and growth of the plant, differentiation of primary and secondary plant body: epidermis: stomatal ontogeny, cuticle and epidermal appendages; secretory structure
4. Phylogeny and evolution of vascular and mechanical tissue elements: origin and development of sclereids and fibres, phylogeny of xylem and phloem elements, wood anatomy: vascular cambium and its seasonal activity.
5. Anatomical variations with ecology: leaf and root anatomy in ecological perspectives; hydraulic architecture of plant
6. Anatomy in applied science: anatomy and pollution, anatomy in forensic science, archaeology and climatology.
7. Let's sum up
8. Suggested Reading
9. Assignment

1. Introduction

Plant anatomy or phytotomy is the general term for the study of the internal structure of plants. Originally it included plant morphology, the description of the physical form and external structure of plants, but since the mid-20th century plant anatomy has been considered a separate field referring only to internal plant structure. Plant anatomy is now frequently investigated at the cellular level, and often involves the sectioning of tissues and microscopy. We can learn about the architecture of the plant cells, how they are put together, and how they relate to each other.

2. Course Objectives

After completion of the course the learners will be able to:

- To know how Vascular and mechanical tissue elements evolved.
- Relate the structure and function of the epidermis, stomata and secretary structure

- An understanding on anatomical variations with ecology
- Be able to distinguish between leaf and root anatomy in ecological perspectives
- To know how origin and development of sclereids and fibres
- Apply anatomy in forensic sciences, archaeology and climatology

3. Meristems and differentiation: meristems and growth of the plant, differentiation of primary and secondary plant body: epidermis: stomatal ontogeny, cuticle and epidermal appendages; secretory structure.

Plant Anatomy

The word **anatomy** has derived from 'ana' meaning asunder (into pieces), and 'temnein' meaning to cut. The term is also known as **internal morphology**. It is the branch of botany which deals with the internal structures and organization of plant body.

Meristems:

In The early stages of the development of the embryo, all the cells undergo division, but with further growth and development cell division and multiplication become restricted to special parts of the plant in which the tissues remain embryonic in character and the cells retain the ability to divide.

A **meristem** is a localized region of tissue which, by cell division, adds new cells to a plant or plant part. In the shoots of vascular plants the activity of meristems results in an increase in length and/or diameter, and following cell growth and differentiation, formation of the various mature tissue regions of the axes as well as the formation of organs such as leaves, cone scales, sporophylls, stipules, flower parts, etc. Some meristems are **self-perpetuating**, and thus can be considered to be "permanent" meristems. Most apical meristems and the vascular cambium are meristems of this type and, as a result of their activity, provide vascular plants with their mode of indeterminate growth.

The term **meristem** (from the Greek *merismos*, division) emphasizes cell division activity as a characteristic of a meristematic tissue. Living tissues other than the meristems may be induced to produce new cells, but the meristems maintain such activity indefinitely, for they not only add cells to the plant body but also perpetuate themselves; that is, some of the products of cell division in the meristems do not develop into adult cells but remain meristematic. Those cells that maintain the meristem as a continuing source of new cells are referred to as the **initiating cells**, or **meristematic initials**, or simply initials. Their products, which after a variable number of cell divisions give rise to the body cells, are the **derivatives of the initials**. One can also say that a dividing cell is a precursor of derivatives. A given initial in a meristem is a precursor of two derivatives, one of which is a new initial and the other a precursor of body cells.

Characteristics of Meristem Tissue:

1. Ability to grow and divide,
2. Small immature cells,
3. Isodiametric, rounded, oval or polygonal,
4. Absence of intercellular spaces.
5. Walls are thin, elastic and made of cellulose,
6. Nucleus conspicuous,
7. Cytoplasm dense,
8. Vacuoles absent or very small,
9. Crystals absent,
10. Endoplasmic reticulum small,

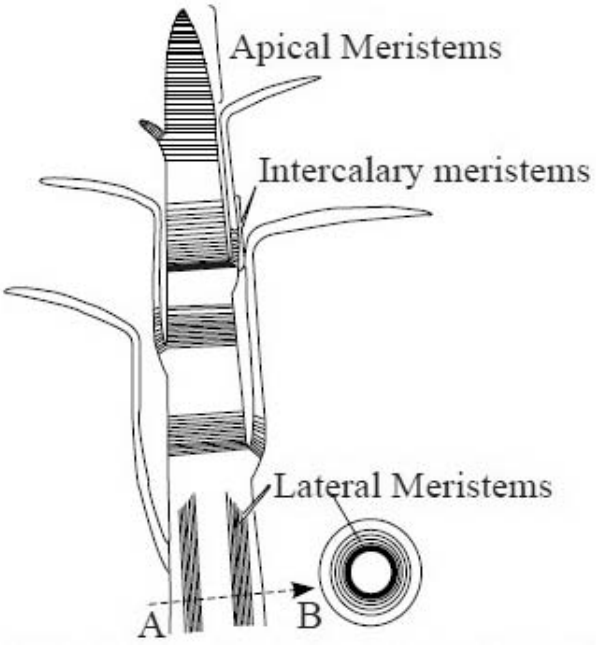
11. Pro-plastids are present instead of plastids.
12. Mitochondria have simple struc-ture.
13. Rate of respiration is very high,
14. There is large scale synthetic activity,
15. There is little reserve food, and
16. Cells of the cambium are, however, slightly different.
17. They possess large vacuoles and are elongated.

Classification of meristems

According to the position of the meristems in the plant body they are divided into the following types:

(a) Apical meristems: Meristems located at the apices of main and lateral shoots and roots.

(b) Lateral meristems: Meristems arranged parallel with the sides of the axis, usually that of stem and root. The vascular and cork cambia are lateral meristems.



(c) Intercalary meristem: This term refers to meristematic tissue derived from the apical meristem and continuing meristematic activity some distance from that meristem. The word intercalary implies that the meristemis inserted (intercalated) between tissues that are no longer meristematic. The best known examples of intercalary meristems are those in internodes and leaf sheaths of monocots, particularly grasses.

According to their origin and development they are the followings:

Promeristem or Primordial Meristem:

Promeristem is the very fundamental stage of meristem action, the region of formation of new organs and tissues. It may be called the earliest embryonic condition consisting of young initials and their derivatives.

All the cells possess the characters of true meristematic cells, viz., diameters alike, dense cytoplasm, large nuclei, proplastids, absence of ergastic matters and thin walls

Primary Meristem:

Primary meristems are composed of cells which are direct descendants of embryonic cells and which have all through retained meristematic nature. So primary meristem may be called a later developmental stage.

Chief primary meristems are those located at the tips of stem, root, and appendages. Primary meristems build up the primary body of the plants.

Secondary Meristem:

Primary meristems gradually differentiate into permanent tissues. Some living permanent cells may regain the power of cell division. They consti-tute the secondary meristem, as they originate from permanent cells.

The cork cambium or phellogen, arising from epidermis, cortical and other cells during secondary increase in thickness, is an example of secondary meristem.

It is to be noted that primary meristems are responsible for the building up of the primary body of the plant; and the secondary meristems, formed later, add new cells to the primary body with definite purposes like effective protection and repair.

The subjacent meristematic tissues are classified according to the tissue systems that are derived from them, namely into **protoderm**, which differentiates into the epidermis; **procambium** (also called provascular tissue), which gives rise to the primary vascular tissues; and the **ground meristem**, precursor of the fundamental or ground tissue system.

According to their plane of cell division they are the followings:

The **mass meristem** grows by divisions in all planes and produces bodies that are isodiametric or spheroidal or have no def nite shape. Such growth occurs, for example, during the formation of spores, sperms (in seedless vascular plants), and endosperm. Divisions in various planes are associated with the spheroidal shape of many angiosperm embryos at a certain stage of development.

The **rib meristem** gives rise to a complex of parallel longitudinal files ("ribs") of cells by divisions at right angles to the longitudinal axis of the cell row. This pattern of growth occurs in elongating cylindrical plant parts as illustrated by the cortex of roots and pith and cortex of stems.

The **plate meristem** shows chiefly anticlinal divisions so that the number of layers originally established in the young organ does not increase any further, and a plate-like structure is produced.

Theories of Structural Development and Differentiation of Meristem :

Theories regarding Apical Meristem

Apical cell theory

- It was proposed by **Hofemister** and supported by **Nageli** (1858).
- The formation of shoot apex from a single superficial cell that is known as apical cell.
- Apical cell is the structural and functional unit of apical meristem.
- This theory is applicable in algae, bryophytes and pteridophytes and not in phanerogams.

Histogen theory

- It was proposed by **Hanstein** (1868).
- The theory is applicable to both shoot and root apex.
- Apical meristem is differentiated into three zones.
  1. Outer dermatogen — It gives rise to epidermis or epiblema (in root)
  2. Middle periblem — It gives rise to cortex.
  3. Inner most plerome — It forms stele and vascular tissue.
- In root apex there occurs one more histogen, calyptrogen, that is responsible for the formation of root cap.
- This theory is not accepted, because there exists no demarcation between three different histogens in shoot and root apex.

Tunica-corpus theory

- It was proposed by **Schmidt** (1924).
- This theory is advanced and accepted for shoot apical meristem.
- Apical meristem is differentiated into two zones.
  1. Outer Tunica - It is one to two cell layer thick. It divides anticlinally and gives rise to epidermis.
  2. Inner corpus - It divide in all planes (anticlinally and periclinally) and gives rise to cortex and stele. It helps in increasing the volume of the plant.

Meristematic Activity and Plant Growth

Meristems and their meristematic derivatives are concerned with growth in the broad sense of the term, meaning irreversible increase in size, including volume and surface. In multicellular plants growth is based on two processes, cell division and cell enlargement. The recent derivatives of meristematic initials produce other derivatives by cell division, and the successive generations of derivatives increase in size. Cell enlargement becomes dominant over cell division and, in time, replaces it entirely. When the derivatives farthest from the meristem initials cease to divide and to expand, they acquire the characteristic specific for the tissues in which they are located; that is, the cells differentiate and eventually mature.

Although cell division as such does not contribute to the volume of the growing entity (Green, 1969, 1976), addition of cells is a primary requirement for growth of a multicellular organism. Cell division

and cell expansion are different stages of the growth process, in which cell enlargement determines the final size of the plant

Epidermis

The term **epidermis** designates the outermost layer of cells on the primary plant body that covers the leaves, flowers, roots and stems of plants. It is derived from the Greek epi, for upon, and derma, for skin. It forms a boundary between the plant and the external environment. The epidermis serves several functions: it protects against water loss, regulates gas exchange, secretes metabolic compounds, and (especially in roots) absorbs water and mineral nutrients. The epidermis of most leaves shows dorsiventral anatomy: the upper (adaxial) and lower (abaxial) surfaces have somewhat different construction and may serve different functions. Woody stems and some other stem structures produce a secondary covering called the **periderm** that replaces the epidermis as the protective covering. In roots the epidermis may have a common origin with cells of the root cap or differentiate from the outermost cell layer of the cortex. The difference in origin of the epidermis in shoots and roots has convinced some investigators that the surface layer of the root should have its own name, **rhizodermis, or epiblem**.

The epidermis is usually one layer of cells in thickness. In some leaves the protodermal cells and their derivatives divide periclinally (parallel with the surface), resulting in a tissue consisting of several layers of ontogenetically related cells. (Sometimes only individual cells of the epidermis undergo periclinal divisions.) Such a tissue is referred to as a **multiple, or multiseriate, epidermis**. The velamen of the aerial and terrestrial roots of orchids is also an example of a multiple epidermis. In leaves the outermost layer of a multiple epidermis resembles an ordinary uniseriate epidermis in having a cuticle; the inner layers commonly contain few or no chloroplasts. One of the functions ascribed to the inner layers is storage of water (Kaul, 1977). Representatives with a multiple epidermis may be found among the Moraceae (most species of *Ficus*), Pittosporaceae, Piperaceae (*Peperomia*), Begoniaceae, Malvaceae, Monocotyledoneae (palms, orchids), and others (Linsbauer, 1930). In some plants subepidermal layers resemble those of a multiple epidermis but are derived from the ground meristem. These layers are called **hypodermis**.

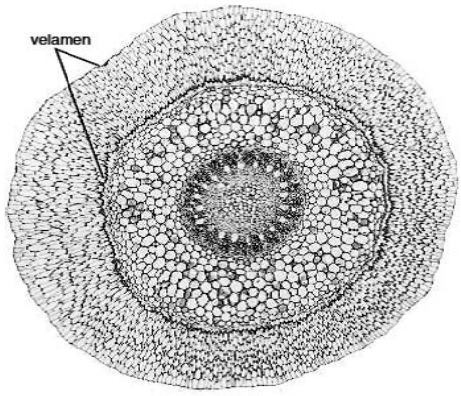


Fig. Transverse section of an orchid root showing the multiple epidermis or velamen
epidermis, or velamen.

The periclinal divisions initiating the multiple epidermis in leaves occur relatively late in development. In *Ficus*, for example, the leaf has a uniseriate epidermisuntil the stipules are shed. Then periclinal divisions occur in the epidermis. During expansion of the leaf, anticlinal divisions also occur and, since these divisions are not synchronized in the different layers, the ontogenetic relationship between the layers becomes obscured. The inner layers expand more than the outer and the largest cells, called **lithocysts**, produce a calcified body, the **cystolith**, composedlargely of calcium

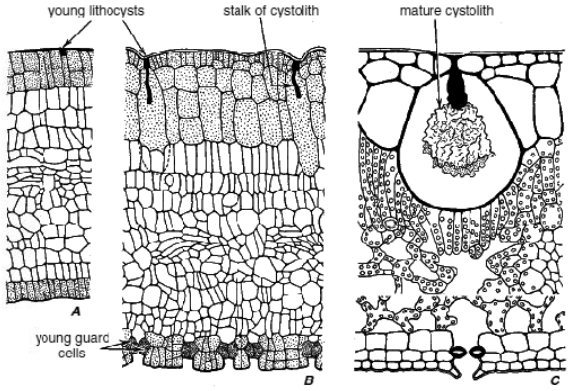


Fig. Multiple epidermis in transverse sections of *Ficus elastica* leaves

carbonate attached to a silicified stalk (Setoguchi et al., 1989; Taylor, M. G., et al., 1993). The stalk originates as a cylindrical ingrowth of the cell wall. The lithocysts do not divide but keep pace with the increasing depth of the epidermis and even overtake it.

Structure of ordinary cell of the epidermis

The ordinary cells of the epidermis vary in shape, size and arrangement, but they are always closely attached to form a compact layer devoid of "intercellular spaces. In the epidermis of petals air spaces may sometimes occur but they are always covered by the cuticle (Eames and MacDaniels, l947), Many epidermal cells are tabular, and in the leaf blade of dicotyledons the anticlinal walls are mostly sinuous. In the stems, and especially in the leaves of many monocotyledons, the epidermal cells are elongated. In-the epidermis of certain seeds (in species of the Leguminosae and in *Punica)* the cells are relatively very elongated in a radial direction and ,are rod-shaped. In certain plants; for instance *Aloe aristata*, the epidermal' cells appear to be hexagonal in surface view but actually they are polyhedral, and according to Matzke (1947), the average number of faces is 10·885. The external wall of the epidermal cells of certain leaves and petals ,is raised in the form of papillae. In certain pteridophytes such papillae are found on the epidermal cell wall facing the mesophyll.

Wall structure:

The epidermal cell wall differs in thickness'-some cells are thin-walled while in others the outer wall is thicker than the other walls. In seeds, scales and certain leaves, e.g. the leaves of conifers, the epidermal cell walls are very thick and lignified.

Primary pit fields are often found in the walls, especially on the radial and inner walls, of the epidermal cells. In the outer epidermal wall of many leaves plasmodesmata are present. These plasmodesmata are termed **ectodesmata** and they may have the appearance of ordinary plasmodesmata.

Cuticle:

The **cuticle**, or **cuticular membrane**, consists predominately of two lipid components: insoluble cutin, which constitutes the matrix of the cuticle, and soluble waxes, some of which are deposited on the surface of the cuticle, the **epicuticular wax**, and others embedded in the matrix, the **cuticular**, or **intracuticular**, **wax**. The cuticle is characteristic of all plant surfaces exposed to air, even extending through the stomatal pores and lining the inner epidermal cell walls of the **substomatal chambers**, large substomatal intercellular spaces opposite the stomata (Pesacreta and Hasenstein, 1999). It is the first protective barrier between the aerial surface of the plant and its environment and the principal barrier to the movement of water, including that of the transpiration stream, and solutes (Riederer and

Schreiber,2001).

In exceptional cases, cuticles are also formed in cortical cells and give rise to a protective

tissue called **cuticular epithelium** (Calvin, 1970; Wilson and Calvin, 2003).

The matrix of the cuticle may consist not only of one but of two lipid polymers, cutin and cutan (Jeffree, 1996; Villena et al., 1999). Unlike cutin, **cutan** is highly resistant to alkaline hydrolysis. Although the cuticles of some species appear to lack cutan (those of the tomato

fruit, *Citrus* and *Erica* leaves), cutan may be the principal or only matrix polymer in some others, notably in *Beta vulgaris*. Cutan has been reported to be a constituent in fossilized plant cuticles, and mixed cutin/cutan cuticles have been reported in a number of extant

species, including *Picea abies*, *Gossypium* sp., *Malus primula*, *Acer platanoides*, *Quercus robur*, *Agave americana*, and *Clivia miniata.*

Most cuticles consist of two more or less distinct regions, the cuticle proper and one or more cuticular layers. The **cuticle proper** is the outermost region, containing cutin and embedded birefringent (cuticular) **waxes** but no cellulose. The process by which it is formed is called **cuticularization**.

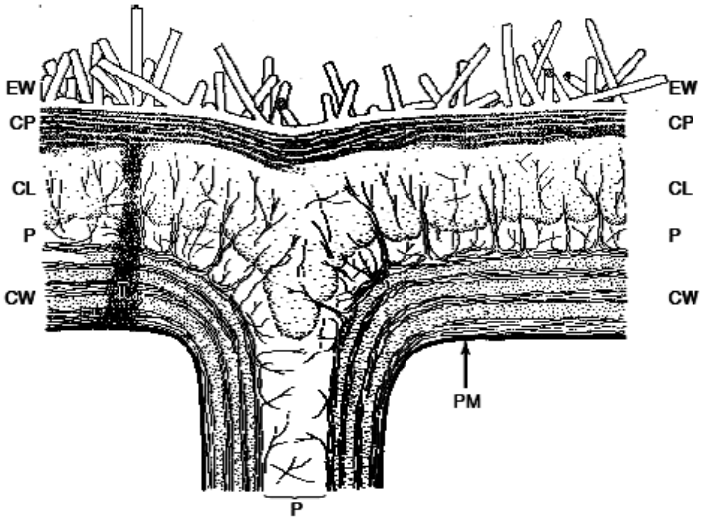


FIGURE 9.6

Generalized structure of a plant cuticle. Details: CL, cuticular layer or reticulate region, traversed by cellulose microfibrils; CP, cuticle proper, showing lamellate structure; CW, cell wall; EW, epicuticular wax; P, pectinaceous layer

The **cuticular layers** are found beneath the cuticle proper and are considered to be the outer portions of the cell wall encrusted to varying degrees with cutin. Cuticular wax, pectin, and hemicellulose may also occur in the cuticular layers. The process by which the cuticular layers are formed is called **cutinization**. Beneath the cuticular layers there commonly is a layer rich in pectin, the **pectin layer**, which bonds the cuticle to the outer walls. The pectin layer is continuous with the middle lamella between the anticlinal walls where the cuticle extends deeply, forming ***cuticular pegs***.

The ultrastructure of the cuticle shows considerable variability. Two distinctive ultrastructural components may be found within the matrix: ***lamellae*** and ***fibrillae***.

Protoplast

The protoplast of the epidermal cells of most plants contains minute leucoplasts and is devoid of chloroplasts. Chloroplasts are found in certain pteridophytes, hydrophytes and in some shade plants. Anthocyanins are found in the vacuoles of the epidermal cells of the petals of numerousflowers, in the leaves of *Zebrinapendula* and red cabbage, in the stems and petioles of *Ricinus* and indifferent organs o(many other plants. Tannins,. mucilage and crystals may be present in epidermal cells .

Special epidermal cells:

Silica and cork cells:

The silica-cells contain silica-bodies which are isotropic masses of silica in the centre of which are usually minute granules.In surface view the silica-bodies may be circular, elliptic, dumbbell or saddle-shaped. The walls of the cork cells are impregnated with suberin and many of them contain solid organic substances. The above short-cells sometimes bear papillae, setae, spines or hairs. Metcalfe (1960) draws attention to the fact that the cork-cells in many plant's contain silica-bodies, and that in certain grasses silica-bodies also occur in some elongated cells. Silica-bodies also occur in specialized epidermal cells of the Cyperaceae and some other monocotyledons

Bulliform cell:

It is a group of outer epidermal cells that can be easily distinguished from the typical epidermal cells by their fan like appearance in cross section and larger sizes. The median cell of bulliform cell is the tallest and the size of the other cells, present on the two sides, diminishes gradually. The cells are thin walled, hyaline and have large vacuole.

The cells contain much water and are devoid of chloroplastids. The cell walls are composed of cellulose and pectic substances; cutin occurs on the outermost wall that is covered by cuticle. They usually form isolated strips that are situated on parallel between the veins. They are present on the outer epidermis of the leaves of Poaceae and other mono-cotyledons except Helobiae.

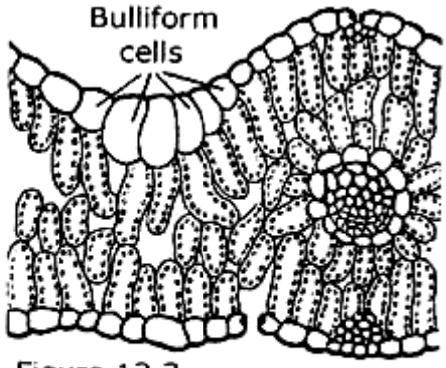
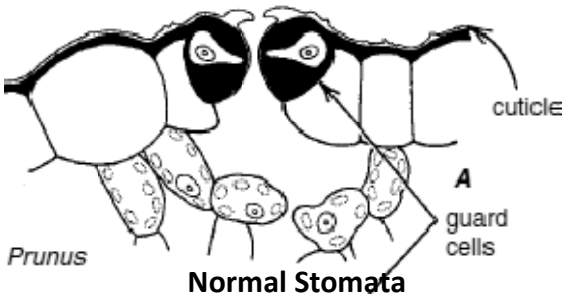


Figure 12.3

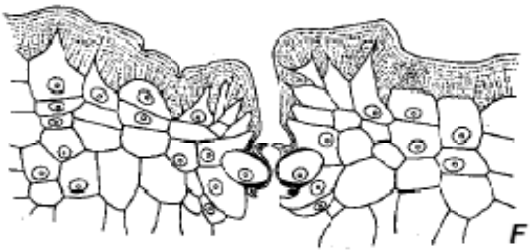
Bulliform cells in the section of *Triticum* leaf.

Stomata:

The continuity of the epidermis is interrupted by minute openings which are intercellular spaces each of which is limited by two specialized cells termed the **guard cells**. The guard cells together with the opening between them constitute the **stoma**. In many plants **subsidiary** or **accessory cells** can be distinguished. These cells differ morphologically from the typical epidermal cells and they constitute two or more cells bordering the guard cells to which they are apparently functionally connected. The subsidiary cells usually develop from protodermal cells adjacent to the stomatal mother cells but they may also develop from sister cells of the mother cell (De Bary, 1877).



Normal Stomata



Sunken Stomata

Stomata are usually found on the aerial portions of the plant and especially on leaves, ordinary stems, and rhizomes., Stomata are absent from roots and the entire plant body of certain parasitic plants that lack chlorophyll, such as *Monotropa* and *Neottia.* In *Orobanche,* however, stomata are found on the stem although this genus is also devoid of chlorophyll.

Stomata are present in some submerged water plants but not in most others. Stomata may be found on petals, staminal filaments (e.g. *Colchicum),* carpels and seeds, but these stomata are usually non-functional. In photosynthesizing leaves stomata may be found on both sides of the leaf or on the lower side only. In certain water plants with leaves that float on the surface of the water, e.g. *Nymphaea,* stomata are found, only on the upper surface of the leaf which is exposed to the atmosphere. In leaves, stomata may occur on both surfaces (**amphistomatic leaf**) or on only one, either the upper (**epistomatic leaf**) or more commonly on the lower (**hypostomatic leaf**). Some examples of stomatal densities (per square millimeter, lower epidermis/upper epidermis), found in Willmer and Fricker (1996) are *Allium cepa* 175/175, *Arabidopsis thaliana* 194/103, *Avena sativa* 45/50, *Zea mays* 108/98, *Helianthus annuus* 175/120, *Nicotiana tabacum* 190/50, *Cornus fl orida* 83/0, *Quercus velutina*

405/0, *Tilia americana* 891/0, *Larix decidua* 16/14, and *Pinus strobus* 120/120. In general, stomatal density is higher in xeromorphic leaves than in leaves of mesomorphic and hygromorphic (hydromorphic) plants (Roth, 1990).

Below the stomata and directed inwards to the mesophyll are large intercellular spaces which are termed **substomatal chambers.** The guard cells of eudicots are generally crescent-shaped

with blunt ends (**kidney-shaped**) in surface view, and have **ledges** of wall material on the outer or both the outer and inner sides. In sectional views such ledges appear like horns. If two ledges are present, the outer ledge delimits a front chamber and the inner ledge delimits a rear chamber. The guard cells are covered with a cuticle. According to a different hypothesis, the radial arrangement of cellulose microfibrils (**radial micellation**) in the guard cell walls plays a more important role in stomatal movement than the differential wall thickening (Aylor et al., 1973; Raschke, 1975). As the dorsal walls of kidney-shaped guard cells move outward with increase in turgor, the radial micellation transmits this movement to the wall bordering the pore (the ventral wall), and the pore opens.

Each guard cell has a prominent nucleus, numerous mitochondria, and poorly developed chloroplasts, in which starch typically accumulates at night and decreases in amount during the day with increasing stomatal opening. The vacuolar system is dissected to variable degrees. The extent of vacuolar volume differs greatly between closed and open stomata, ranging from a very small fraction of cell volume in closed stomata to over 90% in open stomata. In the Poaceae and a few other families of monocots, the guard cells are dumbbell-shaped; that is, they are narrow in the middle and enlarged at both ends.

Ontogeny of stomata

Stomatal development begins with an asymmetric, or unequal, anticlinal division of a protodermal cell. This division results in two cells, one that is usually larger and resembles the other protodermal cells and a second that is usually markedly smaller and contains densely staining cytoplasm and a large nucleus. The smaller of these two cells is called the **stomatal meristemoid**. The mother cell divides to form two cells which differentiate into the guard cells. At first these cells are small and have no special shape but, as they develop, they enlarge and become characteristically shaped. During their development the middle lamella between the two guard cells swells and becomes lens-shaped shortly before the time when it disintegrates to form the stomatal aperture (Ziegenspeck, 1944). Even in those cases in which the mature guard cells are sunken or raised relative to the ordinary cells of the uniseriate epidermis, the guard-cell mother cells and the guard cells are level with the other epidermal cells immediately after their formation. The sinking and the raising is brought about during the maturation of the guard cells. The development of stomata in the leaf continues for a relatively long period during the growth of the leaf.

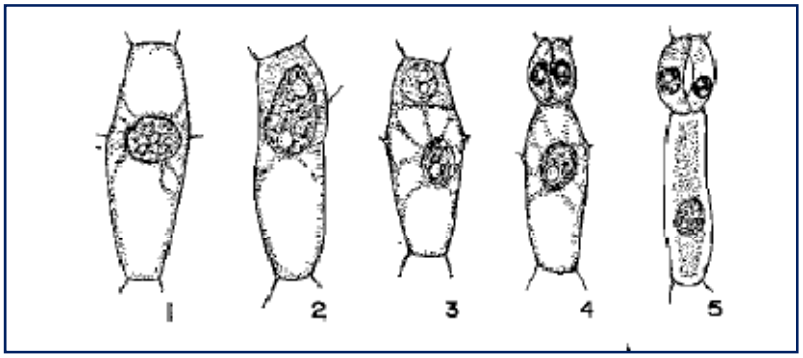


Fig. Ontogeny of stomata of *Allium cepa*

On the basis of the order of the appearance of the stomata all the photosynthesizing organ, two main types of development can be distinguished: (1) that in which the stomata appear gradually in a basipetal sequence, i.e. from the tip of the organ to its base, as is the case in leaves with parallel venation and in the internodes of the articulated species of the Chenopodiaceae; (2) that in which there is no regularity in the appearance of the stomata in the various regions of the growing organ, as is the case in leaves with reticulate venation.

Stomatal types:

On this basis of ontogenetic relationship of subsidiary cells and guard cells, three major categories of stomatal ontogeny have been recognized (Pant, 1965; Baranova, 1987, 1992):

1. **Mesogenous**, in which all of the subsidiary or neighboring cells have a common origin with the guard cells.
2. **Perigenous**, in which none of the subsidiary or neighboring cells has a common origin with the guard cells.
3. **Mesoperigenous**, in which at least one of the subsidiary or neighboring cells is directly related ontogenetically to the guard cells and the others are not.

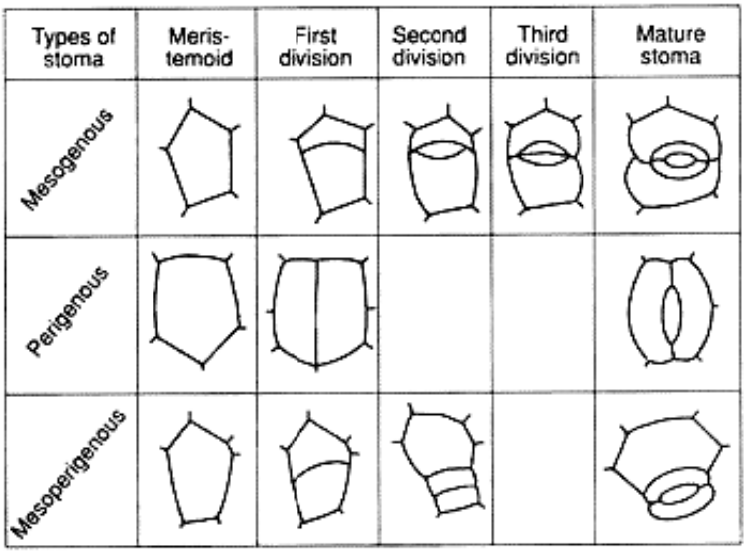


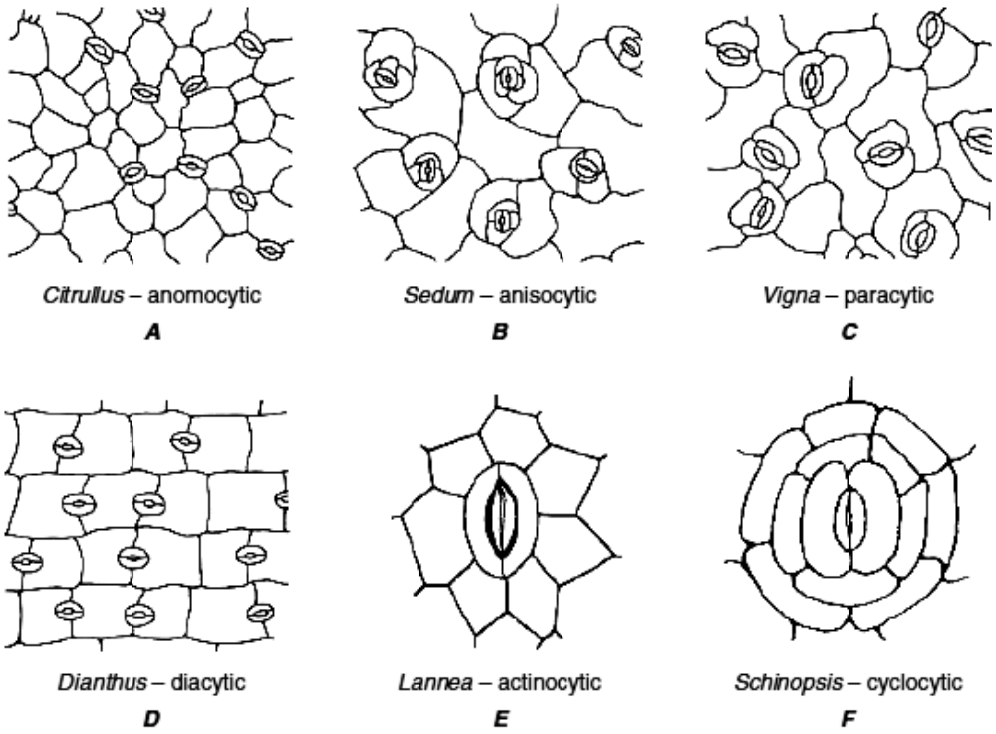
Fig. 5.76 : Diagrammatic representation of ontogenetic types of stomata

Morphologically, four main types of stomata have been distinguished in the dicotyledons, on the basis of the arrangement of the epidermal cells neighbouring the guard cells (Metcalfe and Chalk, 1950).

1. **The Ranunculaceous or anomocytic type** in which the guard cells are surrounded by a certain number of cells that do not differ in size and shape from the other epidermal cells. This type is common in the Ranunculaceae, Geraniaceae, Capparidaceae, Cucurbitaceae, Malvaceae, Scrophulariaceae, Tamaricaceae and Papaveraceae.
2. **The Cruciferous or anisocytic type** in which the guard cells are surrounded by three unequally sized subsidiary cells. This type is common in the Cruciferae, in Nicotiana, Solanum, Sedum and others.
3. **The Rubiaceous or paracytic type** in which each guard cell is accompanied by one or more subsidiary cells, the longitudinal axes of which are parallel to that of the guard cells and aperture. This type is common in the Rubiaceae, Magnoliaceae, most species of the Convolvulaceae and Mimosaceae, some genera of the Papilionaceae, such as Ononis, Arachis, Phaseolus and Psoralea, and various species of other families.

4. **Caryophyllaceous or diacytic type** in which each stoma is surrounded by two subsidiary cells, the common wall of which is at right-angles to the longitudinal axis of the stoma, This type is common in the Caryophyllaceae, Acanthaceae and others.

Actinocytic, in which the stoma is surrounded by a circle of radiating cells whose long axes are perpendicular to the outline of the guard cells; **cyclocytic** (encyclocytic), in which the stoma is surrounded by one or two narrow rings of subsidiary cells, numbering four or more; **tetracytic**, in which the stoma is enclosed by four subsidiary cells, two lateral and two polar (terminal), also found in many monocots.



Functions of Stomata:

Stomata are the gateway through which gaseous exchange takes place between the plants' interior and the atmosphere. When the stomata remain open CO_2 and O_2 are exchanged with the atmosphere and at the same time water is lost in the form of vapour from internal tissues of the leaf called transpiration.

Carbon dioxide is required during photosynthesis and oxygen is required for respiration. If the photosynthetic by-product oxygen concentration becomes greater than atmosphere it diffuses out from the stomatal cavity to the atmosphere through the stomata.

Pathogens may infect the foliage through stomata. The stomata are very much sensitive to environmental changes. The stomatal size and frequency are reduced in dry and polluted air and these changes can be criteria for determining air pollution. Thus stomatal characterisctics of certain fern taxa can be used as biomonitors of air-pollution.

Epidermal appendages/ Trichome:

All unicellular and multicellular appendages of the epidermis are designated by the term **trichome**. They may occur on all parts of the plant and may persist throughout the life of the plant part or may fall off early.

Trichomes are usually distinguished from emergences, such as warts and prickles, which are formed from both epidermal and subepidermal tissue and typically are more massive than trichomes. The distinction between trichomes and emergences is not sharp, however, because some trichomes are

elevated upon a base consisting of subepidermal cells. Thus a developmental study may be necessary to determine whether some outgrowths are solely epidermal in origin or both epidermal and subepidermal in origin.

Characteristics:

1. These are the elongations or extensions of epidermal cells.
2. They are found singly or in groups.
3. Trichomes may be unicellular or multicellular. Morphologically, root hair are typical hair.
4. They are highly variable in shape varying from small protuberances of epidermal cells to very complex, multicellular, branched or stellate structures.
5. Hair may consist of either living or dead cells.
6. Hair with living cells contains very little cytoplasm in their cells.
7. They persist throughout the life of a plant part or may fall very soon.
8. Innumerable types of the hair are known, of which some are uniseriate hair, stinging hair, mucilage hair, etc.

Types of trichomes:

Some morphological trichome categories are (1) **papillae**, which are small epidermal outgrowths often considered distinct from trichomes; (2) **simple (unbranched) trichomes**, a large grouping of extremely common unicellular and multicellulartrichomes; (3) **two-** to **five armed trichomes** of various shapes; (4) **Stellate trichomes**, all of which are star-shaped although variable in structure; (5) **scales**, or **peltate**, **trichomes**, consisting of a discoid plate of cells often borne on a stalk or attached directly to the foot; (6) **dendritic (branched) trichomes**,which branch along an extended axis; and (7) **root hairs**.

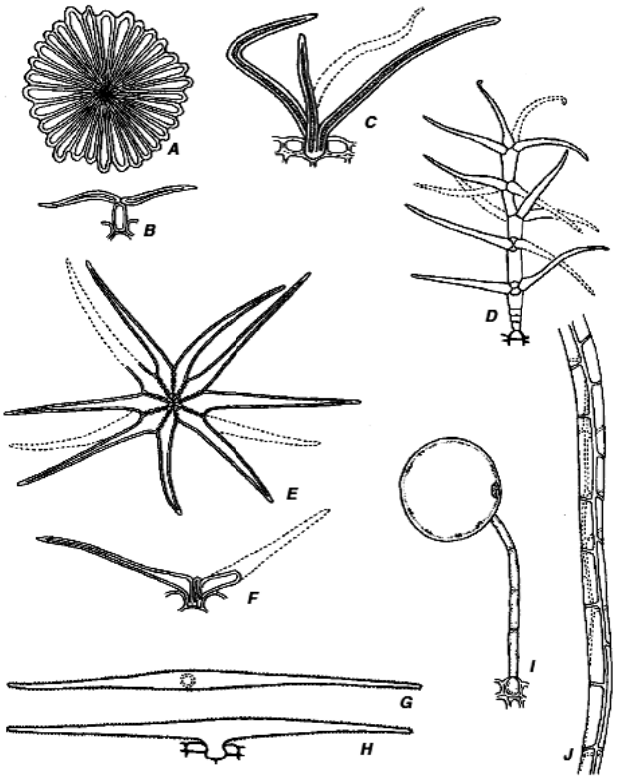


FIGURE 9.24

Trichomes. **A**, **B**, peltate scale of *Olea* in surface **(A)** and side **(B)** views. **C**, tufted, stellate hair of *Quercus*. **D**, dendritic hair of *Platanus*. **E**, **F**, stellate hair of *Sida* in surface **(E)** and side **(F)** views. **G**, **H**, two-armed, T-shaped unicellular hair of *Lobularia* in surface **(G)** and side **(H)** views. **I**, vesiculate hair of *Chenopodium*. **J**, part of multicellular shaggy hair of *Portulaca*. (A–C, I, ×210; D–H, J, ×105.)

In addition there are many specialized types of trichomes such as stinging hairs, pearl glands, cystolith-containing hairs and water vesicles.

Stinging Hairs:

They are one of the most interesting types of the trichomes. It contains a poisonous liquid and consists of a basal bulb like portion from which a stiff, slender and tapering structure is given out. This tapering structure ends in a small knob like or a sharp point.



Stinging Hairs

The tip is usually somewhat oblique, and as the body of an animal or human being comes in its contact with some force, the tip is broken off, and the sharp pointed end readily penetrates the skin of the animal, and fluid is being transferred from the basal knob of the hair to the body of the animal.

Functions of trichomes:

1. Control of transpiration rate
2. Reduction of heating effect of sunlight

Significance for taxonomy:

The type, presence and absence and location of trichomes are important diagnostic characters in plant identification and plant taxonomy. In forensic examination, plants such as Cannabis sativa can be identified by microscopic examination of the trichomes. Although trichomes are rarely found preserved in fossils, trichome bases are regularly found and, in some cases, their cellular structure is important for identification.

Significance for plant molecular biology:

In the model plant *Arabidopsis thaliana*, trichome formation is initiated by the GLABROUS1 protein. Knockouts of the corresponding gene lead to glabrous plants. This phenotype has already been used in genome editing experiments and might be of interest as visual marker for plant research to improve gene editing methods such as CRISPR/Cas9. Trichomes also serve as models for cell differentiation as well as pattern formation in plants

Significance in Defense

Plants may use trichomes in order to deter herbivore attacks via physical and/or chemical means, e.g. in specialized, stinging hairs of *Urtica* (Nettle) species that deliver inflammatory chemicals such as histamine. Studies on trichomes have been focused towards crop protection, which is the result of deterring herbivore. Secrtary structures:

The tissues that are concerned with the secretion of gums, resins, volatile oils, nectar latex, and other substances in plants are called secretory tissues.

External Secretory Structures:

External surfaces of the plant bear several secretory structures of epidermal origin or epi-dermal derivatives or emergences from deeper tissues. These include glands, glandular trichomes, nectaries, osmophores, hydathodes and salt glands.

1. Glandular Trichomes:

The epidermal surfaces of leaves and flowers of many plants contain glandular trichomes and glands. The glandular trichomes consist of a unicellular or multicellular stalk with a head above. The cells of multicellular stalk may be arranged in several rows.

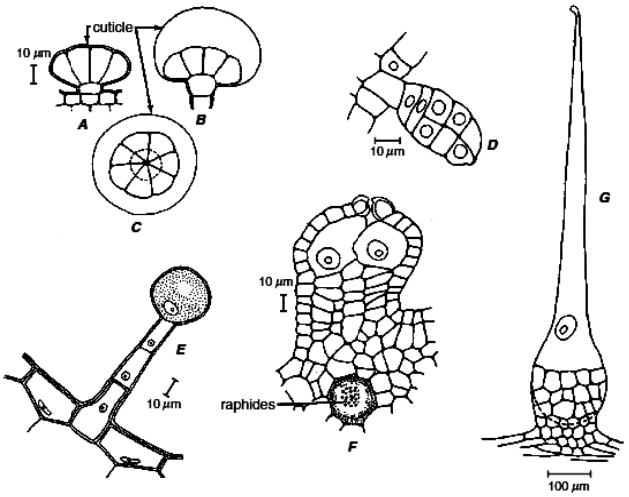


FIGURE 16.1

Secretory structures. A–C, glandular trichomes from the leaf of lavender (*Lavandula vera*) with cuticle undistended (A) and distended (B, C) by accumulation of secretion. D, glandular trichome from leaf of cotton (*Gossypium*). E, glandular trichome with unicellular head from stem of *Pelargonium*. F, pearl gland from leaf of grapevine (*Vitis vinifera*). G, stinging hair of nettle (*Urtica urens*). (From Esau, 1977.)

The unicellular (e.g. *Pelargonium*) or multicellular (e.g. *Callitriche*) heads of the glandular trichomes perform the actual function of secre-tion. The secretion may remain accumulated beneath the cuticle cover of the head such as the volatile oils like camphors, balsams, peppermint oil, resins etc. Glandular trichomes may also secrete nectar and water.

Glandular trichomes also play a role in the defence mechanism of the plant. Certain secretions may act as insect repel-lents. The glandular hairs of tomato and wild potato provide resistance to aphids by secreting sticky exudates that trap the aphids.

2. Nectaries:

Nectary is defined as a gland or any floral part that secretes nectar to attract the pollinators. Sometimes, it may occur on the vegetative organs and is not directly concerned with pollination. These structures are epidermal outgrowths or deeply sunken or at the level of the epidermis of the organ.

The nectariferous tissues may not form any anatomically differentiated structure known as **non-structural nectaries** (e.g., *Dracaena reflexa* leaves, bracts of *Sansevieria zeylanica*, tepals of *Cattleya percivaliana* etc.). Anatomically and morphologically, differentiated nectariferous tissues are called **structural nectaries**. These structures have stalks and specialised parenchymatous secretory cells with cuticle.

The nectary cells are small, thin-walled with dense protoplasts containing dictyosomes, endoplasmic reticulum, small vacuoles and large nuclei. The structures have vascular supplies through which nectar is supplied and accumulated between the cuticle and secretory cells.

Exudation of nectar from nonstructural nectaries takes place through stomata whereas in structural nectaries it takes place trough epidermal cells or trichomes directly to the outside.

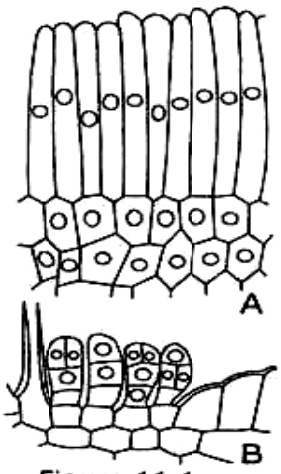


Figure 11.1

Nectary in sectional view. A. *Euphorbia pulcherima*. B. Extrafloral nectary on the stipule of *Vicia*.

The nectar contains glucose, sucrose and fructose as major components. In addition maltose, melobiose, mucilage, proteins, phosphates, mineral ions, organic acids, oxidases, sucrose, vitamins, essential amino acids, etc. are also found to be present in the nectar. The essential amino acids attract the insects for pollination.

The extra floral nectaries are very common in dicots and much less in monocots. It rarely occurs in Poaceae (e.g., *Andropogon* and *Eragrostis*). In dicots it is present on all the organs.

Hydathodes:

Hydathodes are structures that discharge liquid water with various dissolved substances from the interior of the leaf to its surface, a process called **guttation**. The water of guttation is forced out of the leaves by root pressure. Structurally, hydathodes are modified parts of

leaves, usually located at leaf tips or margins, especially at the teeth. In the usual form the hydathode consists of (1) the terminal tracheids of one to three vein endings, (2) the **epithem**, composed of thin-walled, chloroplastdefi cient parenchyma cells located above or distal to the vein endings, (3) a sheath—a continuation of the bundle sheath—that extends to the epidermis, and (4) openings, called **water pores**, in the epidermis.

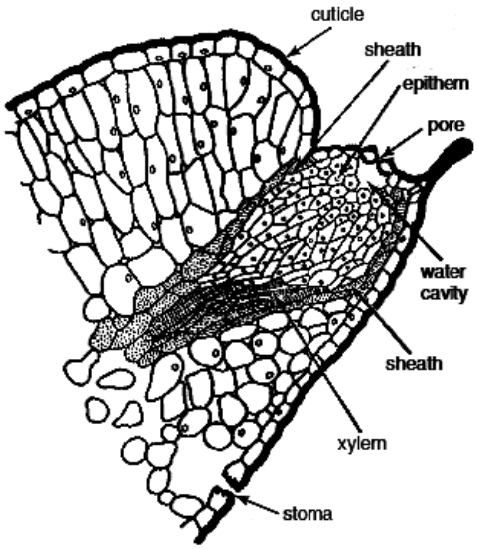


FIGURE 16.5

Hydathode of leaf of *Saxifraga lingulata* in longitudinal section. Tannin-containing sheath cells are stippled.

Colleters:

The term is derived from the Greek **colla**, glue, referring to the sticky secretions from these structures is common on bud scales and young leaves. The fluid they produce is mucilaginous or resinous in nature and insoluble in water. Colleters develop on young foliar organs, and their sticky secretion permeates and covers the entire bud.

The probable function of colleters is to provide a protective coating for the dormant buds and

to protect the developing meristem and young, differentiating leaves or their stipules.

Colleters are not trichomes. They are emergences formed from both epidermal and subepidermal tissues. The colleters are found in the members of Rubiaceae, Apocynaceae.

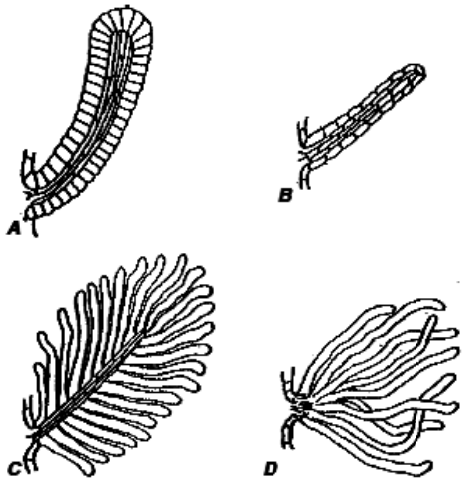


FIGURE 16.17

Different types of colleters. A, the most common standard type. B, the reduced standard. C, the dendroid. D, the brushlike. (From Lersten, 1974a. © Blackwell Publishing.)

Osmophores:

The fragrance of flowers is commonly produced by volatile substances—mainly terpenoids and aromatic compounds—distributed throughout the epidermis of perianth parts (Weichel, 1956; Vainstein et al., 2001). In some plants, however, the fragrance originates in special

glands known as **osmophores**, a term derived from the Greek words *osmo*, odor, and *pherein*, to bear. The term was first used by Arcangeli in 1883 for the fragrant spadix of certain members of the Araceae. The fragrances are attractive to the flowers pollinators. Examples of osmophores are found in Asclepiadaceae, Aristolochiaceae, Calycanthaceae, Saxifragaceae,

Solanaceae, Araceae, Burmanniaceae, Iridaceae, and Orchidaceae. Various floral parts may be differentiated as osmophores, and they may assume the form of flaps, cilia, or brushes.

Osmophores consist of glandular tissue usually several cell layers in depth. The outer layer

is formed by the epidermis, which is covered by a very thin cuticle. Two to five subepidermal layers may differ considerably in density from the subjacent ground tissue or intergrade imperceptibly with it.

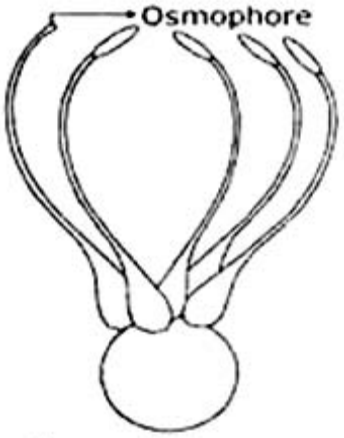


Figure 11.2
Osmophore in the flower of *Ceropegia loranthiflora*

Internal Secretory Structure:

The internal secretory structures may be composed of a single cell or groups of cells. They may occur throughout a tissue (e.g. oils or enzymes) or may be localized in distribution.

Example: castor oil obtained from the endosperm of *Ricinus*; ground nut oil extracted from the cotyledons of *Arachis* etc. Sometimes resin or oil secreting **idioblasts** are formed, e.g. oleo-resin cells are present in the ground tissue of the rhizome of *Zingiber officinale*, the oil cells secreting the aromatic oil occur in the phloem of *Cinnamomum zeylanicum*.

Secretory cavities and ducts:

Secretory cavities and ducts (canals) differ from secretory cells in that they secrete substances into intercellular spaces. They are glands consisting of relatively large intercellular spaces commonly lined by specialized specialized secretory (epithelial) cells. **Secretory cavities** are

short secretory spaces and **secretory ducts** are long secretory spaces. In some plants (*Lysimachia, Myrsine, Ardisia*) resinous material is secreted by parenchyma cells into ordinary intercellular spaces and forms a granular layer along the wall. The contents of the cavities and ducts may consist of terpenoids or carbohydrates or of terpenoids together with carbohydrates and other substances.

Three developmental types of secretory cavities and ducts have been recognized: schizogenous, lysigenous, and schizolysigenous.

Schizogenous cavities and ducts form by a separation of cells, resulting in a space lined with secretory cells composing the epithelium.

Lysigenous cavities and ducts result from a dissolution (autolysis) of cells. In these cavities and ducts the secretory product is formed in the cells that eventually break down and release the product into the resultant space (holocrine secretion). Partly disintegrated cells occur along the periphery of the space.

The development of **schizolysigenous** cavities and ducts initially is schizogenous, but lysigeny occurs in later stages as the epithelial cells lining the space undergo autolysis, further enlarging the space. There is some inconsistency in use of the schizolysigenous category of duct development in those cases in which only occasional epithelial cells

undergo autolysis after the secretory phase. Some investigators consider such ducts as schizolysigenous, but others regard them as schizogenous.

Laticifers:

Laticifers are cells or series of connected cells containing a milky fluid called **latex** (plural, **latices**) and forming systems that permeate various tissues of the plant body. Although the structures bearing latex may be singlecells or series of fused cells, both kinds often produce

complex systems of tube-like growth form in which recognition of the limits of individual cells is highly problematical. The term laticifer therefore appears most useful if applied to either a single cell or a structure resulting from fusion of cells. A single-celled laticifer can be qualified, on the basis of origin, as a **simple**, and the structure derived from union of cells as a **compound laticifer**.

Types:

Articulated laticifers (i.e., jointed laticifers) are compound in origin and consist of longitudinal chains of cells in which the walls separating the individual cells either remain intact, become perforated, or are completely removed. The perforation or resorption of the end walls gives rise to laticifers that are tube-like in form and resemble xylem vessels in origin. Articulated laticifers may arise in both primary and secondary bodies of the plant. **Nonarticulated laticifers** originate from single cells that through continued growth develop into tube-like structures, often much branched, but typically they undergo no fusions with other similar cells. They are simple in origin, and typically arise in the primary plant body.

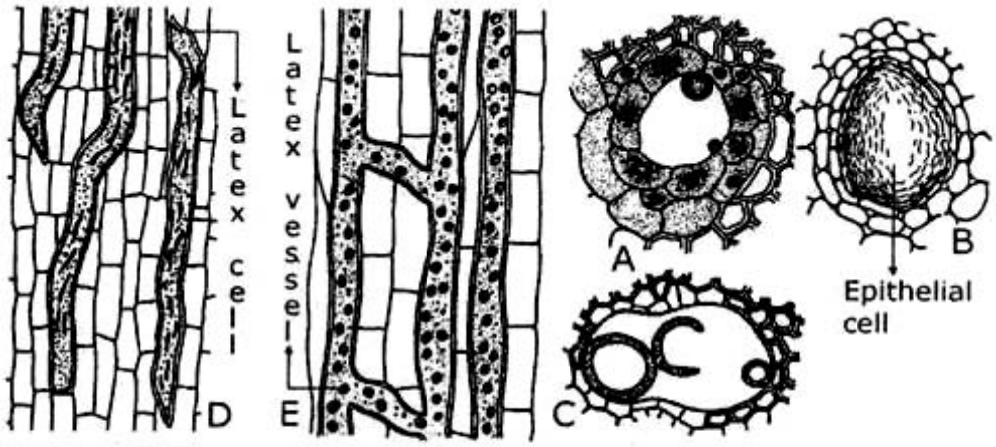


Figure 11.3

Diagrams showing D. latex cell of *Euphorbia pilulifera* (non-articulate), E. latex vessel of *Carica papaya* (articulate) and intercellular spaces : A. Resin duct of *Pinus* (schizogenous), B. oil-cavity of *Citrus* fruit (Lysigenous) and C. Protoxylem lacuna of maize (schizo-lysigenous).

4. Phylogeny and evolution of vascular and mechanical tissue elements: origin and development of sclereids and fibres, phylogeny of xylem and phloem elements, wood anatomy: vascular cambium and its seasonal activity.

Sclereids

Sclereids are short cells with thick secondary walls, more or less isodiametric, strongly lignified, and provided with numerous simple pits. Some sclereids have relatively thin secondary walls, however, and may be difficult to distinguish from sclerified parenchyma cells. The thick-walled forms, on the other hand, may strongly contrast with parenchyma cells: their walls may be so massive as almost to occlude the lumina, and their prominent pits often are ramiform. The secondary wall typically appears multilayered, refl ecting its helicoidal construction. Crystals are embedded in the secondary walls of certain species. Many sclereids retain living protoplasts at maturity. In many plants sclereids appear as **idioblasts**, i.e. as cells which are readily distinguished from the surrounding ceils of the tissue by their size, shape and the thickness of their wall. Idioblastic sclereids are very variable in shape, Sclereids of peculiar shape are found in the leaves of various plants, e.g. in *Camellia, Trochodendron, Nymphaea* and *Olea.*

Types:

Based on shape and size, Sclereids may be classified into -

(1) **brachysclereids**, or **stone cells**, roughly isodiametric or somewhat elongated cells, widely distributed in cortex, phloem, and pith of stems, and in the flesh of fruit; (2) **macrosclereids**, elongated and columnar (rod-like) cells, exemplified by sclereids forming the palisade-like epidermal layer of leguminous seed coats; (3) **osteosclereids**, bone cells, also columnar but with enlarged ends as in the subepidermal layer of some seed coats; and (4) **astrosclereids**, star-cells, with lobes or arms diverging from a central body, often found in the leaves of eudicots. Other less commonly recognized types include **trichosclereids**, thin-walled sclereids resembling hairs, with branches projecting into intercellular spaces, and **filiform sclereids**, long slender cells resembling fibers.

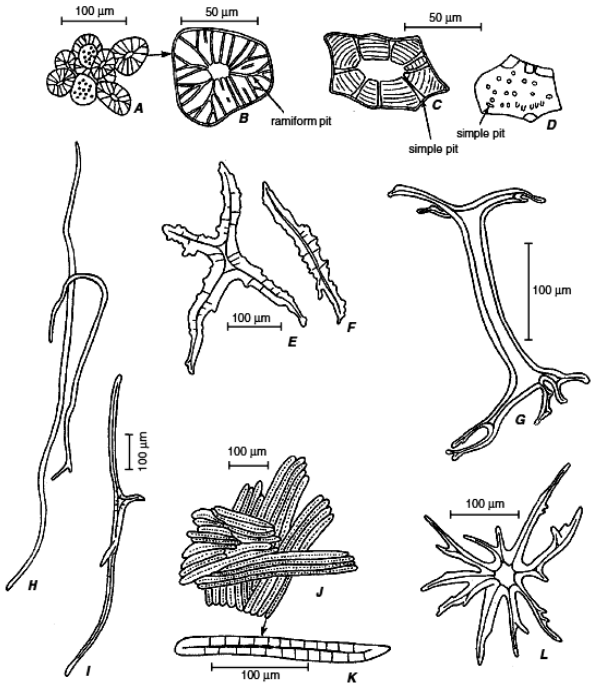


FIGURE 8.10

Sclereids. **A**, **B**, stone cells from fruit flesh of pear (*Pyrus*). **C**, **D**, sclereids from stem cortex of wax plant (*Hoya*), in sectional (C) and surface (D) views. **E**, **F**, sclereids from petiole of *Camellia*. **G**, columnar sclereid with ramified ends from palisade mesophyll of *Hakea*. **H**, **I**, filiform sclereids from leaf mesophyll of olive (*Olea*). **J**, **K**, sclereids from endocarp of fruit of apple (*Malus*). **L**, astrosclereid from stem cortex of *Trochodendron*. (From Esau, 1977.)

Fibers:

A **fiber** is an elongated, tapering sclerenchyma cell with a more or less thick secondary wall. Like sclereids, fibers may be found in various parts of the plant. Fibers are slender, elongated cells, owing to extreme apical elongation during differentiation, and are provided with a multiple-layered secondary wall. The mature fiber wall may be so thick that the cell lumen can be almost or entirely occluded. Most fibers are nonliving cells at maturity, although living fibrous elements are found in the wood of some dicotyledons.

Fibers serve as supporting elements in plant parts that are no longer elongating. The degree of lignification varies, and typicallymthe simple or slightly bordered pits are relatively scarce and slit-like. Many fibers retain their protoplasts at maturity.

According to their position in the plant body, fibres are classified into two basic types - **xylary** and **extraxylary fibres**.

Xylary fibres constitute an integral part of the xylem and they develop from the same meristematic tissues as do the other xylem elements. These fibres are of varied shape in spite of their common origin.

The xylary fibres are classified into:

(1) Libriform fibre, and

(2) Fibre-tracheid on the basis of pit and thickness of the wall.

1. Libriforn fibre:

These fibres are longer in length, have thicker cell wall with simple pit in contrast to tracheids of the plant in which they occur. The pit canal is longer than that of tracheids. The inner aperture of a pit-pair appears slit-like due to wall thickening and is usually formed at right angles to outer aperture.

2. Fibre-tracheid:

This fibre is longer in length, has thicker cell wall with bordered pit than tracheids but shorter in length, has thinner cell wall than libriform fibre of the plant in which they occur. The border of the bordered pit is much reduced than those of tracheids.

As a result the pit chamber becomes smaller than tracheids. The inner aperture appears as slit due to thickening and is usually formed at right angles to outer aperture. Thus the fibre-tracheids are intermediate in all respects between tracheids and libriform fibres.

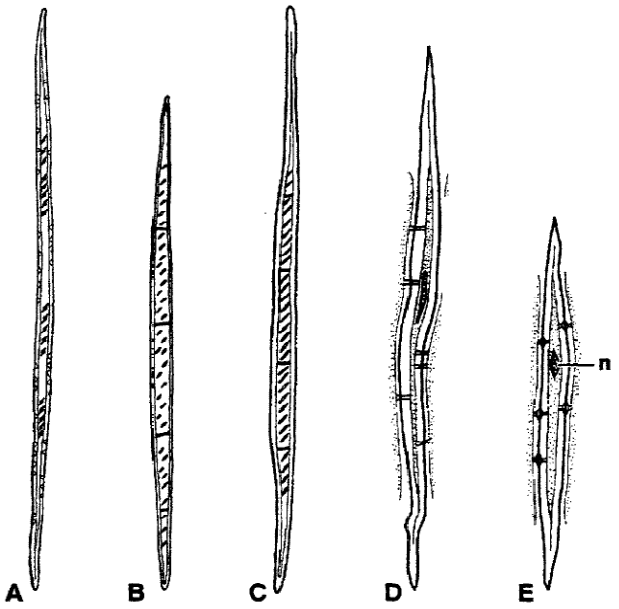


FIGURE 2.26 Xylary fibers. (A) Libriform fiber of white oak, *Quercus alba* (Fagaceae). (B, C) Septate fibers from *Tectona grandis* (Verbenaceae). (D) Living, nucleated libriform fiber of *Suaeda monoica* (Chenopodiaceae). (E) Living, nucleated fiber tracheid of *Teucrium divaricatum* (Labiatae) showing nucleus

Gelatinous or mucilaginous fibre:

These fibres are characterized by either absence or presence of lignin in little amount on the cell wall. Thus gelatinous or mucilaginous fibres differ from other xylary fibres in having less lignin and much a-cellulose on their cell wall, α-cellulose molecules are deposited on inner secondary wall of these fibres instead of lignin.

Gelatinous fibres are found in tension wood of dicotyledon. (Tension wood is one type of reaction wood that is formed in leaning branches or trunk of dicots. The tension wood, i.e. secondary xylem is formed on the upper side of the branch or trunk when they lean due to gravity. These woods usually show eccentric growth rings).

(ii) Extraxylary fibre:

These fibres occur in the cortex, pith and in association with phloem as phloem or bast fibre. They never associate with xylem elements. In monocotyledonous stems the extraxylary fibre may surround the individual bundles-termed bundle-sheath; it may form an uninterrupted cylinder on the ground tissue. These fibres originate partly from ground meristem and the rest from the procambium.

In some dicotyledons (e.g. *Aristolochia*, *Cucurbita* etc.) fibres occur as bands or uninterrupted cylinder on the peripheral side of vascular cylinder and at the innermost layers of cortex. These fibres originate from the ground meristem and termed as pericyclic fibres. The structure, content and shape of these fibres are more or less like xylary fibres.

Among the extraxylary fibers are the **phloem fibers**. Phloem fibers occur in many stems. The flax (*Linum usitatissimum*) stem has only one band of fi bers, several layers in depth, located on the outer periphery of the vascular cylinder. These fibers originate in the earliest part of the primary phloem (the protophloem) but mature as fibers after this part of the phloem ceases to function in conduction. Flax fibers are, therefore, **primary phloem fibers**, or **protophloem fibers**. The stems of *Sambucus* (elderberry), *Tilia* (basswood), *Liriodendron*

(tulip tree), *Vitis* (grapevine), *Robinia pseudoacacia* (black locust), and many others, have both primary phloem fibers and **secondary phloem fibers**, which are located within the secondary phloem.

Two other groups of extraxylary fibers encountered in the stems of eudicots are the cortical fibers and the perivascular fibers. **Cortical fibers**, as the name implies, originate in the cortex. **Perivascular fi bers** are located on the periphery of the vascular cylinder inside the innermost cortical layer (*Aristolochia* and *Cucurbita*). They do not originate as part of the phloem tissue but outside it. Perivascular fibers are commonly referred to as pericyclic fibers.

Function of Fibre:

(i) Fibres are mechanical cells that support the organs in which they occur;

(ii) They protect the inner tissues due to the presence of thick wall;

(iii) Septate fibres are the storage tissue where starch and oils are present;

(iv) Fibres exhibit elastic properties and so can withstand tension

Origin and development Fibers and Sclereids:

Fibers arise from various meristems: those of the xylem and phloem from the procambium and vascular cambium; most extraxylary fibers other than phloem fibers from the ground meristem; and the fibers of some Poaceae and Cyperaceae from the protoderm.

Sclereids also arise from different meristems: those of the vascular tissues from derivatives of procambial and cambial cells; stone cells embedded in cork tissue from the cork cambium, or phellogen; the macrosclereids of seed coats from the protoderm; and many other sclereids from the ground meristem.

The development of the usually long fibers and of branched and long sclereids involves remarkable intercellular adjustments. Primary extraxylary fibers are initiated before the organ has elongated, and they can reach considerable length by elongating in unison with the other tissues in the growing organ. During this period of growth the walls of adjacent cells are so adjusted that no separation of the walls occurs. This method of growth is called **coordinated growth**. The young fiber primordium increases in length without changing cellular contacts whether or not adjacent parenchymatous cells are dividing. The growth of primary extraxylary fibers in unison with the other tissues in the growing organ results in longer fibers commonly being found in longer organs (Aloni and Gad, 1982).

The great length attained by some primary extraxylary fibers is not the result of elongation by coordinated growth only. Somewhat later, the fiber primordium attains additional length by **intrusive growth** . During intrusive growth the elongating cells grow at their apices (**apical intrusive growth**), usually at both ends between the walls of other cells. During elongation, the fiber may become multinucleate as a result of repeated nuclear divisions not followed by formation of new walls. This is especially true of primary phloem fibers.

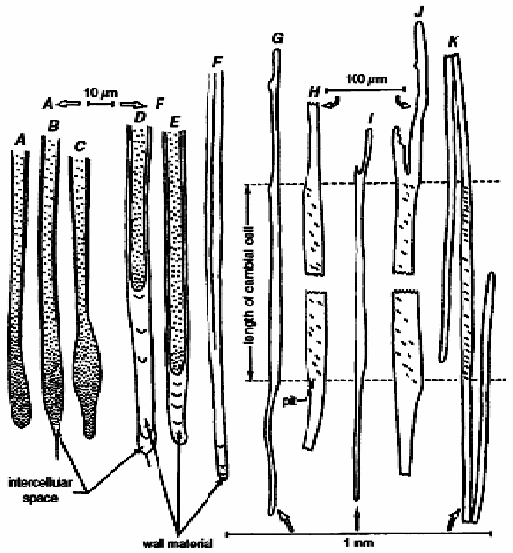


FIGURE 8.15

Apical intrusive growth in fibers from stems. A–F, from phloem of flax (*Linum perenne*). G–J, from xylem and, K, from phloem of *Sparrmannia* (Tiliaceae). H, J, enlarged views of parts of G, I, respectively. A–C, the intrusively growing tips of fibers (below) have thin walls and dense cytoplasm. D–F, the tips of fibers have become filled with wall material after completion of growth. G–K, fibers have extended in both directions from the original position in the cambium (between broken lines). Pits occur only in the original cambial parts. The phloem fiber (K) is considerably longer than the xylem fibers (G, I). (From Esau, 1977; A–F, adapted from Schoch-Bodmer and Huber, 1951; G–K,

The **xylem** is the principal water-conducting tissue in a vascular plant. It is also involved in the transport of solutes, in support, and in food storage. Together with the phloem, the principal food-conducting tissue, the xylem forms a continuous vascular system extending throughout the plant body. As components of the vascular system, xylem and phloem are called **vascular tissues**.

Xylem is a complex tissue as it consists of several types of cells. The most important cells are the **tracheary elements** which are, the non,living cells that are principally concerned with the transport of water and which in certain degree have a supporting function. **Fibers** are present in the xylem where they are mainly concerned with the strengthening of the plant body. **Sclereids** also may be sometimes

present. **Parenchyma** cells which have storage and other functions also occur in the xylem. The xylem of some plants contains laticifers.

The xylem and phloem elongates in developing prgans by the continual differentiation of new elements produced by the **procambium**, which itself is continuously produced by the apical promeristem. The xylem produced by the procambium in the primary body is called·the **primary xylem**. In many plants, after the completion of the formation of the primary body, secondary tissues are developed. The xylem that is produced as a result of the activity of the vascular cambium is called the **secondary xylem**.

In the primary xylem the elements that are completed early, i.e. the **protoxylem**, are distinguished from those completed later, i.e. the **metaxylem**.

Tracheary Elements

The term tracheary element is derived from "trachea," a name originally applied to certain primary xylem elements resembling insect tracheae (Esau, 1961). Two fundamental types of tracheary elements occur in the xylem, the **tracheids** and the **vessel elements, or vessel members**. Both are more or less elongated cells that have lignified secondary walls and are nonliving at maturity. They differ from one another in that tracheids are imperforate cells having only pit-pairs on their common walls, whereas vessel elements also have perforations, which are as lacking both primary and secondary walls through which the vessel elements are interconnected.

A **vessel**, which is also termed a trachea, is built of numerous vessel members that are joined one to the other by their end walls. Vessels are terminated by a vessel member of which the proximal end wall is perforated, whereas the distal end wall is not, i.e. the distal parts of a vessel.

is tracheid-like The part of the vessel element wall bearing the perforation or perforations is called the **perforation plate**. A perforation plate may have a single perforation (**simple perforation plate** and) or several perforations (**multiple perforation plate**). The perforations in a multiple perforation plate may be elongated and arranged in a parallel series (**scalariform perforation plate**, from the Latin scalaris, ladder), or in a reticulate manner

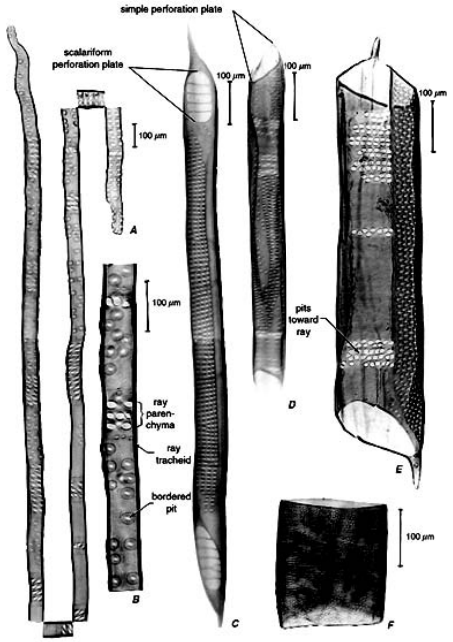
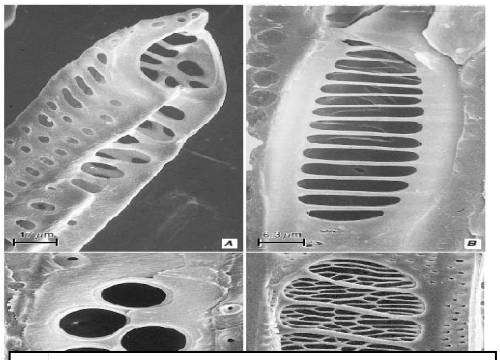


FIGURE 10.2

Tracheary elements. **A**, earlywood tracheid of sugar pine (*Pinus lambertiana*). **B**, enlarged part of **A**. **C–F**, vesse elements of tulip tree, *Liriodendron tulipifera* (**C**), beech, *Fagus grandifolia* (**D**), black cottonwood, *Populus trichc carpa* (**E**), tree-of-heaven, *Ailanthus altissima* (**F**). (From Carpenter, 1952; with permission from SUNY-ESF.)

(**reticulate perforation plate**, from the Latin rete, net), or as a group of approximately circular holes (**foraminate perforation plate**). Perforations generally occur on the end walls, with the vessel elements joined end-on-end, forming long, continuous columns, or tubes, called **vessels**.



SEM of the perforated end walls of vesselelements.**A**Simple perforation plate, **B** Scalariform **C** Foraminate **D** Contiguous scalariform and reticulate.

In many plants the secondary wall thickening of the first-formed xylem (protoxylem) is annular or helical. The 'helical thickening may be single, or more than one helix may be present in a single element. The rings or helices may be arranged in a loose or acdense manner. From an ontogenetic view point ,the annular elements precede the helical elements.In later-formed tracheary elements the helical bands become joined in certain areas giving rise to a ladder-like type thickening; such thickening is termed **scalariform thickening**. In tracheary elements formed at a still later ontogenetic stage the wall thickening is in the form of a network, i.e. **reticulate thickening**. When the openings in the secondary wall of such a network are elongated in a direction perpendicular to the longitudinal axis of the element, the thickening is termed **scalariform-reticulate**

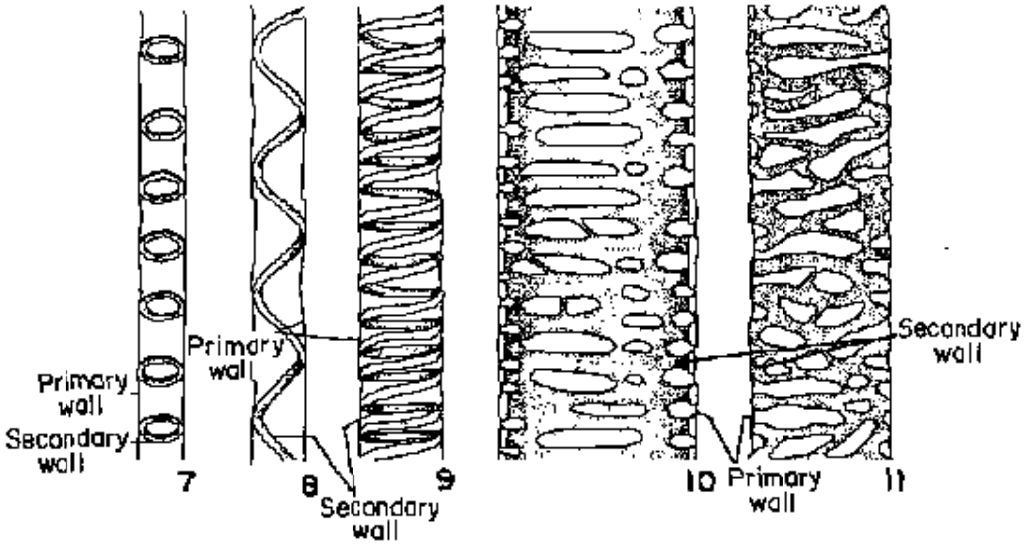


Fig. 7. Annular thickening, 8. Helical thickening. 9. Dense helical thickening, 10. Scalariform thickening. 11. Reticulate thickening.

Simple and bordered pits are found in the secondary walls of tracheids and vessel elements of the latestformed primary xylem and of the secondary xylem. The number and arrangement of these pits are highly variable, even on different wall facets, or surfaces, of the same cell, because they depend on the type of cell bordering the particular wall facet. Usually numerous bordered pit-pairs occur between contiguous tracheary elements (**intervascular pitting**); few or no pit-pairs may occur between tracheary elements and fibers; bordered, half-bordered, or simple pit-pairs are found between tracheary elements and parenchyma cells. In half-bordered pit-pairs the border is on the side of the tracheary element.

The bordered pits in tracheary elements show three main types of arrangement: scalariform, opposite, and alternate. If the pits are elongated transversely and arranged in vertical, ladder-like series, the

pattern is called **scalariform pitting**. Circular or oval bordered pits arranged in horizontal pairs or short horizontal rows characterize **opposite pitting**. If such pits are crowded, their borders assume rectangular outlines in face view. When the pits are arranged in diagonal rows, the arrangement is **alternate pitting**, and crowding results in borders that are polygonal (angular and with more than four sides) in outline in face view. Alternate pitting is clearly the most common type of pitting in eudicots.

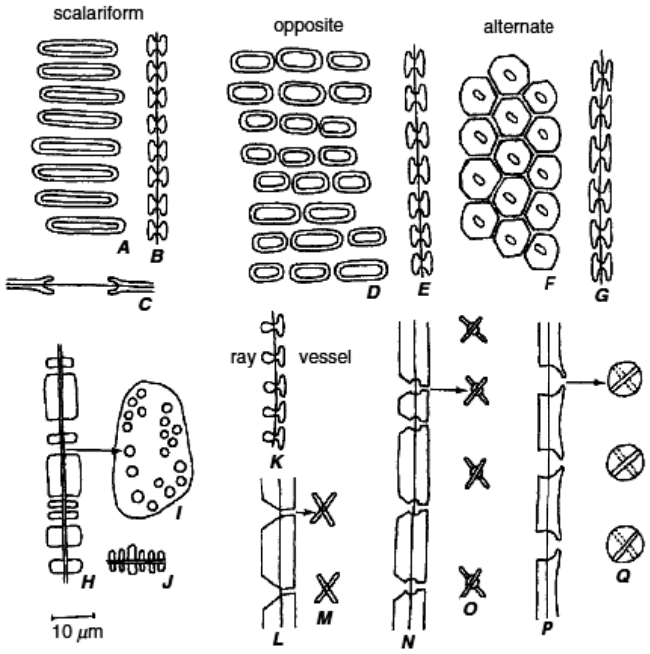


Fig. Pits and patterns of pitting.

Xylem Parenchyma:

Parenchyma that forms one of the constituent of xylem is referred to as xylem parenchyma.

Xylem parenchyma is present in primary and secondary xylem and respectively originates from procambium and cambium. In secondary xylem xylem-parenchyma is also termed as **wood parenchyma** and is present as **axial** and **radial** parenchyma that accordingly occur as parallel and perpendicular to the long axis of organ where they lie.

Xylem Fibre:

Fibre that occurs as element of xylem is called xylem fibre. It is also referred to as xylary fibre and wood fibre.

Xylary fibres are elongated cells the length of which is many times longer than breadth. The two ends of a fibre are tapering to a wedge shape. The cell wall is usually thick. Due to lignin deposition the lumen of fibre becomes narrow.

Phylogenetic specialization of tracheary Elements and fibers:

The xylem occupies a unique position among plant tissues in that the study of its anatomy has come to play an important role with reference to taxonomy and phylogeny. The lines of specialization of the various structural features have been better established for the xylem than for any other single tissue. Among the individual lines, those pertaining to the evolution of the tracheary elements have been studied with particular thoroughness.

The tracheid is a more primitive element than the vessel element. It is the only kind of tracheary element found in the fossil seed plants (Stewart and Rothwell, 1993; Taylor and Taylor, 1993) and in most of the living seedless vascular plants and gymnosperms (Bailey and Tupper, 1918; Gifford and Foster, 1989). The specialization of tracheary elements coincided with the separation of the functions of conduction and strengthening that occurred during the evolution of vascular plants (Bailey, 1953).

In the less specialized state, conduction and support are combined in tracheids. With increased specialization, conducting elements—the vessel elements—evolved with greater efficiency in conduction than in support. In contrast, fibers evolved as primarily strengthening elements. Thus from primitive tracheids two lines of specialization diverged, one toward the vessels and the other toward the fibers.

Vessel elements evolved independently in certain ferns, including the whisk ferns, *Psilotum nudum* and *Tmesipteris obliqua* (Schneider and Carlquist, 2000c; Carlquist and Schneider, 2001), Equisetum (Bierhorst, 1958), Selaginella (Schneider and Carlquist, 2000a, b), the Gnetophyta (Carlquist, 1996a), monocots, and "dicots" (Austrobaileyales, magnoliids, and eudicots). In the eudicots, vessel elements originated and underwent specialization first in the secondary xylem, then in the late primary xylem (metaxylem), and last in the early primary xylem (protoxylem). In the primary xylem of the monocots, origin and specialization of vessel elements also occurred first in the metaxylem, then in the protoxylem; furthermore, in the monocots, vessel elements appeared first in the root and later in stems, inflorescence axes, and leaves, in that order (Cheadle, 1953; Fahn, 1954).

In the secondary xylem of eudicots, species with vessel elements arose from ones with tracheids bearing scalariform bordered pits (Bailey, 1944). Transition from a vesselless to a vessel-containing condition involved loss of pit membranes from a part of the wall bearing several bordered pits. Thus, a pitted wall part became a scalariform perforation plate. Remnants of membranes occur in the perforations of vessel elements of many primitive eudicots and are regarded as a primitive feature in eudicots (Carlquist 1992, 1996b, 2001). The tracheid-vessel transition is not a sharp one; all degree of intermediacy may be found (Carlquist and Schneider, 2002).

The major trends in the evolution of the vessel element are correlated with decrease in vessel element length

1. Decrease in length. The most clearly established trend in evolution of the vessel elements is decrease in length. Longer vessel elements are found in more primitive groups (those with more numerous primitive floral features) and shorter vessel elements in more specialized ones (those with more numerous specialized fl oral features). The evolutionary sequence of vessel element types in the secondary\ xylem of eudicots began with long scalariformly pitted tracheids similar to those found in some primitive eudicots. These tracheids were succeeded by vessel elements of long narrow shape with tapering ends. The vessel elements then underwent a progressive decrease in length. The phylogenetic shortening of vessel elements is a particularly consistent characteristic and has occurred in all vascular plants that have developed vessels (Bailey, 1944). Other trends in vessel element evolution are defi ned by correlation with the decrease in vessel element length.

2. **Inclined to transverse end walls.** As the vessel elements shortened, their end walls became less inclined and fi nally transverse. Thus the vessel elements gradually acquired defi nite end walls of decreasing degree of inclination, in contrast to the tapering ends of tracheids.

3. **Scalariform to simple perforation plates.** In the more primitive state the perforation plate was scalariform, with numerous bars resembling a wall with scalariformly arranged bordered pits devoid of pit membranes. Increase in specialization resulted in removal of the borders and then a decrease in the number of bars until fi nally bars were totally eliminated. Thus a pitted wall part became a scalariform perforation plate, which later evolved into a simple perforation plate bearing a single opening

4. **Scalariform bordered pitting to alternate bordered pitting.** The pitting of vessel walls also changed during the evolution. In intervessel pitting, bordered pit-pairs in scalariform series were

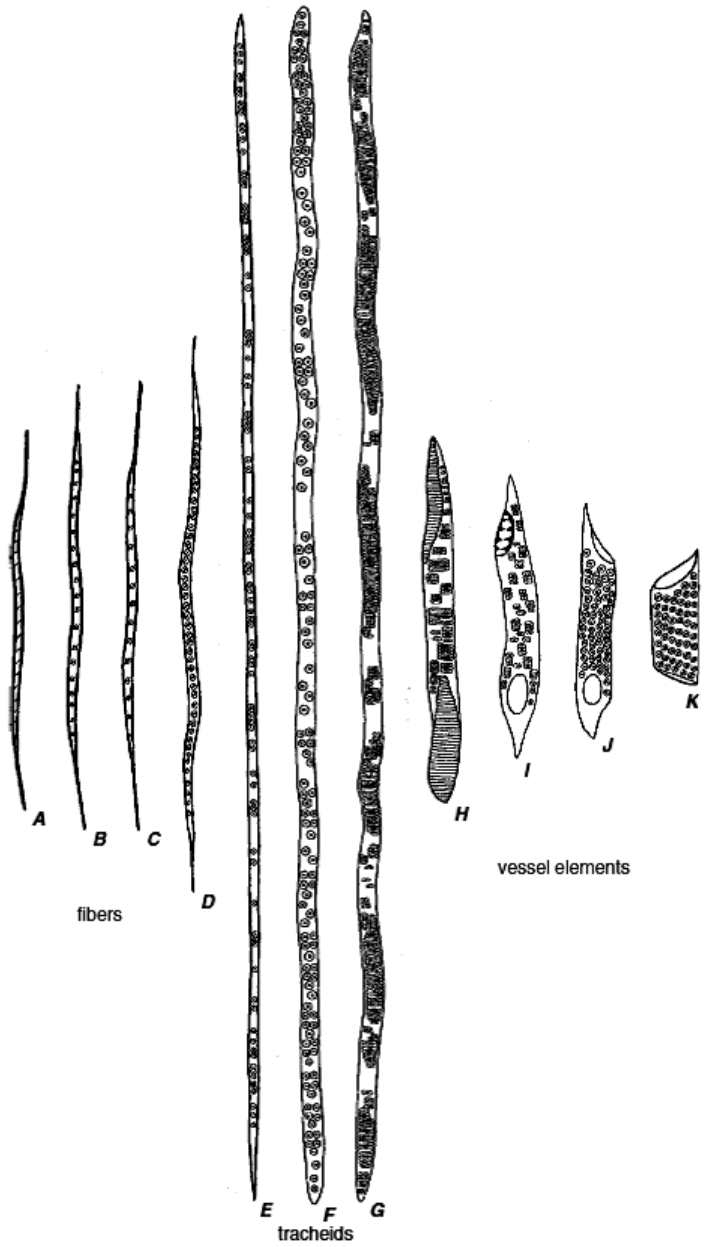


FIGURE 10.14

Main lines of specialization of tracheary elements and fibers. E–G, long tracheids from primitive woods. (G, reduced in scale.) E, F, circular bordered pits; G, elongated bordered pits in scalariform arrangement. D–A, evolution of fibers: decrease in length, reduction in size of pit borders, and change in shape and size of pit apertures. H–K, evolution of vessel elements: decrease in length, reduction in inclination of end walls, change from scalariform to simple perforation plates, and from opposite to alternate pit arrangement. (After Bailey and Tupper, 1918.)

replaced by bordered pit-pairs, first in opposite and later in alternate arrangement. The pit-pairs between vessels and parenchyma cells changed from bordered, through half-bordered, to simple.

5. **Vessel outline from angular to rounded (as seen in transverse section).** In eudicotyledonous vessels, angularity in outline is considered to be the primitive state and roundness a specialized

condition. Interestingly a correlation exists between angularity and narrowness of vessels. Vessels that are rounded in outline tend to be wider.

Presumably phylogenetic specialization of the vessel element proceeded in the direction of increased conductive efficiency or safety, although the relationship between the trends and their adaptive value is not always obvious. For example, there is little agreement on the functional value of decreased vessel element length, although shorter vessel elements are found in eudicots of drier habitats than in related eudicots of wetter habitats (Carlquist, 2001). The adaptive value of the trend from scalariform to opposite to alternate pits appears to be a gain in mechanical strength in the vessel wall rather than safety or conductivity (Carlquist, 1975). Although not as well defined a trend in vessel evolution as others, widening of vessel elements obviously resulted in greater conductive capacity.

The phloem is, thus, a distinctive, important and highly complex tissue through which photosynthate is transported throughout the plant. **Primary phloem** differentiates from provascular tissue and, with primary xylem, is a major component of stem vascular bundles, leaf traces, and the vascular systems of leaves, flower parts, fruits, and seeds.

The primary phloem consists of protophloem and metaphloem. **Protophloem** differentiates earlier and nearer the apical meristem than protoxylem, and in regions that are actively elongating. Consequently (especially in stems) the conducting elements are stretched and often obliterated. **Metaphloem** differentiates later than protophloem, in regions in which growth in length has ceased.

Secondary phloem, like secondary xylem, is derived from the vascular cambium, and is composed of axial and radial systems of cells. The axial system is made up of conducting cells, associated parenchyma cells, companion cells (in angiosperms), and phloem fibers. The radial system consists of phloem rays that are continuous with rays in the secondary xylem.

Sieve elements:

The conducting cells, longitudinally elongate, are collectively called **sieve elements**. There are two types of sieve elements: sieve cells characteristic of gymnosperms, pteridophytes, and other lower vascular plants, and sieve tube members, characteristic of angiosperms. These

cells develop from **sieve element mother cells** which are direct descendants of fusiform cambial initials. Following periclinal, longitudinal divisions, the mother cells in gymnosperms differentiate into functional **sieve cells**, generally with little or no longitudinal

(intrusive) growth. The mature sieve cells, like the fusiform features of sieve elements is the fact that they comprise longitudinal, open, but living, systems through which photosynthate is transported. At maturity, the sieve element protoplast is highly modified, lacking a nucleus and vacuolar membrane, but it retains a functional plasma membrane, and some parietal endoplasmic reticulum, mitochondria,and plastids.

Another distinctive feature of sieve elements is the presence of **sieve areas** in the lateral walls of sieve cells and on the end walls and, in some taxa, on lateral walls of sieve tube members. Sieve areas which are highly specialized and evolutionarily modified primary pit fields consist of groups of **pores** through which the protoplasts of contiguous conducting cells are connected cambial initials from which they are derived, are very long with overlapping ends. By contrast, in dicotyledons, transverse, anticlinal, and/or oblique divisions in the sieve element mother cells lead to columns of superposed **sieve tube members**. Among the distinctive Pores in dicotyledons are often lined with cylinders of **callose** (the carbohydrate, β-1,3-glucan) that enclose the plasma membrane, endoplasmic reticulum, and other cytoplasmic components that traverse the pores. Callose-lined pores have been reported in the Gnetales, but only rarely have been observed in other gymnosperms. It is known that wounding stimulates rapid synthesis of callose, and some evidence indicates that callose cylinders in sieve areas are the result of the stimulation that results from the cutting of a segment of stem to be sectioned. As a sieve element approaches the end of its functional life, callose accumulates in large quantities,

occluding the pores, and may even completely cover the surfaces of sieve areas. Callose in this state is referred to as **definitive callose**.

In sieve tube members sieve areas occur on end walls called **sieve plates**. A sieve plate containing a single sieve area is called a **simple sieve plate** whereas one with several sieve areas is a **compound sieve plate.**

Companion cells

Sieve-tube elements are characteristically associated with specialized parenchyma cells called **companion cells**. Typically, companion cells are derived from the same mother cell as their associated sieve-tube elements, so that the two kinds of cells are closely related ontogenetically. In the formation of the companion cells the meristematic precursor of the sieve-tube element divides longitudinally one or more times. One of the resulting cells, usually distinguished by being larger, differentiates into the sieve-tube element. One or more companion cells may be associated with a single sieve-tube element, and the companion cells may occur on one or more sides of the sieve-tube element wall.

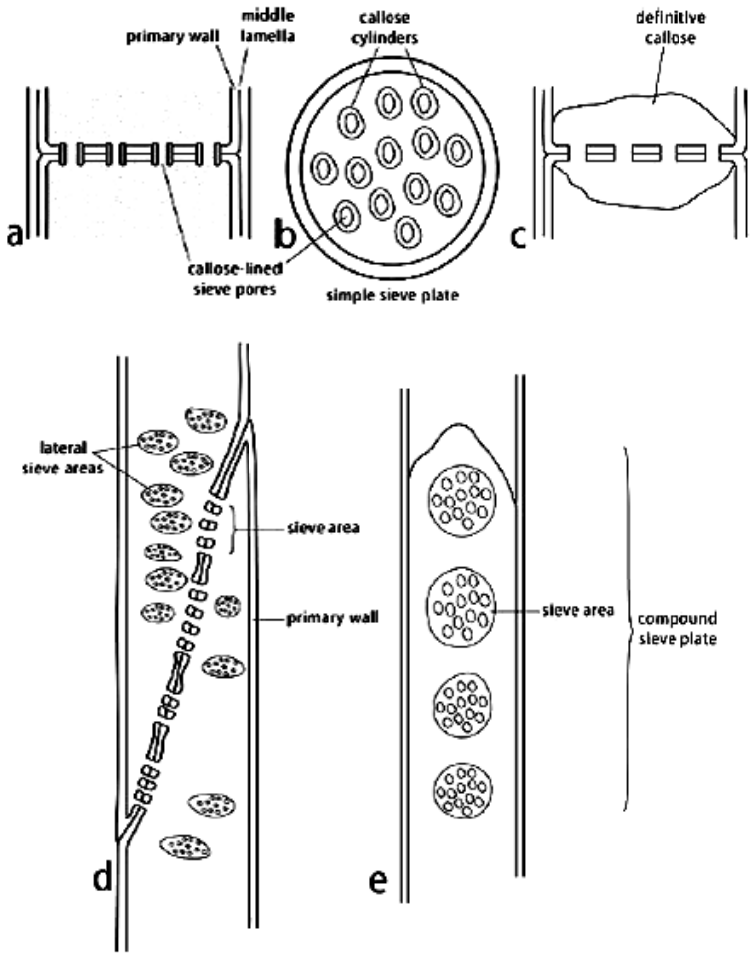


Figure 12.4 (a–c) Diagrams of simple sieve plates, each consisting of a single sieve area. (a) Sectional view of a sieve area. (b) Face view of a sieve area. (c) A sieve area enclosed in definitive callose and, consequently, non-functional. Stippling represents cytoplasmic contents continuous through sieve pores between contiguous sieve tube members. (d, e) Compound sieve plates. To simplify the diagrams callose and cytoplasmic contents are not shown. (d) Sectional view. (e) Face view.

In pteridophytes and gymnosperms companion cells, as described above, do not occur, but cells which stain intensely with cytoplasmic stain are present. These cells are apparently connected physiologically and morphologically to the sieve cell sand have ceen termed **albuminous cells**.

Phloem parenchyma:

The parenchyma cells, other than albuminous and companion cells, which occur in association with phloem, are referred to as phloem parenchyma.

Phloem parenchyma is more or less rectangular or rounded in cross section. In longitudinal section it appears as elongated cell with both ends rounded or pointed; it may also appear as rectangular or cylindrical. In the secondary phloem, parenchyma cells occur in two systems, the axial and the radial systems, and are classified as **axial parenchyma** cells, or phloem parenchyma cells, and **ray parenchyma cells**, respectively.

Phloem fibre:

The fibre, which occurs in association with phloem, is referred to as phloem fibre.

Sometimes the terms bast fibre or bass fibre or basswood or bast wood fibre are synonymously used to mean phloem fibre.

Phylogeny of sieve elements

In the most priinitive form the sieve elements are parenchyma cells that have undergone modifications in connection withi their function. This was followed by the loss of the nucleus. This protoplasmic specialization apparently resulted in the development of the interdependence of the sieve elements and parenchyma cells that retain their nucleus, i.e. the albuminous cells in the gymnosperms and the companion cells in the angiosperms. In the angiosperms the sieve elements and companion cells develop from the same mother cell. The specialization of the sieve elements also involved the development of thick connecting strands. In pteridophyte and gymnosperms these strands are thin and resemble plasmodesmata, while in the angiosperms they are thick and conspicuous. The evolutionary

trends in the shape of the sieve elements and the arrangement of sieve areas have been more thoroughly studied in the monocotyledons (Cheadle and Whitford, 1941; Cheadle, 1948; Cheadle and Uhl, 1948). The specialization, in this group of plants, has taken the following courses: (1) gradual localization of highly specialized sieve areas to the end walls of, the elements; (2) gradual changes in the position of the end wall from oblique to horizontal; (3) gradual change from compound sieve plates to very simple ones; (4) gradual reduction of the sieve areas on the side walls of the elements. In monocotyledons the above investigators also found that the sieve tubes first developed in the. aerial portions of the plants from where the development and specialization spread to the roots. This direction of development is opposite to that of the development of the vessels in the xylem. This phenomenon is understandable from a functional point of view. Features ofthe dicotyledonous sieve-tube members suggest that the phylogenetic development in this group of plants is similar to that of the monocotyledons; but as yet no conclusive research has been done. According to Zahur (1959), the sieve-tube elements in the angiosperms, like the vessel elements, have undergone a decrease in length during the course of evolution.

Vascular cambium

The vascular cambium is a lateral meristem that develops either as longitudinal strands or as a hollow cylinder. In the woody angiosperms and gymnosperms the primary tissues of the stem and root exist for only a relatively short period before they become destroyed or obliterated by the development of the secondary vascular tissues which are produced by the cambium. In many herbaceous angiosperms, and also in most of the recent lower vascular plants, cambium is absent or vestigial.

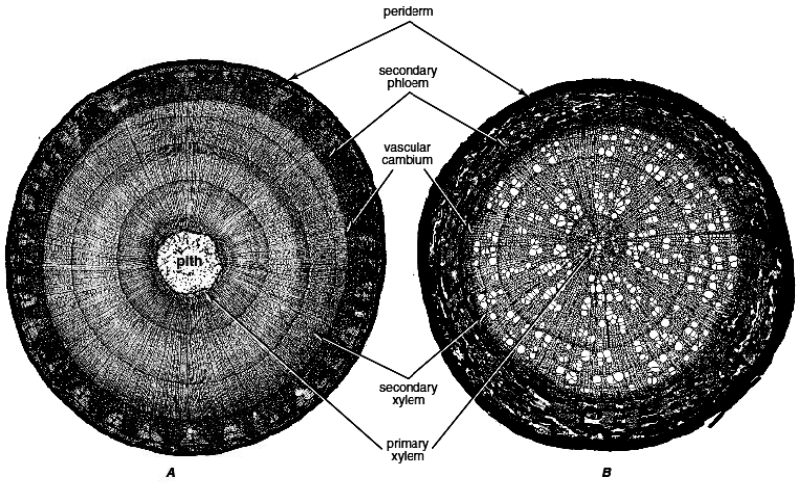


FIGURE 12.1

Transverse sections of *Tilia* stem (A) and root (B), each with periderm and several increments of secondary vascular tissues. The vascular cambium forms a continuous cylindrical sheath about the secondary xylem. (A, ×9.7; B, ×27.)

General development and structure of the vascular cambium

In certain plants, including monocotyledons, all the cells of the procambium undergo differentiation into primary vascular tissues. In almost all the dicotyledons and gymnosperms a portion of the procambium remains meristematic even after the completion of primary growth and develops into the cambium of the secondary body. The cambium that arises within the bundles of primary vascular tissue of the stem is called **fascicular cambium**. The strips of fascicular cambium usually become joined by additional strips of cambium which constitutes the **interfascicular cambium**. The interfascicular cambium is not a continuation of the procambium but develops from the interfascicular parenchyma. Therefore this part of the cambium constitutes a secondary meristem also from the point of view of its origin.

During the growing period the cambial initials together with their immediate derivatives form the **cambial zone**. In a cross-section the cells of the cambial zone are seen to be arranged in radial rows. The cells on either side of this zone gradually widen until they acquire the shape and features of mature phloem and xylem elements.

Morphologically, the vascular cambium contains two types of initials **fusiform initials**, is several times longer than wide; and **ray initials**, is slightly elongated to nearly isodiametric.

The term fusiform implies that the cell is shaped like a spindle. A fusiform cell, however, is an approximately prismatic cell in its middle part and wedge-shaped at the ends.

Types of cambium

Two types of cambium can be distinguished on the basis of the arrangement of the fusiform cells as seen in tangential section.

1. **Storied or stratified cambium** in which the fusiform initials are arranged in horizontal rows so that their ends are approximately at the same level. This type of cambium is found in *Tamarix* and *Robinia*

2. **Non-storied or non-stratified cambium** in which the fusiform initials partially overlap one another and in which they are not arranged in horizontal rows.

The fusiform initials in the second type are longer and this type is the more common.

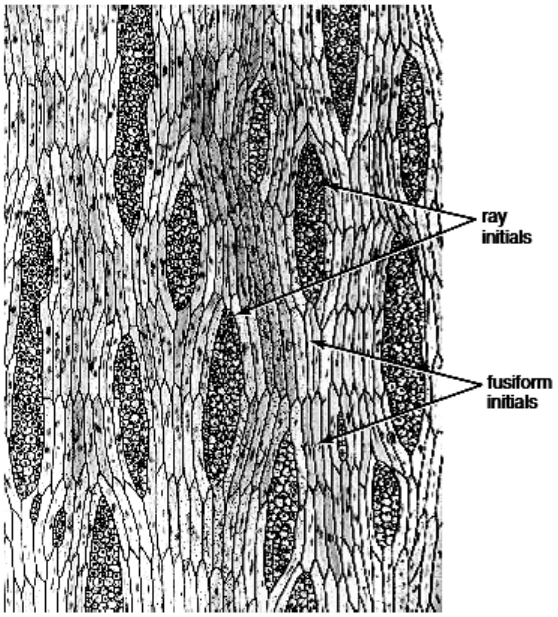


FIGURE 12.3

Storied cambium of black locust (*Robinia pseudoaca-*

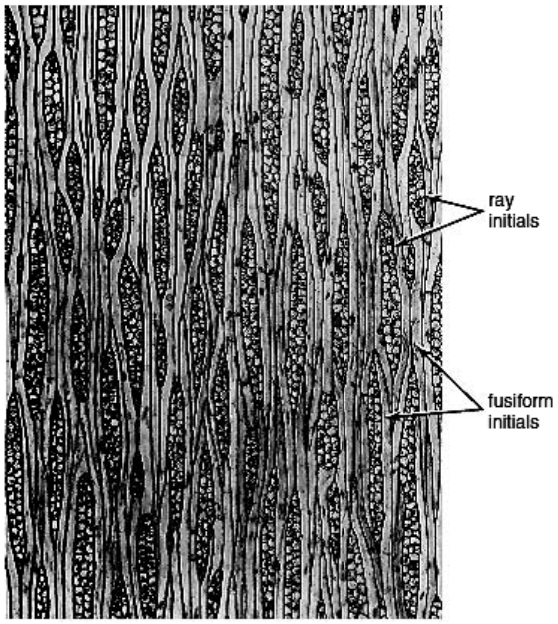


FIGURE 12.4

Nonstoried cambium of apple (*Malus domestica*), as

Seasonal activity of cambium:

There are plants whose cambium is active throughtout the entire life of the plant; i.e., the cambial cells divide continuously and the resulting cells undergo gradual differentiation to form the xylem and phloem elements. This type of activity is usually found in plants growing in tropical regions. Contrary to this, in plants whose origin is in the temperate regions, the cambium ceases its activity with the onset of unfavourable conditions, usually the autumn, and it enters a dormant state which may last from the end of summer till the follewing spring. In spring the cambium again becomes active. From an anatomical point of view the commencement of the cambial activity consists of two stages: (1) the cambial cells become wider radially; and (2) the cells begin to divide. With the enlargement, of the cambial cells their radial walls usually become weakened, so that in this stage the bark of the stems and reets may easily be peeled. In later stages this easy separation of the bark from the xylem is also pessible because of the increase in number, of cells in the cambial zene as a result of the cell divisions. The separatien principally occurs in the regien of the young xylem cells which have already reached their maximum diameter, but which still have thin primary walls.

As has already been mentioned, there are plants in which the cambium is active throughout the year and those in which there is a break, which may be as long as 8 months in duration in the cambial activity. In the Mediterranean and desert regions of Israel it is possible to find both the above two as well as intermediate types (Oppenheimer, 1945; Fahn, 1953, 1955, 1958a, b, 1959a, b, 1962; Fahn and Sarnat, 1963). In these regions the range of temperature is such that the cambium may remain active throughout the year if such activity is in accordance with the hereditary characteristics of the plant. In arid regions, such as these, however, the amount of available-water in the soil is an important factor in the control of cambial activity, and, of course, the general ability to grow depends on this

factor. It is of interest to note that in certain plants, e.g. *Tamarix articulata, Acacia raddiana* and *A. tortilis,* which grow in the desert regions of Israel and whose roots reach those levels that contain a certain amount of moisture even at the end of the dry summer; the cambium is active throughout the year. From the point of view of cambial activity the trees and shrubs growing in Israel may be divided into the following five types.

1. Woody plants, such as *Retama raetam* Webb., *Artemisia monosperma* Del., *Zygophyllum dumosum* Boiss. and *Reaumuria palaestina* Boiss., which all exhibit more or less distinct growth rings, the development of which commences in the early winter months, i.e. between November and January. The cambium in these species is dormant for a fairly long period.

2. Trees and shrubs such as three *Quercus* species, three *Pistacia* species, *Ceratollia siliqua* L., *Tamarix jordanis* Boiss. var. *negevensis* Zoh., *T. galliea* L. var. *maris-mortui* Zoh., and *Calligonum comosum* L'Her. which all exhibit more or less distinct growth rings, the development of which commences in the period March to May, i.e. in the spring.

3. Shrubs, such as *Anabasis articulata* Moq. and *Salsola rosmarinus* Solms-Laub., which are intermediate between the first 'two groups in that the commencement of the growth-ring production is in February.

4. Trees, such as *Eucalyptus camaldulensis* Dehn. and *Tamarix aphylla* Karst., in which the formation of the early wood starts in September (August), .i.e. toward the end of the dry summer season. In *Eucalyptus* the late wood, which consists of one or two bands of flattened fibres two or three layers thick, is produced during the spring or in early summer, and the cambium is inactive or almost so during July-August.

5. Trees and shrubs, such as *Acacia tortilis* Hayne, *A. 'raddiana* Savi, *A. cyanophylla* Lindl. and *Thymelaea hirsuta* Endl., in which there are no growth rings and in which the same type of wood is produced throughout the year.

In *Eucalyptus camaldulensis* the annual growth ring was seen to be produced in September which coincides with the spring of Australia, where this plant.is indigenous.

Secondary xylem:

The **secondary xylem** is formed by a relatively complex meristem, the vascular cambium, consisting of vertically elongated fusiform initials and squarish or horizontally (radially) elongated ray initials. The secondary xylem is therefore composed of two systems, the **axial** (vertical) and the **radial** (horizontal) systems an architecture not characteristic of the primary xylem. In the angiosperms the secondary xylem is commonly more complex than the primary in having a wider variety of component cells. Secondary xylem comprises various elements-tracheids, vessel members, different types of fibres, parenchyma cells, xylem ray cells, and sometimes secretory cells.

Basic structure of secondary xylem

The most distinctive feature characterizing the secondary xylem is the existence of two systems of elements which differ in the orientation of their longitudinal axes - one system is vertical and the other horizontal. The horizontal system comprises the xylem rays, and the vertical or axial system, the tracheary elements, fibres and wood parenchyma. The living cells of the rays and of the axial system are usually interconnected, so that it is possible to speak of a continuous system, of living cells. This system is generally connected with the living cells of the pith, phloem and cortex.

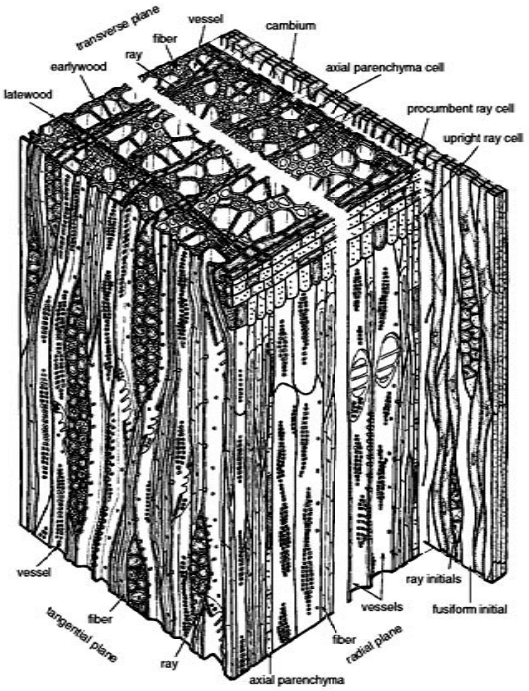


FIGURE II.1

Block diagram of vascular cambium and secondary xylem of *Liriodendron tulipifera* L. (tulip tree), a woody angio-

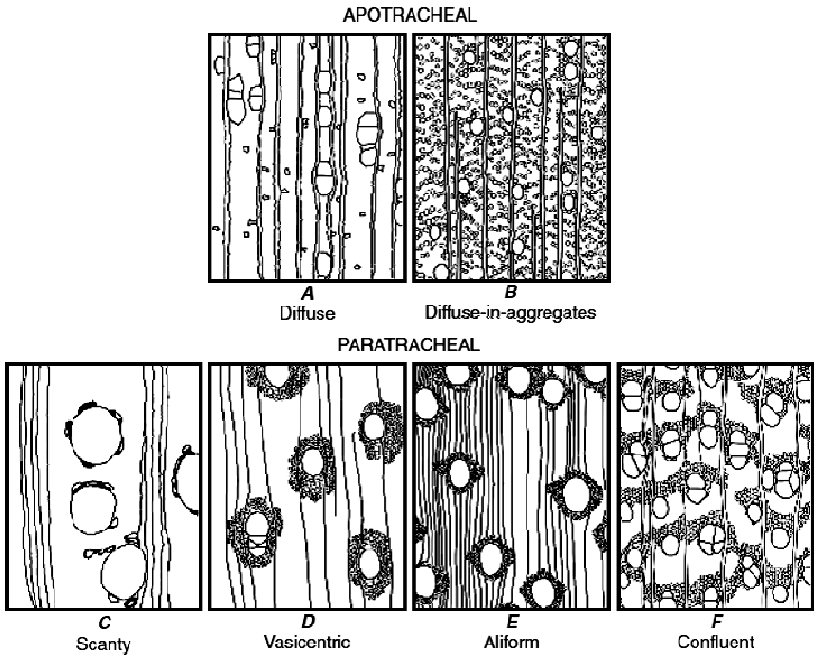
The secondary xylem elements are more or less similar to those occurring in primary xylem with abundant larger pitted vessels and shorter fusiform xylem parenchyma cells meant for storage of starch or fat. Tannins, mineral crystals etc., are also frequently present in these cells. Secondary wall may be absent in xylem parenchyma, if present, the pits between them and tracheary elements are bordered, half-bor-dered or even simple. Secondary xylem parenchyma may occur abundantly in some species or may be reduced in number or entirely absent. The characteristics of the secondary taxonomic characteristic in recent years.

If they occur in association with the vessels, they are called paratracheal and, if they occur indepen-dently, they are called apotracheal. The former are of different types based on their distribution. The most common type is the vesicentric para-tracheal parenchyma which forms entire sheaths, appearing circular or elliptical in cross- section, around the vessels.

Apotracheal parenchyma is considered as primitive and, through its different evolutionary types, advanced to the paratracheal forms culminating in highest developed vasicentric type.

Table 5.13 : Difference between Apotracheal parenchyma and Paratracheal parenchyma

	Apotracheal parenchyma	Paratracheal parenchyma
1.	The axial parenchyma of secondary xylem is situated independent of vessel.	The axial parenchyma of secondary xylem is distinctly associated with the vessel.
2.	It may be : (a) Diffuse: parenchyma cells appear as single cell or as small uniseriate band throughout growth ring (*Quercus*). (b) Banded: parenchyma cells appear as concentric rings (*Hicoria*). (c) Boundary : parenchyma appears at the beginning or end of the growth ring (*Magnolia, Ceratonia* etc.).	It may be : (a) Scanty : the parenchyma cells do not form a continuous sheath surrounding the vessel (*Acer*). (b) Vasicentric : the parenchyma cells form a continuous sheath around the vessel of different width (*Tamarix*). (c) Aliform : vasi-centric parenchyma extends laterally as wings (*Acacia*).



The secondary xylem fibres have thick walls with reduced bordered pits. The two main types of fibres are the fibre tracheids, and the libriform fibres. The former have pits with poorly deve-loped borders, whereas the latter possess simple pits resembling the phloem fibres. Both these fibres are the important mechanically supporting elements of xylem.

Rays:

The ray parenchyma cells of the angiosperms vary in shape, but two fundamental forms may be distinguished**: procumbent** and **upright**. Procumbent ray cells have their longest axes oriented radially and upright ray cells have their longest axes oriented vertically, or upright. Ray cells that appear square in radial sections of the wood are called **square ray cells**, a modification of the upright.

In the angiosperms, rays composed of one kind of cell are called **homocellular**, and those containing procumbent and upright cells, **heterocellular.**

In contrast to the predominantly uniseriate rays of conifers, those of the angiosperms may be one to many cells wide; that is, they may be **uniseriate** or **multiseriate** (multiseriate rays two cells wide

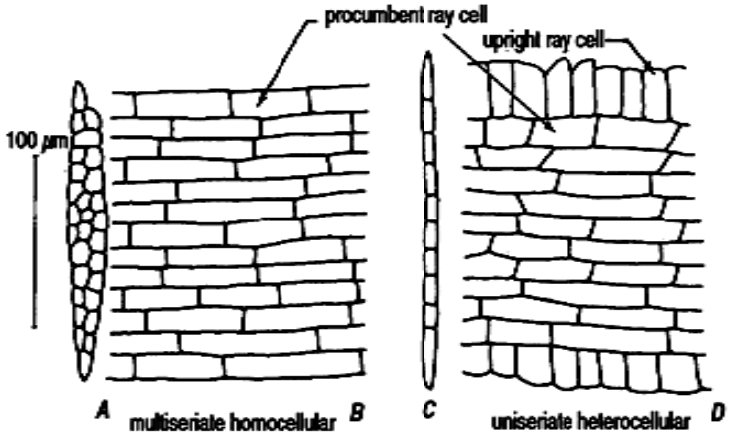


FIGURE 11.18

Two types of rays as seen in tangential (A, C) and radial (B, D) sections. A, B, *Acer saccharum*; C, D, *Fagus grandifolia*. (From Esau, 1977.)

commonly are called **biseriate rays**; and range in height from one to many cells (from a few mm to 3 cm or more). The multiseriate rays frequently have uniseriate margins.

Storied and nonstoried woods

The more or less orderly radial seriation of cells of the secondary xylem, as seen in transverse sections, is a result of the origin of these cells from periclinally, or tangentially, dividing cambial cells. Radial sections also reveal the radial seriation; in such sections the radial series of the axial system appear superimposed one upon the other in horizontal layers, or tiers. The tangential sections are varied in their appearance in different woods. In some, the horizontal layers are clearly displayed, and such wood is called **storied**, or **stratified**, wood (*Aesculus, Cryptocarya, Diospyros,Ficus, Mansonia, Swietenia, Tabebuia, Tilia*, many *Asteraceae* and *Fabaceae*). In others, the cells of one tier unevenly overlap those of another. This type of wood is called **nonstoried**, or **nonstratified**, wood (*Acer, Fraxinus, Juglans, Mangifera, Manilkara, Ocotea, Populus, Pyrus, Quercus, Salix*, conifers).

Growth Rings

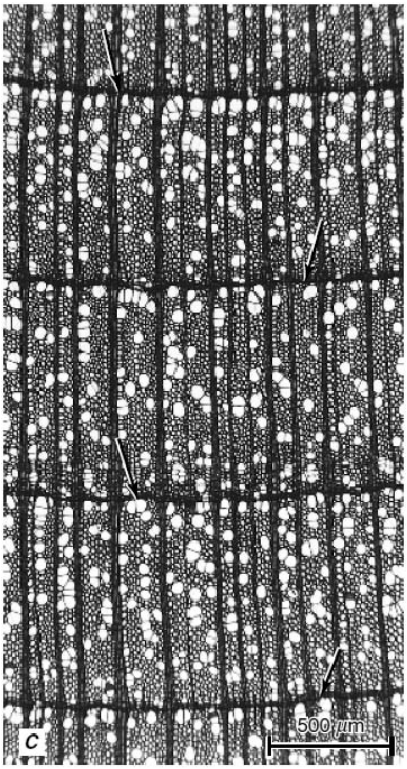
The periodic activity of the vascular cambium, which is a seasonal phenomenon in temperate regions related to changing day lengths and temperatures, produces **growth increments**, or **growth rings**, in the secondary xylem. If such a growth layer represents one season's growth, it may be called an **annual ring**. Abrupt changes in available water and other environmental factors may be responsible for the production of more than one growth ring in a given year. Additional rings may also result from injuries by insects, fungi, or fire. Such an additional growth layer is called a **false annual ring** and the annual growth increment consisting of two or more rings is termed a **multiple annual ring**. In very

suppressed or old trees the lower portions of the stem or of some branches may fail to produce xylem during a given year. Thus, although the age of a given portion of a woody branch or stem can be estimated by counting the growth rings, the estimates may be inaccurate if some rings are "missing" or if false annual rings are present. Trees that exhibit continuous cambial activity, such as those in perpetually wet tropical rainforests, may lack growth rings entirely (Alves and Angyalossy-Alfonso, 2000). It is therefore difficult to judge the age of such trees.

The annual ring is formed due to the forma-tion of different types of wood or secondary xylem in different periods of the year. Spring is the most favourable season for plant growth and the wood formed in this season is less dense and is made of vessels with wider diameters.

Due to the rapid rate of photosynthesis in this favorable season there appears a high demand of water for photosynthate transport and, therefore, larger and wider vessels in secondary xylem are formed in that season. This is called the **early wood or spring wood**.

In summer, the rate of photosynthesis gradu-ally decreases mainly due to high temperature and water scarcity, and thus the secondary xylem formed in this season is dense and made of nar-rower, compact and lignified vessel elements. This part is called **late wood or summer wood.**



Growth rings of tulip tree (*Liriodendron tulipifera*) wood

In some tropical trees distinct annual rings are formed due to periodic cambial activity in alternate wet and dry seasons. Well- marked rings are found in the important timber yielding plants like *Tectona grandis* (teak), *Dalbergia sissoo* (sissoo), *Albizzia lebbeck* (sirish), *Salmalia malabarica* (silk cotton), etc.

The early wood is more conspicuous than the late wood due to the formation of wider and larger vessels. The vessels in the early wood are of unequal diameter and the largest ones show a ring-like arrangement in transverse section. This type of wood is called **ring porous wood**.

Sometimes the vessels of uniform diameter are found to be distributed uniformly throughout the wood. But when there is a gradient of distribution in size from smaller to larger ones through-out the growth ring it is called **diffuse porous wood**. Phylogenetically ring-porous wood is considered more advanced than the diffuse porous wood as intermediate forms are observed between them.

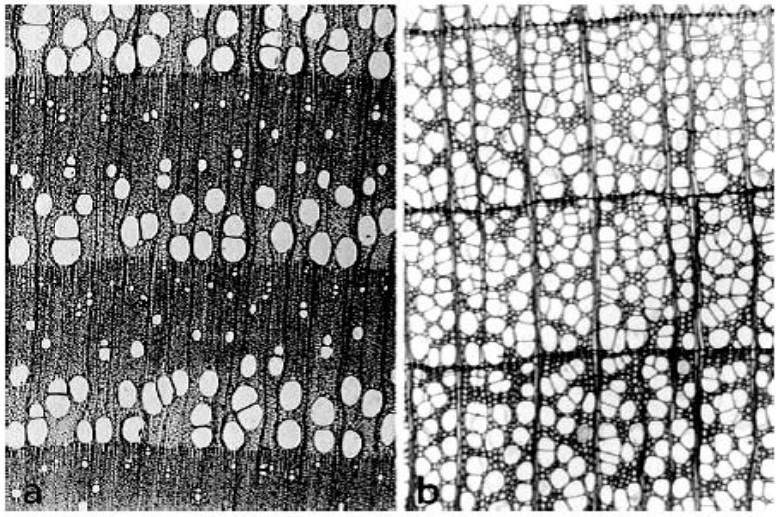


Figure 11.24 Distribution of vessels in secondary xylem as seen in transverse section. (a) Ring-porous secondary xylem in *Fraxinus* sp. (ash). (b) Diffuse-porous secondary xylem in *Tilia americana*. Magnification (a) and (b) × 58.

Tyloses:

Tyloses (singular: tylosis) are the balloon-like protrusions into the tracheary ele-ments of the secondary xylem in certain plants. These are developed by the adjacent xylem parenchyma and ray parenchyma cells. Such ingrowths may be developed in the primary xylem also. Tyloses are the enlarged protrusions of the pit membranes of the half-bordered pits from a living parenchyma cell into a vessel or a tracheid lumen.

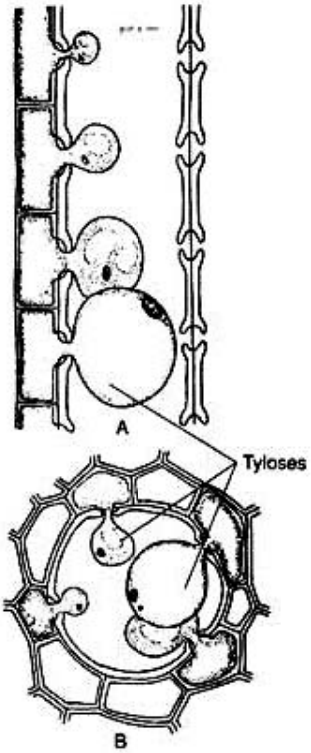


Fig. 5.133 : Secondary xylem in T.S. showing diffuse porous (A) and ring porous (B) wood

Often they are so large that the lumen of the vessel or tracheid is almost blocked. A tylosis contains a nucleus with a certain amount of cytoplasm coming from the living cell. Starch, resin and other matters may also be pre-sent in it. The initial thin walls of tyloses may undergo lignification later leading to the forma-tion of sclereids. A single tylosis may divide and form a multiple structure.

Tyloses are commonly found in many angiospermic families and abundantly found in the species of *Tinospora, Morus, Catalpa, Quercus, Juglans* etc. Normally they develop in the heart wood (previously formed centrally located portion of wood) to give extra mechanical support and to prevent from collapsing the vessel elements by the pressure developed by the secondary growth by blocking the lumen.

Tyloses not only prevent the flow of sap through the vessels but also the movement of fungal hyphae and prevent from spreading of diseases. The sap movement, however, takes place through the newly formed secondary vessel ele-ments. Tyloses are also known to occur in the sap wood beneath the wounds as well as in connec-tion with some diseases.

Occurrence of tyloses has also been repor-ted in some herbaceous plants like *Coleus, Carina, Cucurbita* etc.

Resin ducts

Resin ducts are developed in the vertical or both vertical and horizontal systems of a large number of gymnosperms. The ducts develop schizogenously between resin-producing parenchyma cells which then form the epithelium of the duct. Sometimes a resin duct may become blocked by the enlargement of tbe epithelial cells; such structures are termed **tylosoids**. Differences exist in the thickness and lignification of the cell wall of the epithelial cells in the various conifer genera. In those genera, where theresin-secreting cells have thick, lignified walls and in which these cells die after one season, relatively little resin is produced. In those plants where the epithelial cells are thin walled and function during several seasons a large amount of resin is produced. Resin ducts, the epithelial cells of which have lignified walls, occur in *Abies* and *Cedrus*, for example, while in *Pinus* the secretory cells are thin walled and not lignified.

Tylosoid:

The epithelial cells in certain gymnosperms may block the resin duct by the enlargement and intrusions into them looking like tyloses, called the tylosoids (e.g., *Pinus*).

In angiosperm (e.g., *Vitis, Bombax* etc.) parenchyma proliferates into the neighboring sieve tubes in a tylose-like manner. These proliferations are also known as tylosoids. These tylosoids of gymnosperm and angiosperm differ from tyloses in not protruding through pits.

Sap Wood and Heart Wood:

Two types of wood formation are prevalent in woody species after secondary growth – the outer nascent secon-dary xylem is the **sap wood or alburnum**, and the centrally placed older hard portion is referred to as **heart wood or duramen**. The former is lightcolored containing some living cells in addition to the tracheary elements and fibres.

This part actually carries on the water and solute conduction in addition to mechanical support and storage of food. The hard central dark colored heart wood mainly consists of dead elements representing merely a solid mechanical column.

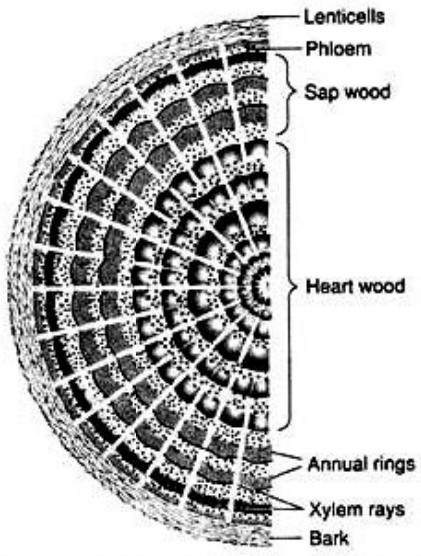


Fig. 5.134 : Secondary growth in stem in T.S. showing sap wood and heart wood

The sap wood is gradually transformed into heart wood through certain changes like:

1. The loss of protoplasts in the living cells.

2. Reduced water contents.

3. Withdrawal of the food matters from the cells.

4. Formation of tyloses to block the lumens of the tracheary elements.

5. Considerable lignification of the walls of the parenchyma cells.

6. Infiltration of substances like gums, resins, oils and particularly tannins and colouring matters on both the walls and lumen of the elements.

Finally, the heart wood becomes more com-pact, strong and dark-colored. The heart wood is commercially more valuable than the sap wood but the latter is functionally more important. The heart wood yields good quality of timber due to its durability and resistance to decay. The dye haematoxylin is obtained from the heart wood of *Haematoxylum campechianum*.

The living xylem elements of sap wood in course of time become dead and finally converted to heartwood. However, in *Sequoia sempervirens* the ray parenchyma of heart wood may remain alive for hundred years.

The ratio between the quantity and the degree of difference of heart wood and sap wood varies with different conditions of growth. In *Abies, Picea* etc. the heart woods are not well differentiated. *Taxus* and *Morus* possess thin sap wood while *Fagus* and *Acer* contain thick sap wood.

5. Anatomical variations with ecology: leaf and root anatomy in ecological perspectives; hydraulic architecture of plant

Ecological Anatomy

The plants which characteristically grow in certain ecological niches often show a type of structure which is believed to be adapted to that particular environment. In the course of evolution, many species have become adapted in both structural and physiological features to habitats with an excessive water supply.

Plants that live wholly or partly submerged in water or in very wet places are known as **hydrophytes** (Gr. hudor, water; phyton, plant). The greatest numbers of plants grow under average conditions of moisture and temperature. Plants of habitats that usually show neither an excess nor a deficiency of water are known as **mesophytes** (Gr. mesos, middle; phyton, plant). Plants that grow habitually where the evaporation stress is high and the water supply low show characteristic adaptations to a decrease in water content. They are known as **xerophytes** (Gr. Xeros, dry; phyton, plant).

Ecological leaf anatomy

The variations in plant structure that are commonly affected by environmental factors are particularly strongly expressed in the morphology and anatomy of leaves. Smooth leaves with entire margins and apical drip tips are more common in lowland tropical rain forests. Leaf areoles of tropical and xeric climate plants tend to be smaller and with fewer freely ending veinlets than those of plants from cooler areas with adequate rainfall.

Sun and Shade Leaves

The level of illumination that a leaf receives during development is perhaps the single most influential environmental factor affecting mature leaf structure. Structurally, leaves vary with respect to both the light intensity and light quality to which they are exposed during development. Within the same individual, leaves produced in bright light conditions (**sun leaves**) tend to be smaller and thicker and to have increased mesophyll tissue per unit area and higher density of stomata, veins, and chlorophyll when compared to leaves exposed to shade (**shade leaves**). The differences in internal leaf structure are related to blade thickness and the amount and distribution of palisade and spongy mesophyll tissue. These structural variations are associated with regulating light and CO_2 profiles within leaves and maximizing photosynthetic efficiencies. Sun leaves fix carbon faster than do shade leaves. Low light can also cause a reduction in mean vessel density. The differences in anatomy and physiology between sun and shade leaves are often reflected in species differences in shade tolerance, successional status, and drought aversion.

In sun leaves, the mesophyll zone usually contains an increased number of compact palisade layers, and each successive layer has greater palisade cell elongation. The foliar cuticle is often thicker than in shade leaves, and hairs, if present, are abundant. In these leaves, the well-developed cuticle may reflect light rays and protect the underlying tissues from excessive radiation. Sun leaves typically possess more numerous stomata than shade leaves and, as a result, transpire more abundantly and perhaps benefit from a cooling effect.

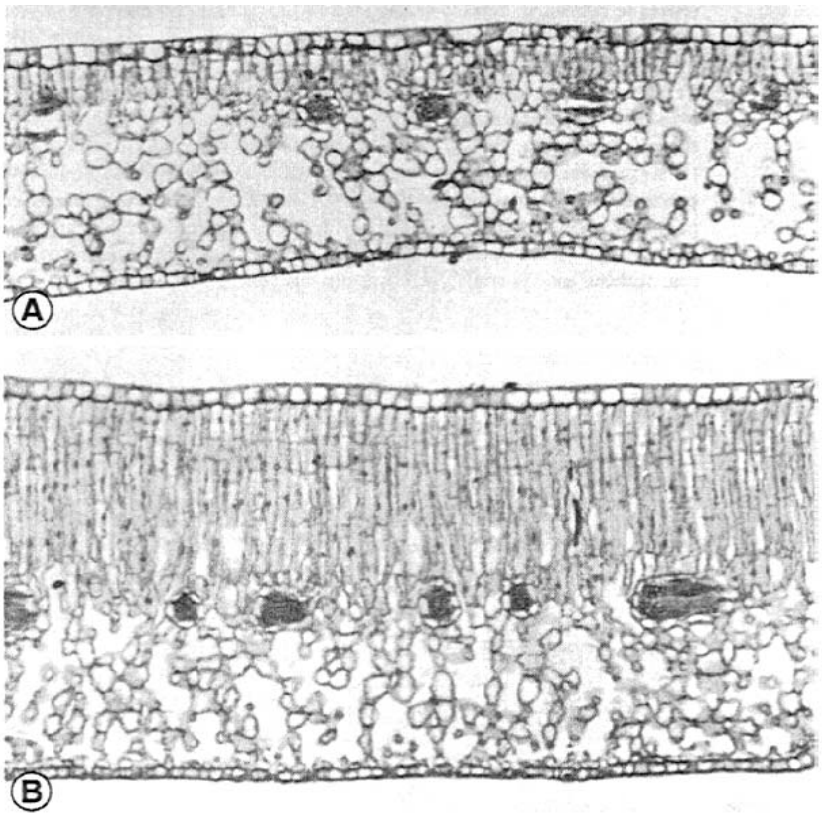


FIGURE 8.2 Differences in internal structure between a shade leaf (A) and sun leaf (B) of *Prunus*

Xeromorphic Leaves

In addition to physiological differences, xerophytic plants typically possess one or more striking morphological and anatomical modifications to water stress, many of these are associated with leaf structure.

Plants that live in habitats where the supply of available water is deficient have been classified into broadly defined categories on the basis of the different adaptive strategies they have developed:

1. **Drought-escaping species** are plants with a compressed growth cycle that complete vegetative growth and reproduction within the short period of time when conditions are favorable.
2. **Drought-evading species** are plants that are able to reduce water loss, or compensate for water loss, by possessing some specialized structural feature or set of features, such as an extensive root system.
3. **Drought-enduring species** are plants that are able to survive even when water uptake cannot take place or is severely reduced. This group includes species with a number of highly divergent specializations such as **temporary leaf loss, changes in leaf angle and the rolling or folding of leaf blades, or permanent adaptations such as succulence or leaves reduced**

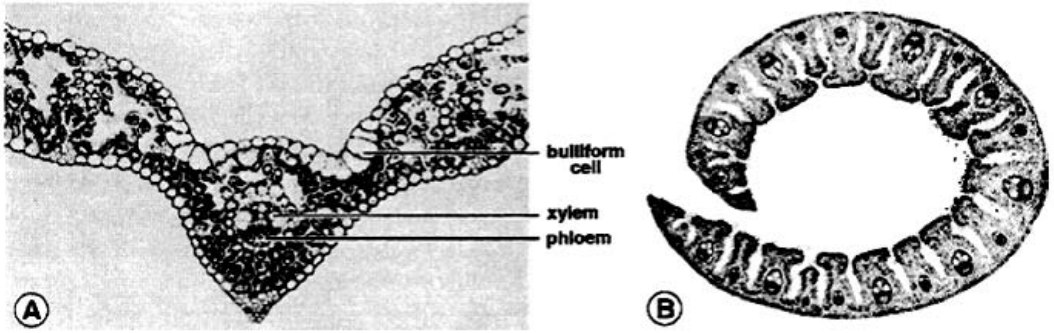


FIGURE 8.4 Transverse sections of grass leaves. (A) *Poa* leaf showing enlarged bulliform cells in the upper epidermis. (B) Beachgrass (*Ammophila breviligulata*) leaf showing curled condition following water loss

to spines. The leaves of some xerophytic grasses contain enlarged epidermal cells that store water and occur in dispersed longitudinal rows. These cells are called **bulliform cells** and under conditions of water deficit they lose turgor and thus constrict in upon themselves, causing the grass lamina to fold or roll inward edge to edge.

The characteristic anatomical features of the xerophytes are as follows:

Epidermis and Thick Cuticle:

Heavy cuticularization and extreme cutinization of the epidermis and even of sub-epidermal cells are common in xerophytes. The thickness of the cuticle shows various gradations. In extreme xerophytes the cuticle may be as thick as, or thicker than the diameter of the epidermal cells. In addition to the presence of thick cuticle, the walls of epidermal cells become cutinized, and sometimes also those of underlying cells. Along with well-developed cutinized layers the epidermal and sub-epidermal cells also become lignified.

For example, in the leaflets of *Cycas*, the lignification may extend even to the palisade parenchyma cells. In certain cases the covering of wax is formed on the epidermis (e.g., *Calotropis*). The epidermal cells are usually radially elongated. In the leaves of *Nerium* and *Ficus*, the epidermis becomes multi-layered. In many xerophytes, in addition to a cutinized epidermis, single to multi-layered hypodermis is also present.

Hairs:

In many xerophytic plants, especially those of alpine regions exposed to strong winds, a covering of matted epidermal hairs on the underside of the leaves prevents water loss. Hairs may also be abundant over the entire aerial part of the plant.

The thick matting of hairs also prevents rapid evaporation through stomata. The xerophytes that possess abundant hairs, on their leaves and stems, are commonly called trichophyllous.

Structure of Stomata:

The reduction of transpiration is of great importance in xerophytes. The xerophytes may possess less stomata, either by reduction of leaf surface or of stomatal number per unit area. To reduce excessive transpiration, usually the stomata that remain sunken in pits are formed.

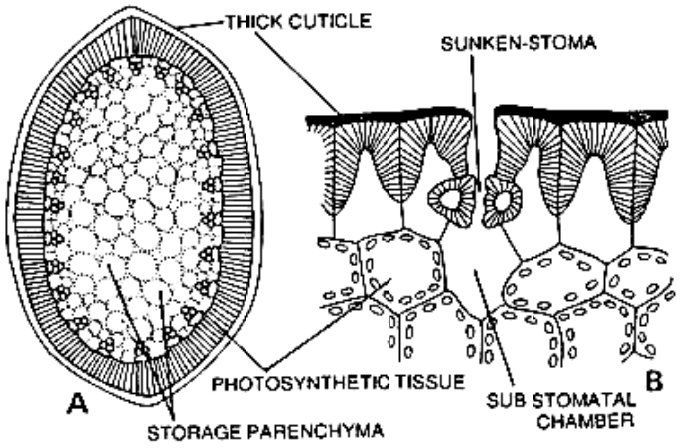


Fig 41.31. Anatomy of xerophytic leaf—*Aloe* (monocot). A, T.S. showing peripheral photosynthetic tissue and central water storage tissue (diagrammatic); B, a portion (detailed) showing thick cuticle, thickening on the radial and outer walls of the epidermal cells and sunken stomata.

Such stomata are commonly known as sunken stomata (e.g., *Pinus, Hakea, Agave*, etc.). In certain cases the stomata are found in groups and they remain confined to depressions found on leaf surface (e.g., *Nerium, Banksia*, etc.). Usually the depressions contain hairs in them which protect the stomata from direct attack of wind gusts.

Sclerenchyma:

The xerophytes commonly have a larger proportion of sclerenchyma in their leaf structure than is found normally in mesophytes. The sclerenchyma is either found in groups or in continuous sheets. For example, in *Banksia*, there is a continuous thin sheet of sclerenchyma between the hypodermis and the mesophyll.

In *Dasylirion*, there is well-developed sclerenchyma below the epidermis. The sheets of sclerenchyma check excessive transpiration to some extent and also give mechanical support to the plant body. The xerophytes that possess heavy sclerification of the leaves are known as **sclerophyllous**.

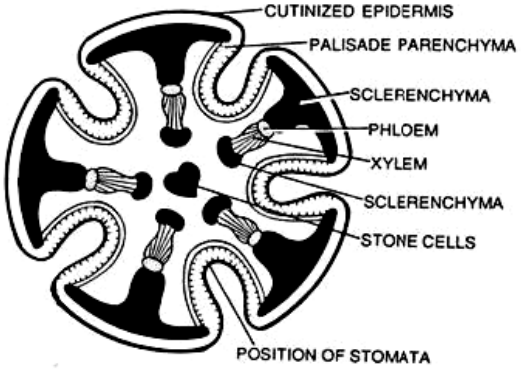


Fig. 41.36. Anatomy of xerophytic leaf. V.S. of leaf of *Hakea sulcata* (sclerophyllous-dicot)—diagrammatic.

Rolling of leaves:

The leaves of many xerophytic grasses roll tightly under dry conditions. In these grasses, the stomata are confined to the ventral surface of the leaf, so that when the leaf edges roll inward, the stomata are effectively shut away from the outside air.

In *Ammophila arenaria*, there is tight upward folding of the leaf and also the sheltered location of the stomata in furrows, greatly reduce air movement over stomatal areas. Special motor cells (hinge) on

the upper surface of the leaf are responsible for the inward rolling of leaves. In the xerophytic grasses, the motor cells are well developed.

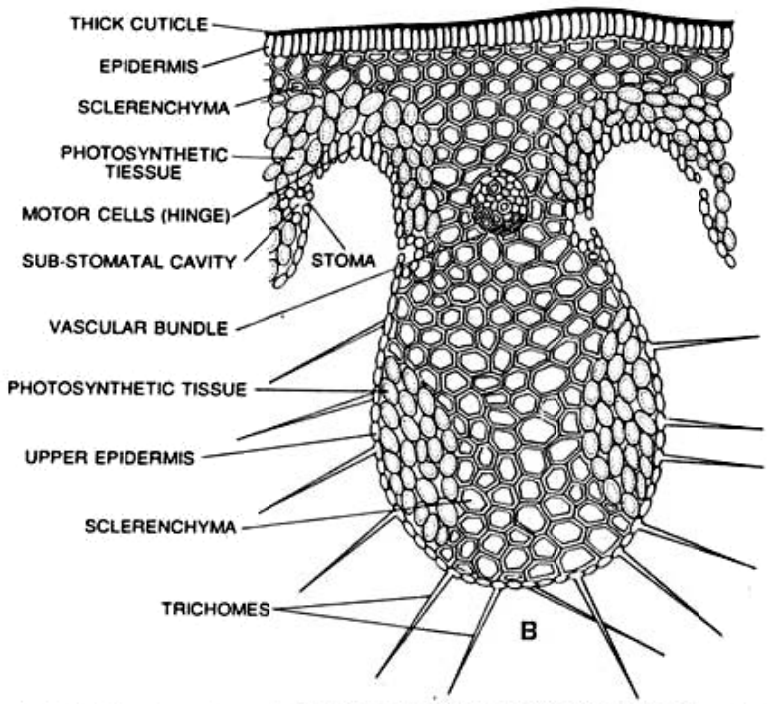


Fig. 41.41. Structure of xerophytic leaf. Part of T.S. of leaf of *Ammophila arenaria*, between two ridges (detailed).

Reduced leaf surface:

In many xerophytes, the reduction of the leaf surface partly prevents water loss because the total exposed surface of the plant body is relatively small as compared with that of normal mesophytes (e.g., *Equisetum, Pinus, Casuarina, Asparagus*, etc.). In such xerophytes the leaves are either scale-like or very small in size. Usually they are not found in the mature

plant, or they persist as small scales or bracts. Xerophytes, with reduced leaves, are known as **microphyllous.**

Needle leaves of gymnosperms:

The needle leaves of gymnosperms are of microphyllous type. In the leaves of many conifers, in addition to reduced leaf surface there are heavy cutinization and sunken stomata. A characteristic illustration of this type is the needle of *Pinus*.

Water storage tissue:

Many fleshy xerophytes possess water storage tissue and mucilaginous substance in them. In leaves such tissues are located beneath the upper or the lower epidermis, or upon both sides of the leaf and sometimes in the centre too. The storage cells are usually large and often thin-walled, as in *Begonia*.

The storage tissue may actually serve as a source or reserve water during drought. The xerophytes that possess fleshy leaves or stems are known as **malacophyllous**.

Abundant palisade parenchyma:

In the stems of many xerophytes, the palisade tissue is present (e.g., *Capparis decidua*). In the xerophytic leaves the palisade is abundant and compactly arranged.

Latex tubes:

In many xerophytic stems and leaves the laticiferous canals are present (e.g., *Calotropis, Euphorbia, Asclepias*, etc.). Due to viscosity of latex the transpiration is reduced to some extent.

Hydrophytes:

Plants grow in water or very wet places. They may be submerged or partly submerged, floating or amphibious. Their structural adaptations are mainly due to the high water content and the deficient supply of oxygen.

The various adaptations are as follows:

(i) The reduction of protective tissue (epidermis here is meant for absorption and not for protection).

(ii) The reduction of supporting or mechanical tissue (i.e., lack of sclerenchyma).

(iii) The reduction of conducting tissue (i.e., minimum development of vascular tissue).

(iv) The reduction of absorbing tissue (roots chiefly act as anchors, and root hairs are lacking).

(v) There is special development of air-chambers (aerenchyma) for aeration of internal tissues.

Epidermis:

In aquatic plants, the epidermis is not protective but absorbs gases and nutrients directly from the water. The epidermis in the typical hydrophyte has an extremely thin cuticle, and the thin cellulose walls permit ready absorption from the surrounding water.

Commonly the chloroplasts are found in epidermal cells of leaves, especially when the leaves are very thin; these chloroplasts utilize the weak light under water for photosynthesis. In submerged plant, stomata are not present, and exchange of gases takes place directly through cell walls. The floating leaves of aquatic plants have abundant stomata on the upper surface.

Lack of sclerenchyma:

Submerged plants usually have few or no sclerenchymatous tissue and cells. The water itself gives support to the plant, and protects it to some extent from injury. The thick walls of tissues, their density and the presence of collenchyma in some plants give some rigidity.

The strands of sclerenchyma occasionally occur, especially along the leaf margins, and increase tensile strength. A few star shaped idioblasts or sclereids are present, which give mechanical support to the body of aquatic plant (e.g., *Limnanthemum, Nymphaea*).

Minimum development of vascular tissue:

In the vascular tissues, the xylem shows the greatest reduction and in many aquatic plants consists of only a few elements, even in the stele and main vascular bundles (e.g., Elatine alsinastrum). In some aquatic plants (e.g., *Potamogeton pectinatus, P. epihydrus*), in the stele and large bundles, and frequently in the small bundles, xylem elements are lacking.

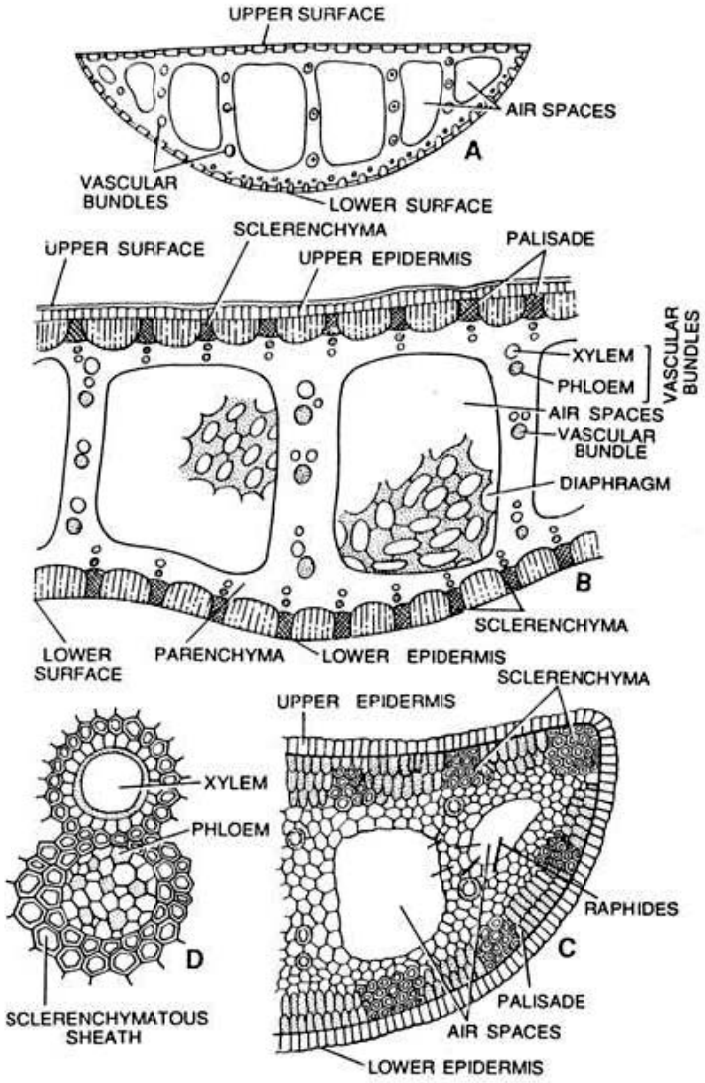


Fig. 41.23. Anatomy of hydrophytic leaf of *Typha* (monocot,). A, T.S. of leaf (diagrammatic); B, detail of a part of A; C, detail of a corner of leaf; D, single vascular bundle.

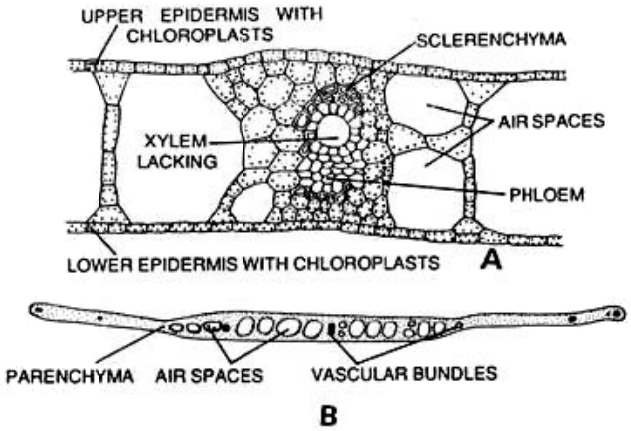


Fig. 41.24. Anatomy of hydrophytes. A, cross section of submerged leaf of *Potamogeton epihydrus* (detail); B, diagrammatic representation of the same.

In these plants there is well developed xylem lacuna in the position of xylem. These lacunae resemble typical air-chambers (air spaces). In many aquatic plants, the phloem is fairly well developed as compared with the xylem.

Development of air-chambers:

Chambers and passages filled with gases are commonly found in the leaves and stems of hydrophytes. The air chambers are large, usually regular, intercellular spaces extending through the leaf and often for long distances through the stem (e.g., *Potamogeton, Pontederia*).

The spaces are usually separated by partitions of photosynthetic tissue only one or two cells thick. The chambers prepare an internal atmosphere for the plant. These air- chambers on the one hand give buoyancy to the plant for floating and on the other they serve to store up air (oxygen and carbon dioxide).

The carbon dioxide that is given off in respiration is stored in these cavities for photosynthesis, and again the oxygen that is given off in photosynthesis during the daytime is similarly stored in them for respiration. The cross partitions of air passages, known as **diaphragms**, prevent flooding.

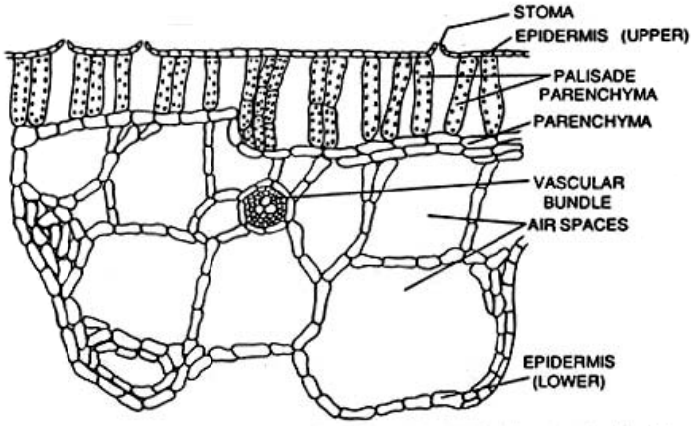


Fig. 41.28. Anatomy of hydrophytie leaf. V.S. of aquatic leaf of *Victoria regia* (dictot.) detail.

Another specialized tissue frequently found in aquatic plants that gives buoyancy to the plant parts on which it occurs is aerenchyma. The presence of this type of aerenchyma is characteristic of species of *Decodon* and *Lythrum.*

Halophytic Leaves

Halophytic plants, or halophytes, are those species able to tolerate saline environments. This adaptation is sometimes considered a special type of xerophytism because of the nature of the environments they inhabit. Halophytic species often show a diversity of structural and physiological adaptations that include succulent leaves with specialized "colorless" storage cells or "window cells" that increase the plants capacity for salt accumulation. Some species have secretory cells or tissues, enlarged tracheids terminating vein endings, structures such as salt glands and salt hairs, and a hypodermis.

The leaves of the salt-tolerant maritime mangrove (e.g., *Avicennia germinans*) contain large, tightly packed, multiple rows of specialized hypodermal cells that play a probable role in salt accumulation and storage. Because salt cannot be allowed to accumulate without limit, many halophytes (including

mangroves) have special surface glands and hairs to remove NaCl from the underlying mesophyll cells and then actively secrete it on the leaf surface. The **salt glands** of *Avicennia* are formed in shallow pits on the upper leaf surface, whereas on the lower surface they are not sunken. Individual glands consist of two to four highly vacuolated basal cells, a cutinized stalk cell, and a minimum of eight terminal cells that are covered by a thin, perforated cuticle that is separated from the cell wall apically. All gland cells are interconnected by plasmodesmata. Salt is deposited in the subcuticular cavity of the gland and then ultimately reaches the leaf surface through the cuticular pores. The structurally complex salt glands of the facultative halophyte *Limonium gmelini* (Plumbaginaceae) are composed of 16 glandular cells overlying 4 collecting cells that are connected to mesophyll cells by plasmodesmata. A layer of cutin surrounds the collecting cells except at pore interruptions.

Other halophytes such as *Atriplex* (Chenopodiaceae) have salt hairs on the surface. These specialized hairs are composed of an enlarged terminal bladder cell that contains a large salt-accumulating and salt-storing central vacuole.

Epiphytic Plants

One of the more interesting examples of structure and function relationships are found among some species of the monocot family Bromeliaceae. All members of the family possess trichomes to some extent; however, the leaf surfaces of the epiphytic subfamily Tillandsioideae are covered with a dense layer of elaborate **absorbing trichomes**. These plants have a xeromorphic structure, and the trichomes facilitate the rapid movement of water and minerals into the shoot. As a result of the trichomes, certain species are able to survive with no roots at all. Individual hairs are peltate structures composed of a multicelled disc of dead cells subtended by a multicellular stalk of living cells. The stalk penetrates the leaf epidermis and functions as a conduit to the mesophyll. The water-absorbing trichomes in bromeliads function in an interesting one-way action. They open to let water in when it rains but close to retain water when it dries out. The roots of these plants, when present, also possess a special covering tissue called **velamen**.

Roots

Xeromorphic Roots

The root systems of certain xerophytes are widely spreading and shallow for optimal water absorption. A short tuberized root is common in some xeric taxa and there is an absence of root hairs under drought conditions. Xeromorphic roots also can be succulent because they contain large water storage cells in the cortex. Some plants from dry regions produce roots that develop a thick, sclerified, and exfoliating bark that cracks and splits off in large sheets. Cactaceae and other succulents conserve water during prolonged dry periods by having few, if any, lateral branch roots; the entire root system may be covered by layers of thick bark. As the soil water potential increases, the dormant root system quickly forms new lateral roots, termed rain-induced roots, that are able to absorb large quantities of water.

Hydromorphic Roots

The roots of aquatic plants range from well developed like those of terrestrial species through various degrees of specialization, including root dimorphism with respect to the presence or absence of root hairs. Because mechanical support and conducting efficiency are no longer necessary, vascular systems are to be ontogenetically and phylogenetically reduced and narrow, with weakly lignified tracheary elements. This reduction in vascular tissue tends to be correlated with the degree of specialization of the plant. Emergent aquatics possess the least reduction in the quantity of conducting tissues, whereas submerged plants have the most reduced. In cases of extreme reduction, the stele can be only 50 lam in diameter and composed of very few sieve tube elements and tracheary elements that are thin-walled and probably vestigial. In hydromorphic roots, the sieve tube elements are commonly distributed in an unusual pericyclic manner. Prominent cortical air spaces are formed by the breakdown or separation of alternate plates of cells and are continuous with those in the stem and leaves. In plants with a well-developed lacunose ground tissue, there is a ready diffusion of oxygen from the stems and leaves down to the roots that are embedded in an oxygen-deficient substrate. The air chambers in aquatic plants are regularly traversed by septa or diaphragms that are solid or porous and sometimes constructed of stellate parenchyma. Diaphragms are thought to serve three functions: (1) to provide strength and stabilization to organs and prevent collapse of the air chambers, (2) to restrict the movement of water when the organ is damaged and internal flooding occurs, and (3) to provide a lateral transport pathway across the cortex.

Aerial Roots

Aerial roots arise from some other aerial organ and function as supporting and protective organs; they also play important roles in translocation and gas exchange. Mangrove species and members of the genus *Pandanus* are noted for aerial root formation. Aerial roots also are characteristic of many epiphytes, such as orchids. Mature orchid roots are composed of three major tissue regions: a central vascular stele, a cortex containing an inner endodermal layer and outer thick-walled exodermis, and an external covering known as the velamen. The specialized velamen tissue arises from the undifferentiated protoderm and at maturity consists of multiple layers of dead cells. The velamen has been assumed to be involved in the uptake, accumulation, and movement of water and salts under the conditions of low mineral and moisture availability that exists in the forest canopy. It appears, however, that the velamen actually performs a number of functions, including adhesion of the root system to its substratum.

Hydraulic Architecture

Vessel element structure and distribution patterns provide a clear example of the significance of ecology on the hydraulic architecture of dicotyledonous trees and shrubs. Statistical analysis reveals a number of trends in specialization related to vessel element morphology and abundance that are directly related to changes in plant habit as well as latitude and altitude of provenance. Figure 8.17A illustrates the positive correlation between vessel frequency and increasing latitude in the genus *Ilex*. A positive correlation also is found between vessel element length and latitude. Figure 8.17B

demonstrates a negative correlation between vessel diameter and increasing latitude. Although the functional significance of this correlation is not clear, it is evident that tropical species of *Ilex* possess longer and wider vessel elements than the temperate representatives of the same species. Similar correlations are known for many other genera. Statistically significant correlations also exist between latitude and other wood anatomical features, such as perforation plate bar number, imperforate tracheary element length, and ray size and frequency.

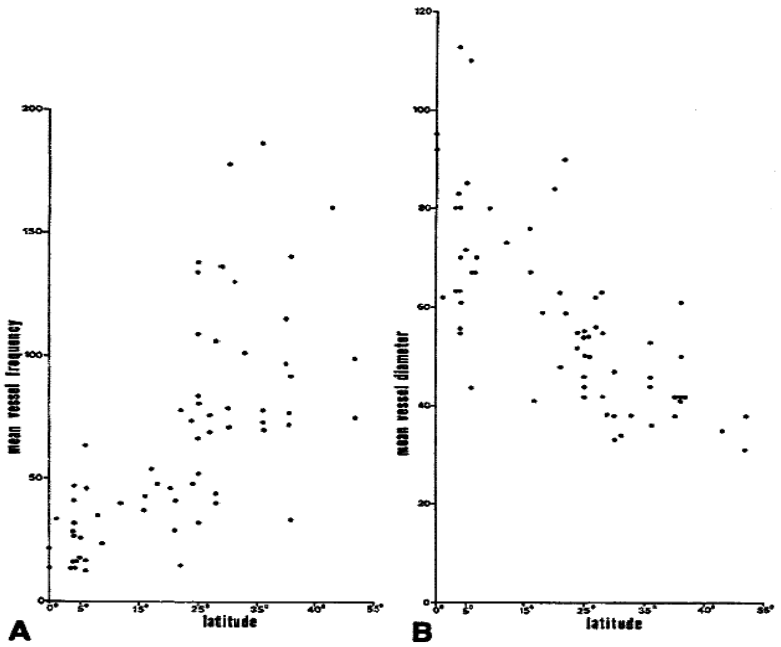


FIGURE 8.17 Correlations between vessel frequency, latitude, and diameter in 64 species of *Ilex* (Aquifoliaceae). (A) Vessel frequency and latitude. (B) Vessel diameter and latitude. Reprinted with permission from Baas (1973), *Blumea* **21**, 193–258.

Habit-related anatomical modification may be superimposed upon phylogenetic advancement. Many trends are the result of complex interrelationships between ecology and plant morphology. In many diverse groups of dicotyledons, features such as vessel element length, tangential diameter, wall thickness, and frequency are known to be under the strong control of one ecological factor, water availability. It has been hypothesized that comparatively long and wide structurally primitive vessel elements are of adaptive value (or are not disadvantageous) only in mesic habitats, where soil moisture is not limiting and foliar transpiration is fairly slow and uniform owing to a constantly high relative humidity. Shorter and narrower but more numerous vessel elements per unit area, with simple perforation plates, have evolved under drier conditions of both low soil moisture and atmospheric humidity. The narrowness of vessels in classically "xeromorphic" dicotyledonous taxa is generally correlated with an increased number of vessels per unit area of xylem transection. These and other anatomical trends can occur among different woody dicot taxa along ecological gradients.

Although these correlations have been made by relating wood structure and plant habitat, their functional significance is not always immediately clear. For example, xylem cavitation, caused by the seeding of air into the vascular system, is a major potential problem for plants. Xylem cavitation occursat species-specific xylem pressures and is correlated with drought tolerance. That is, xeric species cavitate at lower xylem pressure than mesic species. Air seeding occurs at the pit membranes

and xeric species cavitate at lower pressures because they have pit membranes that are less permeable to air seeding. Cavitation pressure and xylem conduit diameter are only weakly correlated. Conifers with small volume and diameter tracheids are not as a group more resistant to cavitation than angiosperms with large vessels. Ring-porous trees are not as a group more vulnerable to cavitation than diffuse-porous trees. The reason for the weak correlation is that cavitation does not depend on conduit size, but on pit membrane structure. Pit membrane structure is more important in cavitation events than conduit size itself.

Some authors have advocated calculating a "vulnerability index" of wood anatomy (VULN), by dividing mean vessel diameter by the mean number of vessels per square millimeter. The lowest indices of vulnerability appear in xerophytic taxa, with narrow but very numerous vessels per unit area. Theoretically, this xylem formulation ("high vessel redundancy") is much safer physiologically than one that consists of wider and fewer vessels per unit area because it will restrict air embolisms to a smaller and more localized portion of the transpiration stream in the event of disruptively high negative pressures, freezing, or other types of injury to the xylem vessels. It also is possible to calculate an index of wood anatomical mesomorphy (MESO), in which the vulnerability index is multiplied by the mean length of the vessel elements.

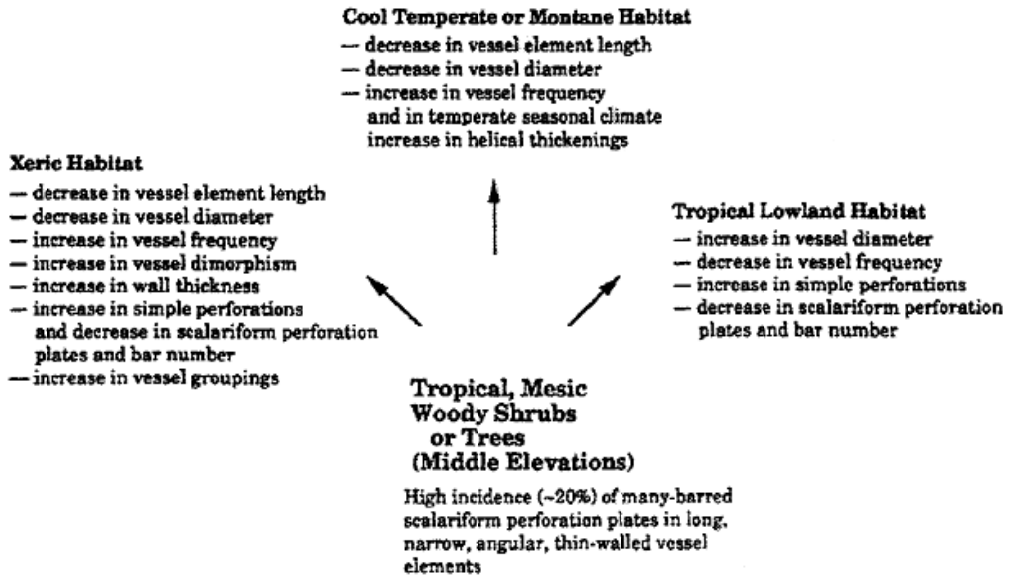


FIGURE 8.18 Major ecophyletic trends of vessel element specialization. Reprinted with permission from Wheeler and Baas (1991). *IAWA Bull. n.s.* **12,** 275–332 (adapted from Dickison).

6. Anatomy in applied science: anatomy and pollution, anatomy in forensic science, archaeology and climatology.

Anatomy in applied science

1. Anatomical Characters of Taxonomic Significance:

It is the prime importance to know exactly to which species a plant specimen belongs. This is necessary for a natural and reliable classification. Most of the plants are classified according to their

macro-morphological features. But an accurate classification results when the information from diverse sources are utilized.

A particular type of hair is constant in a species. This property is used as an aid to identify the different species of Oleaceae, *Ficus* and *Rhododendron* to some extent. Presence of T-shaped hair diagnoses the family Malpighiaceae. Trichomes provide the distinguishing characters within the family Icacinaceae where the genus Ottoschulzia is segregated from Poraqueiba on the basis of trichome characters.

Stoma has been shown to have great value in the taxonomy of several taxa. The distribution, morphology and ontogeny of a stoma are of taxonomic significance. Stoma is absent in roots. In exceptional cases it is reported from Ceratonia siliqua and Pisum arvense seedling roots.

The veins and their innumerable variations in leaf venation pattern provide various characters of taxonomic importance. The anatomical division of Angiosperm into dicotyledon and monocotyledon is based on venation pattern.

Identification of Wood:

The anatomy of a wood sample reveals many characters that help in the identification of plant from which the wood comes. In each country there are several excellent books on wood anatomy. These books provide not only anatomical description of wood of the plants occurring at the particular part of the world, but also give the salient anatomical features that help in the identification of plants.

Anatomy and pollution

Pollutants represent a diverse assortment of materials that are added to the biosphere in quantities so great that they adversely affect the functioning of plants, animals, and humans. Pollutants enter the biosphere as liquids, gases, fine particles (particulates), solids, and radioactive substances. They do serious damage to plants in the form of toxic gases, ozone, acid rain, insecticides, herbicides, and different types of radiation. In the case of liquids and gases, the various pollutants either adhere to the plant surface or enter the leaf through the cuticle or stomata, where physiological and structural responses are elicited.

Knowledge of the following categories of data are required in order to fully clarify the effects of pollution on plants:

1. Age of tissue and plant.

2. Foliar wettability (the attraction of liquid to the leaf surface).

3. Concentration of the pollutant.

4. Length of exposure to the pollutant.

5. Amount and chemical nature of any pollutant entering the leaf by

diffusing through the cuticle or through the stomata.

6. Cuticular characteristics and the deposition of epicuticular waxes.

7. Leaf pubescence.

8. Amount of intercellular space (such as the substomatal chamber).

9. Cell wall thickness.

10. Movement and assimilation of pollutant within the leaf.

11. Physiological factors affecting cellular sensitivity.

12. Environmental factors affecting cellular sensitivity, such as relative humidity, temperature, and light intensity.

Anatomy and Plant Resistance

Leaf morphology can theoretically exert an effect on leaf resistance to harmful substances by altering the depth of the boundary layer. The potential role of pubescence in regulating pollution uptake and in determining the responses of plants to air pollution has been suggested by studies that show considerably higher trichome frequencies and individual hair lengths in healthy plants growing in assumed high-pollution areas, as compared to the same species from low-pollution sites. Results from a number of studies have supported the hypothesis that leaf surface wax can be a major factor in controlling laminar resistance. Because stomata are believed to represent a major site of pollutant penetration in some plants, stomatal structure, frequency, and distribution have been assumed to be significant variables affecting plant sensitivity and overall leaf resistance.

Effects of Pollution on Wood

The relationship between airborne pollutants and forest decline is complex, studies of the anatomical characteristics of wood from pollution-diseased trees indicate that tree ring width patterns often can be used to measure the effects of pollution on tree growth over long time scales. Does the wood from diseased trees show any reduction in overall quality? As a general rule, softwoods such as fir and spruce have been shown to be more sensitive to air pollutants than hardwoods such as beech and oak.
The most common structural alterations associated with pollution in forest communities are an overall suppression of tree growth and a reduction in the xylem growth ring width as the result of a reduction in cambial activity. Pollution-induced growth suppression also can result in the formation of discontinuous rings, as well as in the occurrence of missing rings.

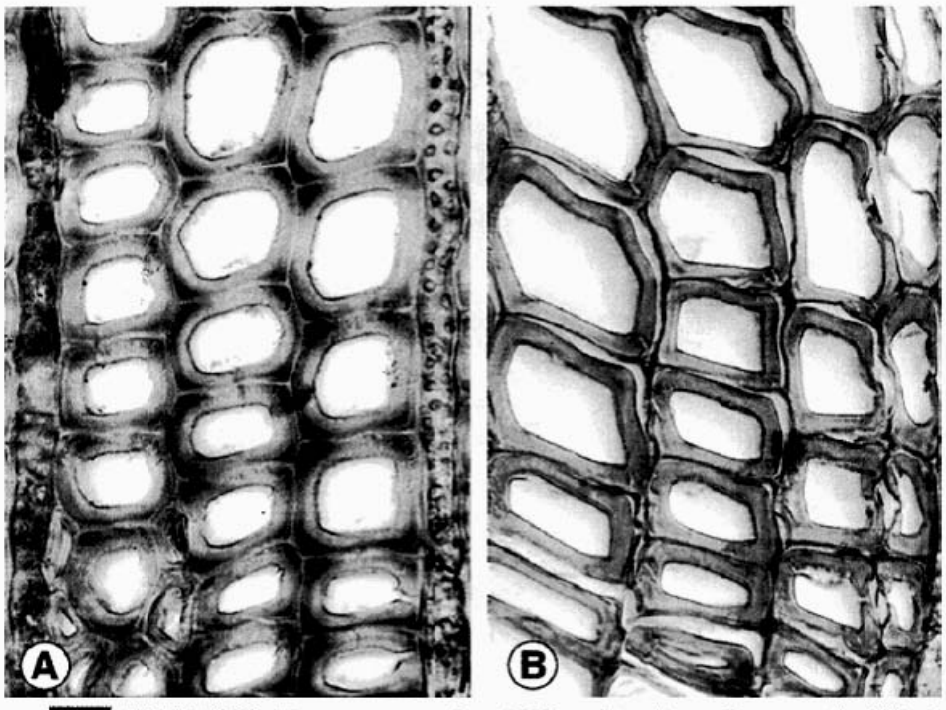


FIGURE 8.24 Transverse sections of wood of *Abies religiosa* (Pinaceae) from nonpolluted (A) and polluted areas (B) of Mexico. Atmospheric pollution resulted in thinner walled tracheids. Courtesy of Bernal Salazar Sergio and Teresa Terrazas.

Gaseous Air Pollutants

The effect of Destructive atmospheric phytotoxins such as sulfur dioxide (SO_2), hydrogen fluoride (HF), and nitrogen dioxide (NO_2) upon leaf structure can be dramatic and varied and often result in significant reductions in growth biomass and in acute and chronic tissue damage. Native vegetation or plants experimentally fumigated with hydrogen flouride or sulfur dioxide typically show shrinkage and eventual collapse of leaf mesophyll cells. Sulfur dioxide is injurious to plant tissues in concentrations as low as 0.3 to 0.5 ppm. Gaseous air pollutants enter the leaf primarily through the stomata. Within the mesophyll, the gas goes into solution on the wet mesophyll cell walls and forms toxic ions or acids. Some pollutants cause a decrease or total loss of cell membrane semipermeability in the vicinity of the stomata. Any redistribution or distortion of chloroplasts results in a reduction in net photosynthesis, which can be followed by the collapse of both lower and upper epidermal cells. Peroxyacyl nitrates specifically cause spongy mesophyll cells to collapse and be replaced by air pockets. This tissue damage produces a symptom known as **leaf glazing** on either the lower leaf surface or both surfaces of the lamina in some plants. The resulting damage is usually visible as areas of chlorosis or yellowing on the lamina.

Ozone Injury to Plants

Ozone is a major component of smog and is known to cause serious injury to plants even after only short exposure at very low concentrations of 0.1 to 0.3 ppm. Young, expanding leaves are the most susceptible to pollution injury. Ozone enters the leaf through stomata, and leaves sensitive to ozone reportedly show a rapid and progressive series of structural changes, ending in cellular collapse. Palisade parenchyma cells are especially affected following the disruption of the cell membrane. In addition to these responses, ozone stress in leaves can produce increased hair density and thickened epidermal cell walls. Lipid droplets and tanninlike depositions can increase in the mesophyll vascular bundle sheath cells of some leaves.

Acid Rain

Exposure of leaves to acid rain produces a number of histological alterations and injuries in the form of surface lesions, smaller mesophyll cells, fewer intercellular spaces, and the collapse of epidermal, palisade, and spongy mesophyll cells. A diverse collection of plants has reacted to simulated acid precipitation by forming foliar galls. These develop through a combination of cell enlargement (hypertrophy) and cell proliferation (hyperplasia) and become raised above the leaf surface. In plants that have been subjected to simulated acid rain events, most lesions develop near trichome and hydathode bases, near stomata, and along veins. Liquid acid rain appears to enter through the cuticle. In this sequence, therefore, cellular damage is most often initiated at the epidermis but then develops internally with more pollutant exposure.

Carbon Dioxide

CO_2 enrichment studies have shown leaves of different species to undergo changes in stomatal density and strong morphological modification, particularly in leaf shape. Experiments also have revealed significant increases in leaf thickness under enriched CO_2 conditions. In some plants, the increase in lamina thickness can be correlated with the formation of an additional palisade layer. Increased CO_2 levels also result in decreases in stomatal frequencies.

Ionizing Radiation

Radioactive substances emit three classes of ionizing radiation: alpha radiation with comparatively little penetrating power, beta radiation with an intermediate degree of penetration, and short wave

length gamma radiation that is similar to X-rays and that has considerable penetrating power. It has long been recognized that the meristematic cells forming the shoot apex are extremely sensitive to radiation and readily undergo cytological and genetical alterations.

A variety of responses have been reported in plants exposed to ionizing radiation. These include the loss of apical dominance and abnormal branching patterns. Leaves of irradiated plants typically exhibit marked and variable changes in color, form, and texture. The size of the lamina is frequently reduced, along with an increase in pubescence. Leaves often show an increased rate of abscission, irregular blade structure including tissue necrosis, and distorted venation. Modifications can be observed in the arrangement and size of palisade cells, in the enlargement of the spongy mesophyll cells, and in an increase in intercellular space as compared with control individuals. Distortion in the vascular tissue, most notably the xylem, also has been described. Increases and decreases in the amount of vascular tissue have been noted. Following exposure to acute gamma radiation, the foliage of Sequoia has been described as gnarled and swollen, with abnormal mesophyll and vascular tissue. There also can be an extensive development of transfusion tissue and an abnormal pattern of resin duct formation.

Ultraviolet B Radiation

Ultraviolet (UV) light rays are the extremely short wave length component of sunlight. Leaves subjected to high levels of UV-B undergo structural changes as well as (1) damage to DNA to proteins and membranes, (2) alterations in transpiration and photosynthesis, and (3) striking changes in growth. These structural changes include curling to reduce the leaf area exposed to UV. Epidermal cells can either collapse or increase in size and become thicker walled, along with undergoing a decrease in numbers of stomata and an increase in the thickness of the entire lamina. The damaged mesophyll is characterized by a marked elongation of palisade cells. However, an exceptionally thick-walled and lignified epidermis can weaken the severity of these UV-B effects on the underlying mesophyll cells.

Anatomy in Forensic Investigation:

Forensic botany, or botanical evidence suitable for a law court, has proven to be useful in the following ways: (1) as scientific evidence for resolution of court cases involving property dispute, fraud, and matters of legal responsibility or obligation; (2) as scientific evidence in criminal cases offered to prove than an alleged crime has been committed; (3) as scientific evidence in murder, accidentaldeath, or questioned death cases offered in connection with establishing the cause or time of death; and (4) as scientific evidence offered in connection with identifying the perpetrator of a crime or establishing the location of a crime.The application of forensic science is indispensable in investigating a crime. Forensic science has many disciplines and forensic botany is one of them. Forensic botany encompasses many sub- disciplines, which include plant anatomy, plant systematic (taxonomy and species identification), palynology (the study of spores and pollen) etc. They refer the use of plant materials in solving crimes or resolving other legal problems.

Plants remains are present everywhere. In a crime scene they may occur in the form of macroscopic pieces (ex. wood, twigs, leaves, flowers, fruits, seeds etc.) or microscopic forms (ex. pollen, spores,

trichomes, cell walls in stomach contents etc.). The morphological and anatomical diversity expressed by plant species provide characters to identify plant parts. Species identification is a prerequisite in analyzing botanical evidences for casework.

Analysis of anatomical samples:

Cell shape and orientation of certain structures within a cell can be helpful in classification of a species. In order to learn about plant anatomy and specific plant structures within the plant body plan, it is important to take a practical approach. In the following sections we describe examples of in expensive laboratory exercises that can be performed as training for forensic botanists. Plant anatomy provides characters such as trichomes, stomata, cuticular pattern, leaf venation, wood anatomy, growth rings etc. to aid in species identification and in performing physical matches of evidence. The identified plant materials help the investigators of criminal cases to determine whether a suspect was present in a crime scene, in which season the crime occurred, how long the body has been buried, whether the body has been moved etc.

Plant Anatomical Features used in Forensic Botany

Since 1930s: became increasingly more common in forensic applications (Lane et al., 1990).

Cell wall: particularly important for two reasons:

◊it is not easily digested by most organisms and therefore persists when other plant features are destroyed (Bock and Norris, 1997)

◊ the size, shape, and pattern of cell walls is often taxon-specific (Lane et al., 1990).

Unique cell types: sclereids, trichomes- are useful in identifying botanical material (Lane et al., 1990).

Tissue organization of different plant parts (Willey and Heilman, 1987).

Wood characteristics (secondary tissue organization)

Roots: Frequently found with exposed or shallowly buried bodies

Plant roots, like their above-ground counterparts, exhibit annual growth rings that can be useful in pinning down the post-mortem interval, or at least the time since the body came to be at the location where it was found.

Three ways in which the roots can be used to date the remains or otherwise characterize the burial site:

(1) **Examine root development after it has been damaged.** When a grave is dug or the ground otherwise disturbed, roots can be damaged but still continue growing. If the meristematic zone is damaged, no secondary xylem cells can be produced, leaving a permanent lesion. The number of growth rings laid down after the lesion indicates the number of years since the damage occurred.

(2) **Examine roots in direct contact with the remains.** Roots in contact with the bones or personal effects of the deceased can be cross-sectioned at the point of contact and the annual rings counted, establishing a minimum time frame for time since death. The contact must be penetrative, i.e. the roots must be growing through clothing or bones, in order for the interpretation to be valid.

(3) **Examine branch growth.** Annual longitudinal growth of the roots, in addition to radial growth, can be estimated, and a time frame determined from the length of a root from its point of contact with the remains to its distal end.

Stomach contents:

Characteristic cell types from food plants can be used to identify a victim's last meal (Bock and Norris, 1997). knowledge about which can be useful in determining the victim's whereabouts or actions prior to death

Some of these cell types include (Dickison, 2000):

Sclereids (pears)

Starch grains (potatoes and other tubers)

Raphide crystals (pineapple)

Druse crystals (citrus, beets, spinach)

Silica bodies (cereal grasses and bamboos)

Time since death can be approximated by the state of digestion of the stomach contents

It normally takes at least a couple of hours for food to pass from the stomach to the small intestine

A meal still largely in the stomach implies death shortly after eating, while an empty or nearly-empty stomach suggests a longer time period between eating and death (Batten, 1995).

However, there are numerous mitigating factors to take into account:

- the extent to which the food had been chewed
- the amount of fat and protein present
- physical activity undertaken by the victim prior to death
- mood of the victim
- physiological variation from person to person.

All these factors affect the rate at which food passes through the digestive tract.

A Case Study:

In a case where a young woman had been stabbed to death, witnesses reported that she had eaten her last meal at a particular fast food restaurant.

Provenance

Her stomach contents did not match the limited menu of the restaurant, leading investigators concluded that she had eaten at some point after being seen in the restaurant.

The investigation led to the apprehension of a man whom the victim knew, and with whom she had shared her actual final meal (Dickison, 2000).

Tree ring analysis (Dendrochronology) is a common technique for dating masterworks by European painters, many of which were painted directly on wood.

Given that the samples are in good condition, analysts can pinpoint the exact year when the tree, from which the wood for the painting was taken, was cut down.

Case Study

A Peter Paul Reubens painting originally dated 1616 was shown to be at least 10 years younger, and a painted wall panel recovered from a house in Switzerland in the 1970s was determined to have been painted on spruce harvested in 1497 (Schweingruber, 1988).

Dendrochronology techniques are useful in determining the provenance of wooden art objects and musical instruments

In one case, two violins forming part of an inheritance were claimed to have been made by Antonio Stradivari.

The sounding-boards of the instruments were x-rayed and compared to standard curves for spruce from the Alpine region of northern Italy, where Stradivarius is known to have worked.

The oldest rings from the samples dated to 1902 and 1894 respectively for the two violins.

Furthermore, these oldest rings were not the outermost rings of the wood from which the violins were constructed.

Allowing for a period of seasoning before the wood could be used to make the instruments, analyses showed that the violins could not have been made before 1910.

Given that Stradivari did his best work at the turn of the 17th century, the instruments were deemed to be fakes (Schweingruber, 1988).



The Kidnapping of Charles Lindbergh, Jr. (March 01, 1932)

A critical piece of evidence in the case was a crude homemade wooden ladder left at the scene. Xylotomist Arthur Koehler of the United States Forest Service undertook a meticulous examination of the ladder and when the case finally came to trial four years later, offered the first botanical testimony ever to be heard and accepted in American courts.



The ladder had been constructed in three sections, presumably for ease of transport

Koehler identified each side rail and rung with a number and identified each piece to species. Through careful examination of the characteristic milling marks left on each piece and comparisons with local mills, he was able to trace all components of the ladder back to their respective retail sources.

He also noted distinctive marks left on the wood by a dull, nicked hand plane. Of particular interest was rail #16, a piece of low-grade pine which had four distinctive square nail holes. It was also relatively unweathered.

The low grade of the wood, the nail holes, and its unweathered condition suggested that particular piece of wood had been removed from some interior construction.

Without a suspect however, progress on the case was slow. In September of 1934, some of the notes used to pay the ransom were used at a gas station by Bruno Hauptmann, a carpenter who lived in the Bronx, New York City. He was arrested when $14 600 of the ransom money was found in his garage.

Upon searching the attic for more ransom money, police noticed that one of the floorboards was eight feet shorter than the others. The square nail holes in rail 16 lined up exactly with holes in one of the attic floor beams, and the annual ring pattern of rail 16 matched that of the short floorboard. A hand plane recovered from Hauptmann's garage was indeed dull and damaged, and made marks identical to those on the ladder and on a homemade shelf in the Hauptmann garage.

Hauptmann was convicted of kidnapping and murder and was executed on April 3rd, 1936.

Wood: in archaeology

Identify Archaeological Plant Remains:

The wood-anatomy of present- day-plant provides characters to identify the fragmentary wood. These characters also enable to identify the wood and charcoal preserved in sites from antiquity.

A burnt wood or charcoal sample is collected from the site of excavation. Microscopic slides are prepared and examined thoroughly. The observation shows that the very delicate features like perforation plate and lateral wall pitting are still retained. The wood anatomy of archaeological sample is compared with that of present-day-wood and thus their identity can be detected.

After authentication it can be decided whether it was selected for burning purpose only. It may happen that the plants composed the vegetation of that area at that time. The plants, as they grew locally, were obtainable at ease and so were selected for burning purposes.

A wood is best preserved in those localities where continuous wet or dry conditions prevail. A fluctuating dry and wet atmosphere encourages the growth of pathogenic microorganisms that may attack the wood thus causing the wood to decay. It is not yet definitely known when the first use of wood began. It is assumed that the practice of using wood started in Old Stone Age for digging purposes in search of food.

For the first time, actual evidences of using wood have been recorded from the sites of New Stone Age. But no sites at this age in India produce any evidence of the use of wood. The Indus Valley civilization revealed the uses of wood for many purposes. The different use of wood was also recorded from the archaeological excavation of the proto-historic period in India namely, the Bronze Age civilization of Harappa and the Copper Age civilization of Hastinapura.

It has been reported that the Harappans used the wood of *Cedrus deodara* (Deodar) and *Dalbergia latifolia* (Rosewood) for making coffins. These durable and scented woods are still in use after thousands of years for the same purpose. *Zizyphus* was used as wooden mortar for pounding grains. This wood has the property of shock absorbing and the Harappans were quite aware of the fact.

Dalbergia sissoo (Sissoo) and *Holarrhena antidysenterica* (Kurchi) —these two timber-yielding plants were found in Hastinapura. These plants provide good fuel woods. It is not known whether these were used as firewood or charcoal. It is assumed that the Copper Age civilization was aware about the woods that have high calorific value.

In Iron Age the species of *Quercus* were used in making buildings and boats. At the site of exacavation at Brigg at South Humberside a boat made up of *Quercus* wood was preserved. It was interesting to find that the main logs of the boat were sewn together with twigs of *Salix*.

The twigs were twisted and passed through the regularly made holes on the timbers. No nails were used to hold the timbers. Corylus were well preserved in waterlogged condition in Somerset at Bronze Age. These were used in building track ways across swampy grounds.

Apart from wood and charcoal other archaeological plant remains are also preserved. As for example a sandal was preserved in ancient Egypt. Anatomical studies reveal that it is composed of *Cyperus papyrus* and *Borassus* sp.

7. Let's sum up

- Plant anatomy is the branch of botany which deals with the internal structures and organization of plant body.
- A meristem is a localized region of tissue which, by cell division, adds new cells to a plant or plant part. In the shoots of vascular plants the activity of meristems results in an increase in length and/or diameter, and following cell growth and differentiation, formation of the various mature tissue regions of the axes as well as the formation of organs
- The term epidermis designates the outermost layer of cells on the primary plant body that covers the leaves, flowers, roots and stems of plants. In seeds, scales and certain leaves, e.g. the leaves of conifers, the epidermal cell walls are very thick and lignified.
- The continuity of the epidermis is interrupted by minute openings which are intercellular spaces each of which is limited by two specialized cells termed the guard cells. In many plants subsidiary or accessory cells can be distinguished. These cells differ morphologically from the typical epidermal cells and they constitute two or more cells bordering the guard cells to which they are apparently functionally connected.
- All unicellular and multicellular appendages of the epidermis are designated by the term trichome. They may occur on all parts of the plant and may persist throughout the life of the plant part or may fall off early.
- The tissues that are concerned with the secretion of gums, resins, volatile oils, nectar latex, and other substances in plants are called secretory tissues.
- Sclereids are short cells with thick secondary walls, more or less isodiametric, strongly lignified, and provided with numerous simple pits. A fiber is an elongated, tapering sclerenchyma cell with a more or less thick secondary wall.
- The xylem is the principal water-conducting tissue in a vascular plant. It is also involved in the transport of solutes, in support, and in food storage. Together with the phloem, the principal food-conducting tissue, the xylem forms a continuous vascular system extending throughout the plant body.
- The vascular cambium is a lateral meristem that develops either as longitudinal strands or as a hollow cylinder. The secondary xylem is formed by a relatively complex meristem, the vascular cambium, consisting of vertically elongated fusiform initials and squarish or horizontally elongated ray initials.

- The variations in plant structure that are commonly affected by environmental factors are particularly strongly expressed in the morphology and anatomy of leaves. Smooth leaves with entire margins and apical drip tips are more common in lowland tropical rain forests

- In the case of liquids and gases, the various pollutants either adhere to the plant surface or enter the leaf through the cuticle or stomata, where physiological and structural responses are elicited.

- The morphological and anatomical diversity expressed by plant species provide characters to identify plant parts. Species identification is a prerequisite in analyzing botanical evidences for casework.

8. Suggested Readings

1. Fahn, A. Plant Anatomy (4th ed.), 1990, Wiley Eastern.

3. Metcalfe, C.P. & Chalk, L. Anatomy of Dicotyledons, Vol. I & II, London Press, Oxford

4. Dickison, W.C. Integrative Plant Anatomy, 2002, Academic Press

5. Eames, A.J. & Mac. Daniels, L.H. An Introduction to Plant Anatomy, Latest Ed., McGraw Hill

6. Esau, K. Anatomy of Seed Plants (2nd ed.), 1977, John Wiley & Sons

7. Cutter, E.G. Plant Anatomy: Experiment and Interpretations, Part I & II, Edward Arnold

8. Beck, C.B. An Introduction to plant Structure and Development (2nd ed.), Cambridge University Press

9. http://www.biologydiscussion.com/

10. https://en.wikipedia.org/

9. Assignment

1. What are Paracytic stomata? Write down the ontogeny of stomata
2. Distinguish between primary and secondary plant body.
3. Describe application of anatomy in the field of forensic sciences.
4. Explain hydraulic architecture of plant.
5. Describe anatomical features of ecological grous of plant.
6. What are tyloses?

7. Name the associated structure of companion cell.
8. Name the conductory elements of xylem.
9. What is annual ring?
10. Name the meristem which is responsible for growth in diameter of the cortex.
11. What is intrafascicular cambium?
12. Mention the different types of meristems.
13. What are sclereids?
14. What are trichomes?
15. Where are bulliform cells present?
16. What constitutes the stomatal apparatus?
17. Differentiate between spring wood and autumn wood.
18. Describe the phylogeny of xylary elements.
19. Write a short note on wood anatomy.

All the materials are self writing and collected from e-book, journals and web sites

POST GRADUATE DEGREE PROGRAMME (CBCS)

IN

BOTANY

SEMESTER - II

Course: BOOCT 2.1

(Plants in human welfare)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia

West Bengal, India

COURSE PREPARATION TEAM

Dr. Bapi Ghosh
Assistant professor
Department of Botany, DODL
Kalyani University

Dr. Pallab Kumar Ghosh
Assistant professor
Department of Botany, DODL
Kalyani University

Dr. Sudipta Roy
HOD & Associate professor
Department of Botany
Kalyani University

Dr. Zahed Hossain
Associate professor
Department of Botany
Kalyani University

Dr. Sudha Gupta
Assistant professor
Department of Botany
Kalyani University

Dr. Malay Kr. Adak
Assistant professor
Department of Botany
Kalyani University

Dr. Neera Sen Sarkar
Assistant professor
Department of Botany
Kalyani University

June, 2019

Directorate of Open and Distance Learning, University of Kalyani

Published by the Directorate of Open and Distance Learning,
University of Kalyani, Kalyani-741235, West Bengal and Printed by
Printtech, 15A, Ambika Mukherjee Road, Kolkata-700056

All right reserved. No part of this work should be reproduced in any form without the permission in writing from the Directorate of Open and Distance Learning, University of Kalyani.

Authors are responsible for the academic contents of the course as far as copyright laws are concerned.

Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2017 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Sankar Kumar Ghosh, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every member of PGBOS (DODL), University of Kalyani. Heartfelt thanks is also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and co-ordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

Self Learning Materials (SLMs) have been published by the Directorate of Open and Distance Learning, University of Kalyani, Kalyani-741235, West Bengal and all the copyright reserved for University of Kalyani. No part of this work should be reproduced in any from without permission in writing from the appropriate authority of the University of Kalyani.

All the Self Learning Materials are-self writing and collected from e-book, journals and websites.

Director
Directorate of Open and Distance Learning
University of Kalyani

SYLLABUS

COURSE–BOOCT 2.1

Plants in human welfare

(Full Marks–75)

Course	Group	Details Contents Structure		Study hour
BOHCT 2.1	Plants in human welfare	**Unit 1. Know your plants -I**	1. Know your plants-Algae and Fungi	1
		Unit 2. Know your plants -II	2. Know your plants-Bryophyte and Pteridophyte	1
		Unit 3. Know your plants -III	3. Know your plants-Gymnosperms and Angiosperms	1
		Unit 4. Exploitation of microbes	4. Exploitation of microbes- Virus and Bacteria	1
		Unit 5. Mushrooms	5. Mushrooms – their uses and cultivation	1
		Unit 6. Environmental clean-up	6. Environmental clean-up by plants	1
		Unit 7. Stress in plants I	7. Stress in plants- Water	1
		Unit 8. Stress in plants II	8. Stress in plants- heat and cold	1
		Unit 9. Genetically modified crops I	9. Genetically modified crops- Introduction and steps	1
		Unit 10. Genetically modified crops II	10. Genetically modified crops- Examples	1
		Unit 11. Exploitation of plant resources	11. Exploitation of plant resources	1
		Unit 12. Conservation of plants I	12. Conservation of plants-Biodiversity and conservation type	1
		Unit 13. Conservation of plants II	13. Conservation of plants-IUCN categories, MAB, CITES and Hotspot	1
		Unit 14. Plants in forensic science I	14. Plants in forensic science-Introduction and Analysis of samples	1
		Unit 15. Plants in forensic science II	15. Plants in forensic science-Case histories	1
		Unit 16. IPR	16. Intellectual Property Rights	1

Content

COURSE – BOOCT 2.1
Plants in human welfare

	Page No.
Unit 1. Know your plants -I	1-50
Unit 2. Know your plants -II	50-73
Unit 3. Know your plants -III	73-81
Unit 4. Exploitation of microbes	81-84
Unit 5. Mushrooms	84-93
Unit 6. Environmental clean-up	93-97
Unit 7. Stress in plants I	97-99
Unit 8. Stress in plants II	99-103
Unit 9. Genetically modified crops I	104-108
Unit 10. Genetically modified crops II	108-117
Unit 11. Exploitation of plant resources	117-140
Unit 12. Conservation of plants I	140-147
Unit 13. Conservation of plants II	147-149
Unit 14. Plants in forensic science I	150-163
Unit 15. Plants in forensic science II	163-168
Unit 16. IPR	168-185

COURSE – BOOCT 2.1
Plants in human welfare

Hard Core Theory Paper **Credit: 4**

Content Structure

1. Introduction
2. Course Objective
3. Know your plants
4. Exploitation of microbes
5. Mushrooms – their uses and cultivation
6. Environmental clean-up by plants
7. Stress in plants
8. Genetically modified crops
9. Exploitation of plant resources
10. Conservation of plants
11. Plants in forensic science
12. Intellectual Property Rights
13. Let us sum up
14. Suggested Reading
15. Assignment

1. Introduction

Biology is the youngest of the formalized disciplines of natural science. Progress in physics and chemistry proceeded much faster than in Biology. Applications of physics and chemistry in our daily life also have a higher visibility than those of biology. However, twentieth century and certainly twenty-first centuries has demonstrated the utility of biological knowledge in furthering human welfare, be it in health sector or agriculture. The discovery of antibiotics, and synthetic plant -derived drugs, anesthetics have changed medical practice on one hand and human health on the other hand. Life expectancy of human beings has dramatically changed over the years. Agricultural practices, food processing and diagnostics have brought socio -cultural changes in human communities.

2. Course Objectives

After completion of the course the learners will be able to:

- describe the Mushroom cultivation process and their uses;
- explain the Characteristics of different plant groups;
- discuss the role of plant in forensic science;
- explain the concepts of genetically modified crops;
- Identify IUCN categories plant.

3. Know your plants

Plants are multicellular and mostly photosynthetic organisms which found essentially everywhere, both in water and on land.

The aquatic plants include red, brown and green algae and the land plants include mosses, ferns, gymnosperms and angiosperms. The angiosperms or flowering plants are dominated the earth the last 70 million years. There are over 3, 00,000 angiosperm species growing on the earth which show enormous diversity in size and form. In India there are about 45,000 plant species are known that represent about 7% of the world's flora. Nearly, 4900 species of angiosperms are endemic to India.

Algae:

The term algae (Latin — seaweeds) was first introduced by Linnaeus in 1753, meaning the Hepaticeae. The algae comprise of a large heterogeneous assemblage of plants which are diverse in habitat, size, organisation, physiology, biochemistry, and reproduction.

It is an important group of Thallophyta (Gr. Thallos — a sprout; phyton — a plant), the primitive and simplest division of the plant kingdom. The orderly system-atic study of algae is called Phycology (Gr.phycos — seaweeds; logos — study or discourse).

Defining algae

One of the most frustrating questions often asked in the study of algae is: What is an alga? Perhaps the **frustration arises fromthe inability to define concisely all that is presently encompassed by the term, without seeming exceedingly ambiguous**. Algae (plural, from the Latin alga which means seaweed) both historically and currently have been variously defined.

Stearn (1992) gave the literal translation as 'seaweed, a thing of little value', a definition that is entertaining in its quaintness, but not exactly informative. **For the algae are both more and less than seaweeds.** More, in that the term is now also applied to a large number of unicellular as well as thallose organisms occupying a variety of habitats that include freshwater and terrestrial, as well as marine. Less, in that the term 'seaweed' sometimes encompasses marine angiosperms, *i.e.* the seagrasses that are more closely related to trees than they are to any of their photosynthetic co-inhabitants of the marine environment.

Authors have attempted to define the algae by exclusion:

Entwisle *et al*., (1997) have defined algae as 'simple photosynthetic organisms not included among the mosses, liverworts (and other bryophytes) or the vascular plants'.

Ragan (1998) defines algae as 'cyanobacteria plus photosynthetic eukaryotes and their immediate relatives, but excluding green plants'.

Some have adopted a vividly circular explanation, e.g. 'those organisms studied by phycologists' (Entwisle & Huisman, 1998), phycologists being those scientists who study algae!

Yet others take a historical view, essentially including those organisms traditionally regarded as algae and their close relatives.

Papenfuss (1955) defined the algae as, '...plants [in which]... reproductive organs lack a primarily produced sterile jacket of cells', but qualified this by giving the Charophyta as an exception.

Bold & Wynne (1978) amended the definition to include organisms that, when unicellular, may function directly as gametes, when multicellular producing either unicellular or multicellular gametangia; in the latter case every gametangial cell is fertile. This definition separates the algae from other green plants, but only serves to define the group partially (excluding as it does the many asexual taxa).

Although an accurate definition of the algae appears to be elusive, the reason for our seeming inability to define what we are talking about is more straightforward, for the term is applied to an artificial cluster of unrelated or distantly related groups of organisms. In fact, it is imperative that this aspect of the 'algae' be stressed if there is to be any understanding of their place in the overall scheme of life. So what do we mean when we talk of algae? **In essence it is a term of convenience, one that describes a concept rather than a taxonomic group.** If pushed, we would describe the algae, and please note the ***intentional vagueness***, as **'mostly simply constructed, mostly photosynthetic, plant-like organisms and their close relatives'**.

Characteristics of Algae:

1. Algae are chlorophyll-bearing autotrophic thalloid plant body.
2. Almost all the algae are aquatic.
3. The plant body may be unicellular to large robust multicellular structure.
4. The multicellular complex thalli lack vascu-lar tissue and also show little differentiation of tissues.
5. The sex organs are generally unicellular but, when multicellular, all cells are fertile and in most cases the entire structure does not have any protection jacket.
6. The zygote undergoes further development either by mitosis or meiosis, but not through embryo formation.
7. Plants having distinct alternation of genera-tions. Both gametophyte and sporophyte generations — when present in the life cycle are independent.

Occurrence of Algae:

The algae are ubiquitous (present every-where) in distribution, i.e., they are found in fresh water as well as marine water, on soil, on rock, as epiphytes or parasites on plants and animals, in hot springs, in desert, on permanent snow-fields etc. Based on habitat the algae may be categorized as:

1. Aquatic Algae:

Aquatic algae may be fresh water (when salinity is as low-as 10 ppm) or marine (when salinity is 33-40%). Again, certain algae grow in brackish water which is unpalatable for drinking, but less salty than sea water. The fresh water algae usually grow in ponds, lakes, tanks, ditch-es etc.

The very common fresh water algae are *Chlamydomonas, Volvox, Ulothrix, Chara, Oedogonium, Spirogyra, Nostoc, Oscillatoria* etc. Some of the very common marine algae are *Sargassum, Laminaria, Ectocarpus, Polysiphonia, Caulerpa, Bangia, Padina* etc.

2. Terrestrial Algae:

Some algae are found to grow in terrestrial habitats like soils,' rocks, logs etc. The algae that grow on the surface of the soil are known as saprophytes. Many blue-greens, on the other hand, grow under the surface of the soil, and are called cryptophytes.

The algae growing in the desert soil may be typified as endedaphic (living in soil), epidaphic (living on the soil surface), hypolithic (growing on the lower surface of the stones on soil), chasmolithic (living in rock fissures) and endolithic algae (which are rock penetrating).

The common terrestrial members are *Oscillatoria sancta, Vaucheria geminata, Chlorella lichina, Euglena* sp., *Fritschiella* sp. and *Phormidium* sp.

3. Algae of Remarkable Habitats:

1. Halophytic Algae (or Eurhaline):

They grow in the highly concentrated salt lakes, and include *Chlamydomonas ehrenbergli, Dunaliella* and *Stephanoptera* sp.

2. Symbiotic Algae:

They grow in association with fungi, bryophytes, gymnosperms or angiosperms. The best examples of symbiotic algae found in association with fungi are *Nostoc*, *Gloeocapsa*, *Rivularia*; the members of Cyanophyceae and *Chlorella, Cytococcus*, *Pleurococcus*; the members of Chlorophyceae.

This symbiotic association consis-ting of algae and fungi is called lichen. *Nostoc* may also associate with Anthoceros and Anabaena associates with the roots of *Cycas* to form coralloid roots.

3. Cryophytic Algae:

This group of algae grow-ing on ice or snow provides attractive colours to snow-covered mountains. The alpine and arctic mountains become red due to the growth of the Haemotococcous nivalis; green snow in Europe is due to the growth of *Chlamydomonas yellowstonensis*.

Scotiella nivalis and *Raphidonema brevirostri* cause black colouration of snow, whereas Ancyclonema nordenskioldii is responsible for brownish purple colouration.

4. Thermophytes or Thermal Algae:

This group of algae occurs in hot water springs (50- 70°C) where normal life is not possible. Many blue-greens (e.g., *Oscillatoria brevis, Synechococcus elongates, Heterohormogonium* sp.) are grown in such hot springs.

5. Lithophytes:

They grow on the moist surface of stones and rocks, e.g., *Nostoc,. Gloeocapsa, Enteromofpha, Batrachospermum* etc.

6. Epiphytic Algae:

They grow on other plants including other algal members.

These are:

i. *Ptilota plumosa* and *Rhodymenia pseudopalmatta* on *Laminaria hyperborean*, ii. Diatoms on *Oedogonium, Spirogyra* etc.

Blue-green algae like *Nostoc, Oscillatoria*, diatoms like *Achnanthes* etc. grow on different bryophytes.

Algae like *Cocconis, Achnanthes* etc. grow epiphytically on *Lemna*, an aquatic angiosperm. Alga like *Trentepohlia* grows on the barks of different angiospermic plants, and is very common in Darjeeling (India).

7. Epizoic Algae:

The algae growing on animals like fish, snail etc. are called as epizoic, e.g., *Stigeoclonium* are found in the gills of fishes.

8. Endozoic Algae:

They grow in the tissues of animals, e.g., *Zoochlorella* sp. is found in *Hydra viridis*.

9. Parasitic Algae:

Some algae grow parasitically on different plants and animals.

These are:

a. *Cephaleuros* (Chlorophyceae) is para-sitic and grows on the leaves of various angiosperms, such as tea (*Camellia sinensis*), coffee (*Coffea arabica*), *Rhododendron*, *Magnolia* and pepper (*Piper nigrum*). The most important one is *Cephaleuros virescens*, which causes Red rust of tea.

b. *Rhodochytrium* (Chlorophyceae) grows on ragweed (*Ambrosia*) leaves.

10. Psammon:

The algae which grow in sandy beaches are called psammon, e.g., *Vaucheria, Phormidium* etc.

Thallus Organisation in Algae:

Thalli of algae show a range of organisation starting from unicellular form to highly organised multicellular habit where the plant body is differentiated into root-like, stem-like, and leaf-like structures giving a higher plant-like appea-rance. Their size ranges from a few micron to several metres.

The algal thalli are grouped into the following, based on their organisation:

A. Unicellular Algae:

Unicellular forms of algae are also called acellular algae as they function as complete living organisms. Unicellular forms are common in all the groups of algae except Rhodophyceae, Phaeophyceae and Charophyceae. The unicells may be motile or non-motile.

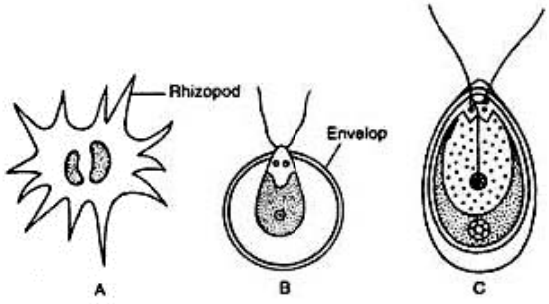


Fig. 3.1 : Unicellular motile algae : A. *Chrysamoeba*, B. *Phacotus*, and C. *Chlamydomonas*

a. The motile unicells are either rhizopodial or flagellated.

The rhizopodial forms lack rigid cell wall and have cytoplasmic projections that help them in amoeboid movement, e.g., *Chrysamoeba* (Chrysophyceae), *Rhizochloris* (Xanthophyceae).

The flagellated unicells resemble the motile gametes and zoospores. The flagella function as the organ of locomotion varying in number and type in different groups. The flagellated unicells are found in many groups of algae, e.g., *Phacotus* and *Chlamydomonas*, of Chlorophyceae. *Euglena* of Euglenophyceae etc.

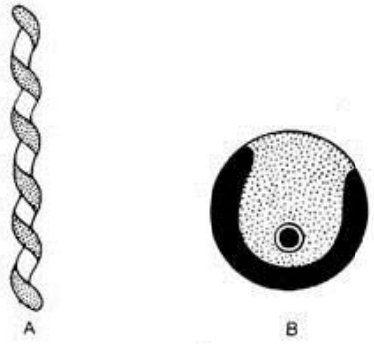


Fig. 3.2 : Unicellular non-motile algae, A. *Spirulina*, and B. *Chlorella*

b. The non-motile cells may be spiral filament as found in *Spirulina* (Cyanophyceae). The coccoid unicellular algae are the simplest forms of algae found in Cyanophyceae, Chlorophyceae etc., e.g., *Gloeocapsa, Chlorella* .

B. Multicellular Algae:

1. Colonial:

The colonial habit is achieved by loose aggregation of cells within a common mucilaginous investment. The cells of these usually remain connected with each other by cytoplasmic threads.

a. Coenobium:

When a colony has a defi-nite number of cells with a definite shape and arrangement, it is called coenobium.

Coenobium may be:

i. In motile form, cells are flagellated and whole coenobium can move by the organised beating action of flagella, e.g., *Volvox*, *Pandorina*, *Eudorina* etc. In Volvox the coenobium is a hollow sphere.

Colonial Algae

ii. In non-motile form, the cells are without flagella, thereby the coenobium is non-motile, e.g., *Scenedesmus*, *Hydrodictyon*.

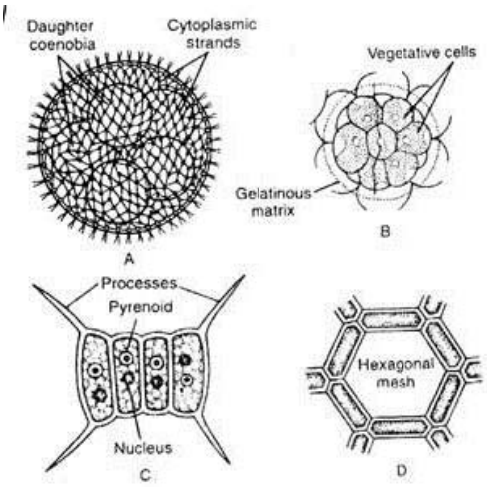


Fig. 3.3 : Colonial algae : A. *Volvox*, B. *Pandorina*, C. *Scenedesmus*, and D. *Hydrodictyon*

b. Aggregated Form:

Unlike coenobium the cells are aggregated irregularly showing a colonial mass of various size and shape.

It is of three types:

i. Palmelloid:

In this type the non- motile cells remain embedded in an amorphous gelatinous or mucilaginous matrix. Each and every cell of the organisation is independent and can perform all the functions as an individual. *Chlamydomonas* and *Chromulina* represent palmelloid as a temporary feature in their life cycle.

But in *Tetraspora* and *Palmodictyon* (Chlorophyceae), *Gleochloris* and *Chlorosaccus* (Xanthophyceae), *Phaeocystis* (Chrysophyceae) and *Microcystis* (Cyanophyceae), the palmelloid habit is a permanent feature.

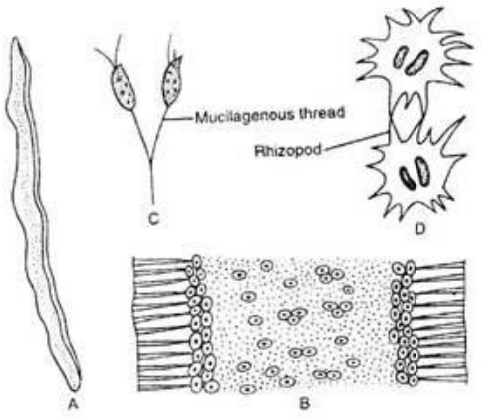


Fig. 3.4 : Aggregated form : A. *Tetraspora*, B. *Tetraspora*, (portion of a colony in T.S.), C. *Chrysodendron* and D. *Chrysidiastrum*

ii. Dendroid:

In this type the number, shape and size of the cell is variable. They look like microscopic trees (e.g., *Prasinocladus, Ecballocystis, Chrysodendron* etc.). A mucilaginous thread is present at the base of each cell, thus showing a sort of polarity.

iii. Rhizopodial:

In this type the cells are united through rhizopodia. e.g., *Chrysidiastrum* (Chrysophyceae).

2. Filamentous:

The filamentous plant body is formed through repeated cell divisions in a single plane and in a single direction, where the cells remain firmly attached to each other — end to end forming a chain or a thread. The fila-ments may be unbranched or branched.

a. Unbranched Filament:

It may be free-floating (e.g., *Spirogyra*) or attached to the substratum (e.g., *Ulothrix, Oedogonium*, etc.). The free-floating unbranched filaments are not differentiated into basal and apical ends. All the cells in the filament are alike. But the Unbranched filaments that remain attached to the substratum are differentiated into base and apex.

All the cells of the filament are similar except the basal attachment cell that is specially modified for the purpose called holdfast or rhizoidal cell.

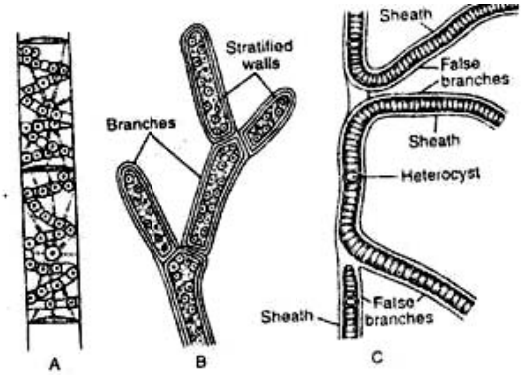


Fig. 3.5 : Filamentous type : A. *Spirogyra*, B. *Cladophora* and C. *Scytonema*

b. Branched Filament:

It is formed when a filament occasionally starts division in a second plane.

It is of two types:

i. Falsely Branched:

The trichomes of blue greens may break either due to death or decay of the intercalary cells. The broken ends emerge out of the mucilaginous sheath in the form of a branch. They do not arise as lateral out-growths, e.g., *Scytonema*.

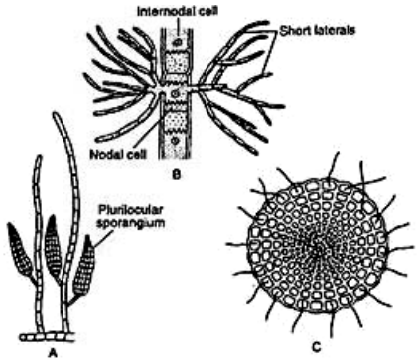


Fig. 3.6 : Branched filament showing heterotrichous habit : A. *Ectocarpus*, B. *Draparnaldiopsis*, and C. *Coleochaete*

Filamentous Type

ii. Truly Branched:

When a cell in the filament occasionally starts division in a second plane, true branch is formed. Thus true branches arise as lateral outgrowths of the main filament. True branches are of the following three types: Simple filament, Heterotrichous habit, and Pseudoparenchymatous habit.

Simple Filament:

In this branching sys-tem the whole thallus remain attached to the substratum by a basal cell and the branches may arise from any cell of the filament except the basal cell, e.g., *Cladophora*.

Heterotrichous Habit:

In this branching system the whole thallus is differentiated into prostrate and erect system. Both the prostrate and erect systems may be well-developed (e.g., *Fritschiella*, *Ectocarpus*). Progressive elimination of the prostrate system is observed in *Draparnaldiopsis*, *Stigoclonium*, oi of the erect system as in *Coleochaete*.

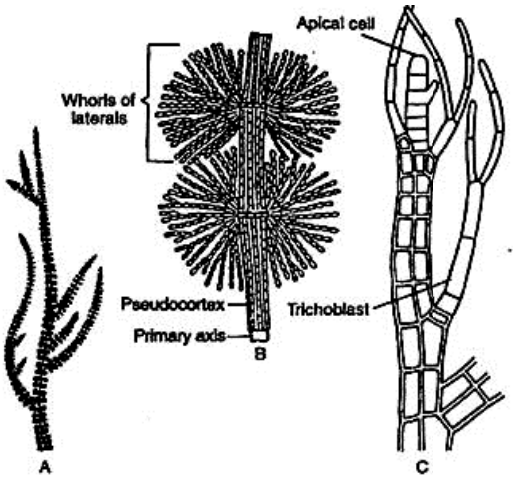


Fig. 3.7 : Pseudoparenchymatous habit : A. *Batrachospermum*, B. *Batrachospermum* (portion of the plant body), and C. *Polysiphonia*

Branched Filament

Pseudoparenchymatous Habit:

If one or more central or axial filaments together with their branches fuse to form a parenchymatous structure, it is called pseudoparenchymatous. thallus. Again, if it is formed by the branches of a single filament it is known as uniaxial (e.g., *Batrachospermum*), or it may be multiaxial where more than one filament are involved (e.g., *Polysiphonia*).

Pseudoparenchymatous Habit

3. Siphonaceous Forms:

In this form the thallus is aseptate and multinucleate i.e., a coenocyte. It may be simple branched (e.g., *Vaucheria*) or may be very elaborate with clear division of labour, differentiated into aerial and subterranean portions (e.g., *Botrydium*).

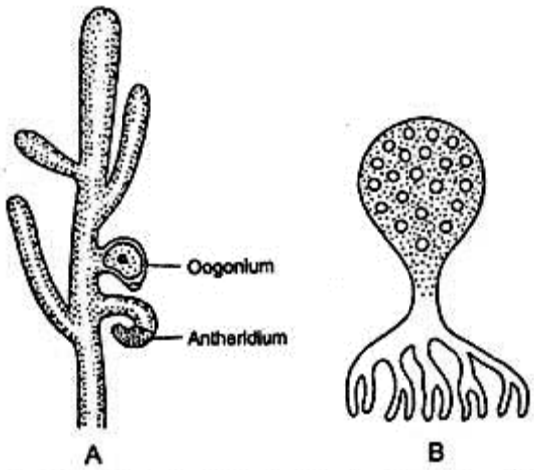


Fig. 3.8 : Siphonaceous algae : A. *Vaucheria*, and B. *Botrydium*

4. Parenchymatous Forms:

When the cells of a filament divide in multidirectional planes, it results the formation of a parenchymatous thallus and ultimately becoming foliose and flat (e.g., *Ulva*), tubular (e.g., *Enteromorpha*, *Scytosiphon*) or complex (e.g., *Sargassum*) structure.

Growth of the parenchymatous thalli may be diffused (when all the cells can divide), intercalary (when the dividing region remain in the intercalary position) e.g., *Laminaria* (, trichothallic (growth by a specialised intercalary meristem at the base of a terminal hair) e.g., *Porphyra* or apical (when one or more well-defined apical cells divide to produce the remainder of the thallus), e.g., *Fucus*.

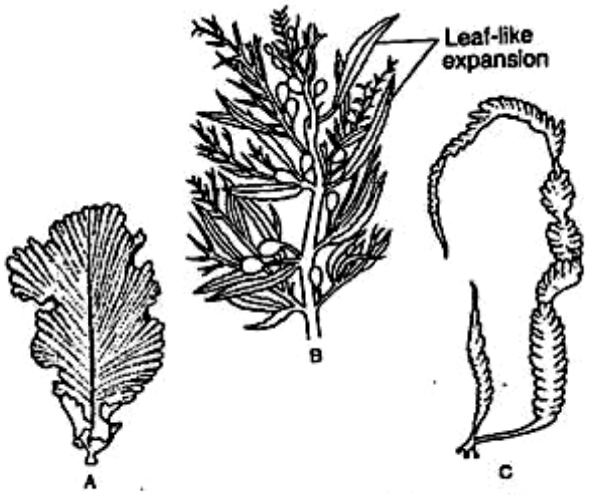


Fig. 3.9 : Parenchymatous algae : A. *Ulva*, B. *Sargassum*, and C. *Laminaria*

Reproduction:

The following points highlight the three modes of reproduction in algae.

1. Vegetative Reproduction:

In this type, any vegetative part of the thallus develops into new individual. It does not involve any spore formation and there is no alternation of generations. It is the most common method of reproduction in algae.

The vege-tative reproduction in algae is of the follow-ing types:

a. Cell division or fission:

The unicellular forms of algae commonly reproduce by this simple process, often called binary fission as found in *Chlamydomonas*, *Synechococcus*, diatoms etc. In this method the vegetative cell divides mitotically into two daughter cells, those finally behave as new individual.

b. Fragmentation:

In this method, the multicellular filamentous thallus breaks into many celled fragments, each of which gives rise to a new individual. The fragmentation may be accidental or by the formation of separation discs or by some other mechanical force or injury. It is found in *Spirogyra*, *Ulothrix*, *Oedogonium*, *Zygnema*, *Cylindospermum* etc.

c. Hormogonia:

This method of vegetative reproduction is found in blue-green algae. The trichomes of blue-green algae break up within the sheath into many-celled segments called hormogonia or hormogones. They

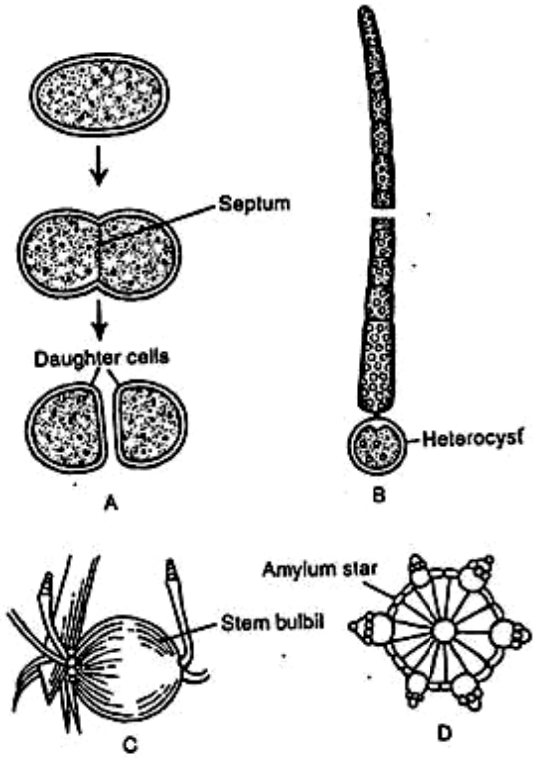


Fig. 3.16 : Vegetative reproduction in algae : A. Cell division (*Synechococcus* sp.) B. Fragmentation of filament (*Cylindrospermum* sp.) C. Stem bulbil (*Chara* sp.) and D. Amylum star (*Chara* sp.)

remain delim-ited by the formation of heterocysts, separation discs or necridia or by the death and decay of intercalary cells of the trichome. Hormogonia are commonly found in *Nostoc, Oscillatoria, Cylindosporium* etc.

d. Formation of Adventitious Branches:

Adventitious branches are formed in different large thalloid algae, which, when detached from the plant body, develop into new individuals (e.g., *Fucus, Dictyota*). Protonema-like adventitious branches are formed from the internodes of *Chara*, stolons of *Cladophora glomareta* etc.

e. Bulbils:

Tuber-like outgrowths are deve-loped due to storage of food at the tip of rhizoids and on the lower nodes of *Chara*, called bulbils. After detachment from the plant body, bulbils grow into new plants.

f. Amylum stars:

Star-shaped aggregation of starch containing cells develops on the lower nodes of Chara. These struc-tures are called amylum stars. When detached from the plant body, they grow into new plants.

g. Budding:

In Protosiphon bud-like struc-tures are formed due to proliferation of vesicles delimited from the parental body by a septum, which, after detachment, grow into a new plant.

Asexual Reproduction:

Asexual reproduction involves the formation of certain type of spores — either naked or newly walled. It is a process of rejuvenation of the protoplast without any sexual fusion. Each and every spore germinates into a new plant. In this method, there is no alternation of generations.

The asexual spores may be of various types:

a. Zoospores:

These are motile naked spores provided with two, four or many flagella and called as bi-, quadri- or multiflagellate zoospores, respectively. Biflagellate zoospores are found in *Chlamydomonas, Ulothrix Ectocarpus* etc., quadriflagellate zoospores are found in *Ulothrix* and multiflagellate zoospores are found in *Oedogonium.*

But the multinucleate and multiflagellate zoospores as found in *Vaucheria* are called synzoospores. Each zoospore has a chloroplast and an eye spot. The zoospores may be either haploid or diploid.

They are formed within the zoosporangium. There may be single zoospore (e.g., *Oedogonium*) or many zoospores (e.g., *Cladophora*) per zoosporangium.

The zoospores are liberated either by the disintegration of the zoosporangial wall or by the formation of an apical pore on the zoosporangium.

b. Aplanospores:

Aplanospores are non-motile spores. These spores are formed either singly or its protoplast may divide to form many aplanospores inside sporangium during unfavourable conditions, especially in drought (e.g., *Ulothrix, Microspora*). The aplanospores may also be formed in certain algae of semiaquatic habitat.

When they appear identical to the parent cell, they are referred to as **autospores** (e.g., *Scenedesmus, Chlorella* etc.). Aplanospores with thickened wall and abundant food reserve are known as **hypnospores** (e.g., *Pediastram, Sphaerella* etc.).

c. Tetraspores:

Diploid plants of some algae (e.g., *Polysiphonia*) produce a special type of haploid aplano-spores, called tetraspores, formed within tetrasporangium. The diploid nucleus of a tetrasporangium divides meiotically to form four haploid nuclei which — with little amount of protoplasm — are deve-loped into four tetraspores. After libe-ration the tetraspores germinate to form male and female gametophytes.

d. Akinetes:

The vegetative cells of certain filamentous algae develop into elonga-ted thick-walled spore-like structures with abundant food reserves, called akinetes (e.g., *Gloeotrichia*). They can tide over the unfavourable conditions. With the onset of favourable condition they germinate into new individuals.

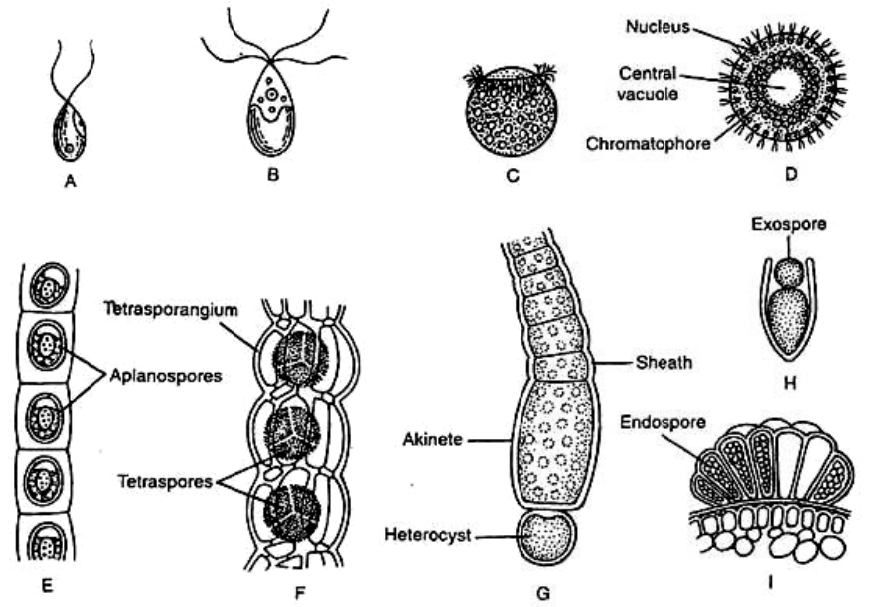


Fig. 3.17 : Asexual spores in algae : A. Biflagellate microzoospore, and B. Quadriflagellate microzoospore of *Ulothrix* sp., C. Multiflagellate zoospore of *Oedogonium* sp., D. Synzoospore of *Vaucheria* sp., E. Aplanospores of *Ulothrix* sp., F. Tetraspores of *Polysiphonia* sp., G. Akinete of *Gloeotrichia* sp., H. Exospore of *Chamaesiphon incrustans*, and I. Endospores of *Dermocarpa prasina*

e. Exospores:

In some algae, spores are regularly cut off at the exposed distal end of the protoplast in basipetal succession, called exospores. These spores aggregate in groups and develop new colonies, e.g., *Chamaesiphon*.

f. Endospores:

These are small spores formed by the divisions of the mother protoplast. They are also called conidia or gonidia. They are set free after the dissolution of mother wail. Without taking rest, the spores germinate direct-ly and develop into a new plant, e.g., *Dermocarpa*.

Sexual Reproduction:

All algae except the members of the class Cyanophyceae reproduce sexually. During sexual reproduction gametes fuse to form zygote. The new genetic set up can develop by the fusion of gametes coming from the different parents.

Types of Sexual Reproduction in Algae

Depending on the structure, physio-logical behaviour and complexity of sex organs, sexual reproductions are of the following five types:

a. Autogamy:

In this process the fusing gametes are developed from the same mother cell and after fusion they form zygote. For the above, plant developed through autogamy does not show the introduction of any new characteristic, e.g., Diatom (*Amphora normani*).

b. Hologamy:

In some unicellular member the vegetative cells of different strains (+ and -) behave as gametes and after fusion they form zygote. It is an inefficient process considering the point of multiplication, but new genetic combi-nations are developed by this process, e.g., *Chlamydomonas*.

c. Isogamy:

It is the process of union, between two gametes which are morpho-logically and physiologically similar — after fusion they form zygote. The gametes are called isogametes. Usually they are flagellate, e.g., *Chlamydomonas eugametos, Ulothrix* etc.

d. Anisogamy:

In this process the uniting gametes are morphologically and physiologically different. The smaller and more active one is the microgamete (male), whereas the larger and less active one is the macrogamete (female), e.g., *Chlamydomonas braunii*.

Deviating from the typical anisogamy, when the uniting gametes show morphological similarity with physiological difference, it is called physiological anisogamy. e.g., *Zygnema, Spirogyra* etc.

e. Oogamy:

It is an advanced process where fertilisation takes place between a small motile (non-motile in Rhodophyceae) male gamete (sperm or antherozoides) with a large non-motile female gamete (egg or ovum). Male gametes develop within antheridium, whereas the female gamete within the oogonium, e.g., *Oedogonium, Vaucheria, Chara, Laminaria, Sargassum, Polysiphonia, Batrachospermum* etc.

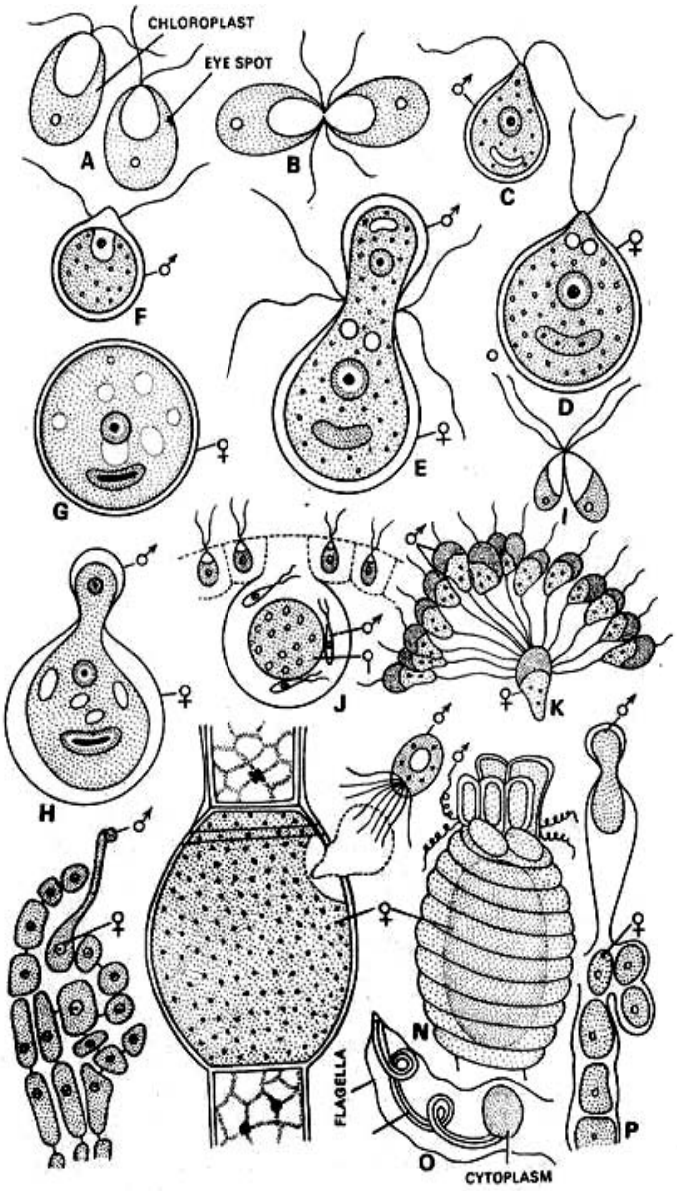


Fig. 3.8. Reproduction. Sexual. A, two gametes of same size (isogametes); B, fusion of same (isogamy); C and D, two gametes of different size (male and female); E, fusion of same (anisogantny); F and G, male and female gametes; H, fusion of same (oogamy); I, anisogamy in *Enteromorpha inteslinalis*; J. oogamy in *Volvox*; K, clump formation in *Ectocarpus*, large female is surrounded by male gametes; L, oogamy in *Oedogonium*; M, advanced oogamy in *Polysiphonia*; N, oogamy, in *Chara*; O, antherozoid of *Chara*; P, oogamy in *Batrachospermum*.

Life Cycle:

The following points highlight the four main patterns of life cycle in algae. The patterns are: 1. Haplontic Life Cycle 2. Diplontic Life Cycle 3. Diplohaplontic Life Cycle 4. Triphasic Life Cycle.

1. Haplontic Life Cycle:

The plant body is gametophyte (haploid) and sporophyte (diploid) stage is represented only by zygote. The gametophytic plant develops haploid gametes in the gametangium. The fusion between gametes results the formation of zygote, the only diploid stage i.e., sporophytic phase of the life cycle.

The zygote undergoes meiotic division and forms four meiospores. These meiospores develop into haploid plants. The alternation of generations can be interpreted by chromosome number.

This life cycle is also known as monogenic life cycle. This type of life cycle is found in majority of Chlorophyceae like *Chlamydomonas, Ulothrix, Oedogonium, Spirogyra, Chara* etc. and all members of Xanthophyceae.

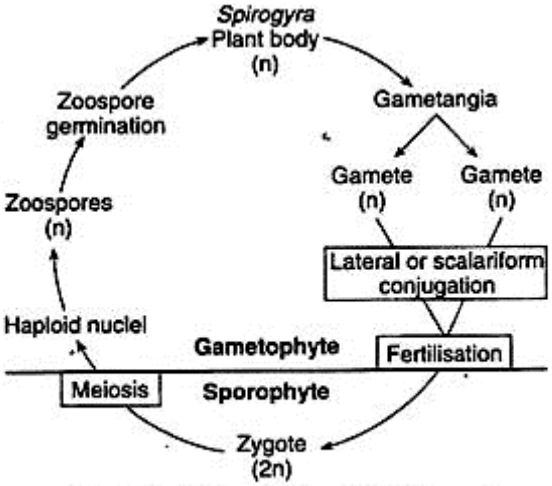


Fig. 3.19 : Haplontic life cycle (*Spirogyra*)

2. Diplontic Life Cycle:

The plant body is sporophyte and develops sex organs. Sex organs produce gametes by meiosis. The gamete only represents the gametophytic stage. The gametes undergo fertilization immediately and form zygote. The zygote does not undergo meiosis and give rise to new sporo-phytic plant body.

This type of life cycle is found in majority of the members of Bacillariophyceae, some mem-bers of Chlorophyceae like *Cladophora glomerata*. *Fucus* and *Sargassum* of Phaeophyceae also show this type of life cycle.

3. Diplohaplontic Life Cycle:

In this type the haploid and diploid phases are equally prominent and are represented by two distinct vegetative individuals. They differ only in chromosome number and function. The haploid gametophytic plant reproduces by sexu-al method, while diploid sporophytic plant by asexual process. In this life cycle alternation of two vegetative individuals occurs by sporogenic meiosis and fusion of gametes.

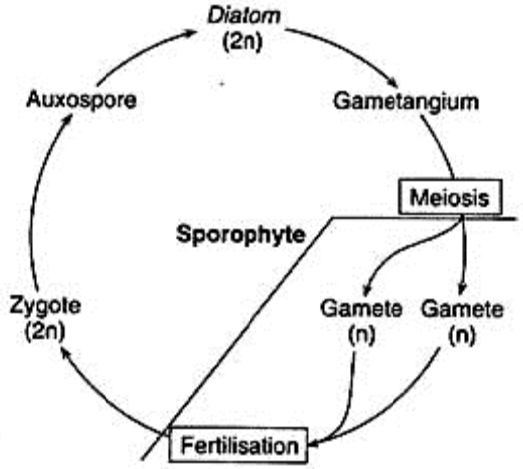


Fig. 3.20 : Diplontic life cycle (Diatom)

It is of two types:

i. Isomorphic or Homologous Diplohaplontic Type:

In this type both sporophytic and gameto-phytic plants are morphologically similar and free living. The gametophytic plant (haploid) pro-duces gametes, undergo sexual reproduction and form zygote. The zygote germinates directly into a sporophytic (diploid) plant. The sporophytic plant forms haploid zoospores by meiosis. These zoospores can develop new gametophytic plant.

This type of life cycle is found in *Clapdohora, Ulva, Draparnaldiopsis* of Chlorophyceae and *Ectocarpus* of Phaeophyceae.

ii. Heteromorphic or Heterologous Diplohaplontic Type.

In this type both sporophytic (diploid) and gametophytic (haploid) plants are morphologically dissimilar.

Generally the sporophyte is complicated and much elaborate, but the gametophyte is simple and small as found in Laminaria of Phaeophyceae. In some cases like *Cutlaria* etc. gametophyte is dominant over sporophyte.

In *Laminaria* the gametophytic plant body is made up of minute filaments which produce gametes. The gametes undergo fusion and form zygote, which germinates directly into a sporophytic plant. The sporophytic plant body is macroscopic and several meters in length.

The sporophytic plant bears zoosporangia and produce zoospores after meiotic division. The haploid zoospores on germination produce haploid gametophytic plant.

4. Triphasic Life Cycle:

In this type, there is succession of three distinct generations.

It is of two types:

i. Haplobiontic Type:

In this cycle the gametophytic (haploid) phase is elaborate, dominant and persists for long time than sporophytic (diploid) phase which is represented only by zygote i.e., haplobiontic type and two successive haploid generations are interrupted only by diploid zygote stage indicate its triphasic nature. This type of life cycle is found in the primi-tive members of Rhodophyceae like *Batrachospermum* and *Nemalion*.

In *Batrachospermum* the gametophytic plant body develops sex organs and produces male (spermatium) and female (egg) gametes. The gametes by fusion form zygote.

The zygote immediately undergoes meiosis and produces another haploid gametophytic plant, the carposporophyte. The carposporophyte develops carposporangium which produces haploid carpospores. The carpospores germinate and develop new free-living gametophytic plant.

So in this cycle, three phases are:

i. Haploid carposporophyte,

ii. Haploid gametophyte, and

iii. Diploid zygote.

ii. Diplobiontic Type:

In this type there is one gametophytic phase and two sporophytic phases indicate its triphasic nature and the sporophytic phase is more elabo-rate and persists for long duration than the game-tophyte i.e., diplobiontic type.

This type of life cycle is found in *Polysiphonia*, a member of Rhodophyceae.

In *Polysiphonia*, the gametophytic phase is represented by two types of gametophytic plant i.e., male and female plant, those bear spermatangium and carpogonium respectively. Later, the spermatangium and carpogonium develop sperms and egg respectively.

The male and female gamete i.e., sperm and egg undergo fusion and form zygote. The zygote (2n) develops into a diploid carpospophytic phase. The diploid carpospores are formed in the carposporophyte.

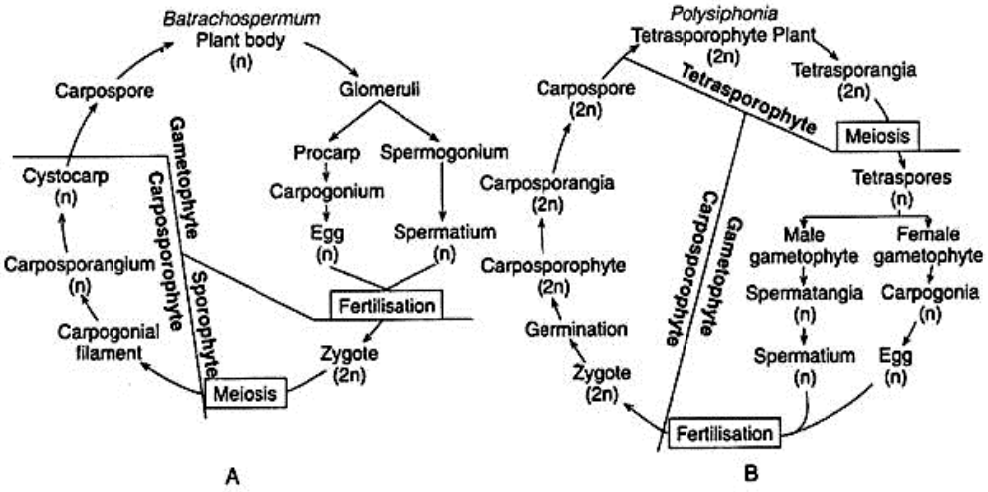


Fig. 3.22 : Triphasic life cycle : A. Haplobiontic type (*Batrachospermum*), B. Diplobiontic type (*Polysiphonia*)

The carpospores on germination develop the diploid tetrasporophytic plants. The tetrasporo- phytic plant develops diploid tetrasporangia each of which produce four tetraspores (n) by meiotic division. They are liberated by splitting of sporangial wall. Out of four tetraspores two produce male gametophyte and the other two into female gametophyte.

So in this cycle, three phases are:

i. Haploid gametophyte,

ii. Diploid carposporophyte, and

iii. Diploid tetrasporophyte.

Classification of algae: Robert Edward Lee (1989)

According to this system of classification, there are four distinct evolutionary groups.

1st Group: Includes the prokaryotic algae. Include the **Cyanophyta**&**Prochlorophyta**

2nd Group: Algae with a chloroplast surrounded only by two membranes of the chloroplast envelope. Include the **Glaucophyta**, **Chlorophyta**&**Rhodophyta**.

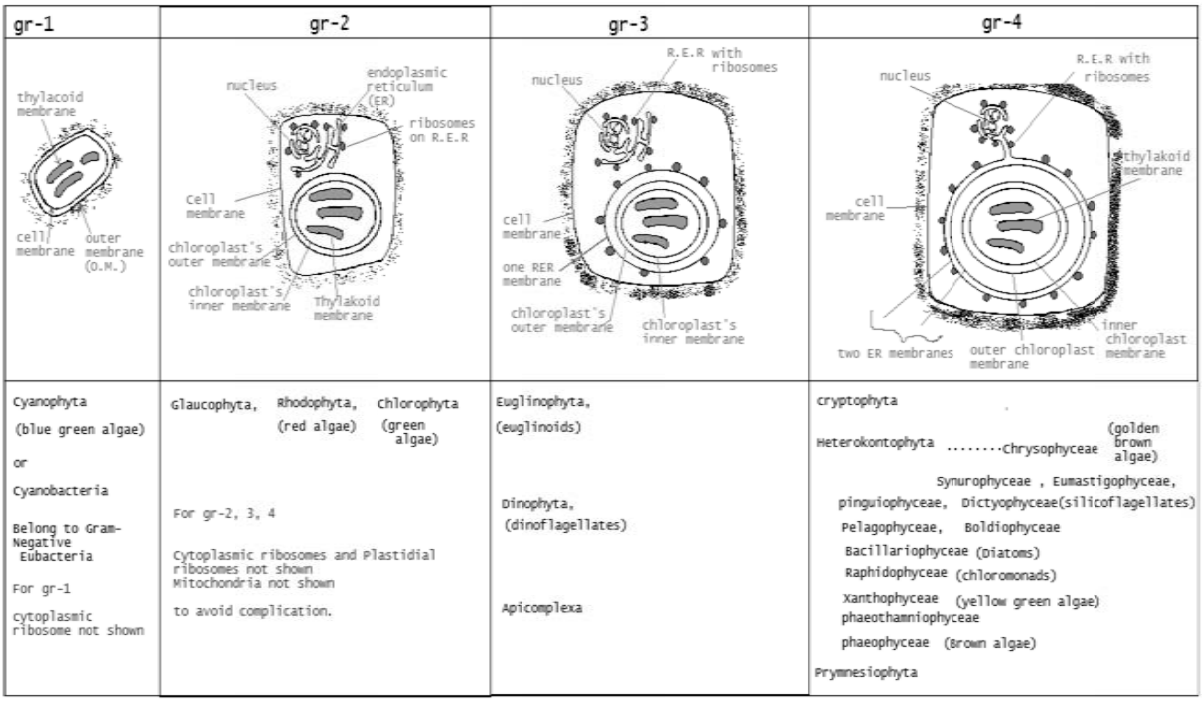
These algae evolved through an evolutionary event hat involved the capture of a prokaryotic algal cell in a food vesicle by a phagocytic non photosynthetic protozoan. Normally the prokaryotic algal cell would have been digested as a source of food by the heterotrophic protozoan. However, the prokaryotic algal cell was retained in the host cytoplasm as an endosymbiont. Such an endosymbiont was of benefit to the host protozoan because the host received some of the photosynthatates from the endosymbiont. It was also of benefit to the endosymbiont because it resided in the more stable and protected environment of the cytoplasm of the host. Eventually, in the process of evolution, the plasma membrane of the endosymbiont became the inner membrane of the chloroplast envelope and the food vesicle membrane of the host became the outer membrane of the chloroplast envelope.

The Glaucophyta represent an intermediate stage in this process, in which the endosymbiotic alga has not completely evolved into a chloroplast. The endosymbiotic bacteria is called the **Cyanelle**, the host **-Cyanome** and the association between the two is called **Syncyanosis**. The algae in the Rhodophyta and the Chlorophyta represent the completion of this evolutionary pathway into mature chloroplasts.

3rd Group: Algae that have the chloroplast surrounded by one membrane of chloroplast endoplasmic reticulum. Include the **Euglenophyta** and **Dinophyta**.

This evolutionary pathway as outlined by Lee (1977) resulted when a chloroplast from an eukaryotic algae was taken up into a food vesicle by a phagocytic protozoan. Normally the protozoan would have digested the chloroplast as a source of food, however, in this case the chloroplast was retained in the cytoplasm of the protozoa as an endosymbiont. The host protozoan benefitted from the association by receiving photosynthatates from the endosymbiont chloroplast. The endosymbiont chloroplast benefitted by the stable environment created by the cytoplasm of the host. Eventually the food vesicle membrane of the host became the single membrane of chloroplast endoplasmic reticulum surrounding the chloroplast.

4th Group: Algae with two membranes of chloroplast endoplasmic reticulum surrounding the chloroplast. Include the **Cryptophyta**, **Chrysophyta**, **Prymnesiophyta**, **Bacillariophyta**, **Xanthophyta**, **Eustigmatophyta**, **Raphidophyta** &**Phaeophyta**.



A brief outline of the classification up to class (with characters) is given below:

Group 1. Prokaryotic algae

1. Phylum. Cyanophyta

Class. Cyanophyceae

1. The individual cells are prokaryotic in nature. The nucleus is incipient type and lacks membrane bound organelles.

2. Both vegetative and reproductive cells are non-flagellated.

3. Cell wall is made up of microfibrils and is differentiated into four (4) layers. The cell wall is composed of mucopeptide, along with carbohydrates, amino acids and fatty acids.

4. Locomotion is generally absent, but where occurs, it is of gliding or jerky type.

5. The principal pigments are chlorophylls a (green), c-phycocyanin (blue) and c-phycoerythrin (red). In addition, other pigments like β-carotene and different xanthophylls like myxoxanthin and myxoxanthophyll are also present.

6. Membrane-bound chromatophores are absent. Pigments are found embedded in thylakoids.

7. The reserve foods are cyanophycean starch and cyanophycean granules (pro-tein).

8. Many filamentous members possess specialized cells of disputed function (supposed to be the centre of N_2 fixation) known as heterocyst.

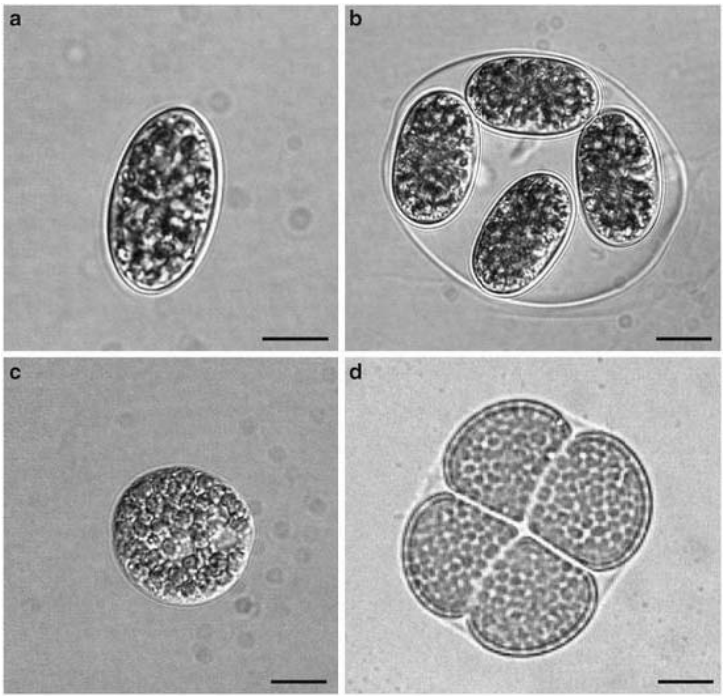
9. Reproduction takes place by vegetative and asexual methods. Vegetative repro-duction takes place by cell division, fragmentation etc. Asexual reproduction takes place by endospores, exospores, akinetes, nannospores etc.

10. Sexual reproduction is completely absent. Genetic recombination is repor-ted in two cases.

Common genus: *Nostoc, Oscillatoria, Gloeocapsa, Gloeotrichia* etc.

Group 2. Eukaryotic algae with chloroplast surrounded only by the two membranes of the chloroplast envelope.

1. Phylum. Glaucophyta:



1. Members of this group have endosymbiotic cyanobacteria (cyanelles) in the cytoplasm instead of chloroplast.

2. Most of the cyanelles in this phylum lack a wall and are surrounded by two mem-branes – the old food vesicle membrane of the cyanome (host) and the plasma membrane of the cyanelle. In course of evolution these two membranes became the chloroplast envelop.

3. Various inclusions of the cyanobacteria like polyhedral bodies, cyanophycean granules, polyphosphate bodies are lost. The cyanome cytoplasm took over the formation of the storage product.

4. A pyrenoid, being differentiated from proteinaceous bodies, is present in the cyanelle.

5. Pigments like chlorophyll a and the phycobiliproteins are present.

Common genus: *Cyanophora, Glaucocystis, Geosiphon* etc.

2. Phylum. Rhodophyta (red algae)

The phylum Rhodophyta has a single class Rhodophyceae.

Class. Rhodophyceae:

1. Plants are generally marine habitat, called sea weeds, grow in various depth. (Out of about 4,000 species, about 200 species are of fresh water).

2. Pit connections between the cells in all of the orders except Porphyridales, Erythropeltidales and haploid phase of the Bangiales.

3. Chloroplast with one thylakoid per band and absence of chloroplast endo-plasmic reticulum.

4. Presence of chlorophylls a and d, phy-cobiliproteins include r-phycocyanin, allophycocyanin, and three forms of phycoerythrins (b, r and c).

5. Floridean starch as a storage product.

6. Lack of flagellated cells.

e.g., *Porphyridium, Rhodosorus, Batrachospermum, Gelidium, Polysiphonia* etc

3. Phylum. Chlorophyta (green algae)

It is divided into 4 clases:

A. Class. Micromonadophyceae:

Important characteristics:

Scaly or naked flagellates with interzonal spindles are persistent during cytokinesis; primi-tive green algae, some of which gave rise to the other classes in the Chlorophyta.

B. Class. Charophyceae (flagella are attached in a lateral position in the cell):

1. Motile cells asymmetrical, two flagella attached in a lateral position in cell.
2. Flagellar root consisting of a broad band of microtubules and a second smaller microtubular root.
3. Multilayered structure (MLS) may be present, rhizoplast absent.
4. Scales are common, outside the motile cells.
5. Persistant interzonal mitotic spindle in telophase.
6. Phragmoplast produces new cross-walls after cell division.
7. Eye-spots usually not present.
8. Sexual reproduction involves the forma-tion of a dominant zygote. Meiosis occurs during zygote germination.

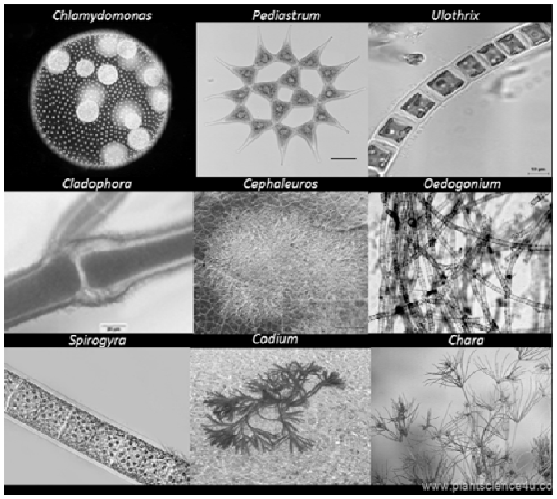
e.g., *Stichococcus, Raphidonema, Klebsormidium, Spirogyra, Zygnema, Coleochaete, Chara, Nitella*.

C. Class. Ulvophyceae (flagella are attached at the anterior end of the cell).

1. Plants are predominantly marine.
2. Flagella attached at anterior end of cell.
3. Externally, the motile cells have near- radial symmetry.
4. Flagella roots consist of 4 cruciately arranged microtubular roots and some-times a rhizoplast.
5. Multilayered structure (MLS) is absent.
6. Scales may be present on motile cells.
7. Presence of persistent interzonal spindle in telophase.
8. Eye-spots are common.
9. Dormant zygote is absent..

Common genera: *Ulva, Ulothrix, Cladophora* etc.

D. Class. Chlorophyceae (flagella are attached at the anterior end of the cell)



1. Plants are predominantly fresh water.
2. Motile cells are with radial or near- radial external symmetry.
3. Flagella are attached at the anterior end of cell.
4. Flagella roots consist of 4 cruciately arranged microtubular roots and some-times a rhizoplast.
5. Multilayered structure (MLS) is absent.
6. Theca is common in motile cells.
7. In telophase, the interzonal spindle col-lapses; phycoplast produces the new cross wall in cell division.
8. Eye-spots are common.
9. Zygote undergoes a dormant period; meiosis occurs when the zygote germi-nates.

e.g., *Chlamydomonas, Volvox, Tetraspora, Palmella, Hydrodictyon, Scenedesmus, Chlorella. Oedogonium, Oedocladium*.

Group 3. Eukaryotic algae:

Eukaryotic algae with chloroplast surrounded by one membrane of chloroplast endo-plasmic reticulum.

1. Phylum. Euglenophyta (euglenoid)

It consists of only one Class:

Class Euglenaceae:

1. They are unicellular.
2. They have chlorophyll a and b.
3. One membrane of chloroplast and endoplasmic reticulum.
4. The nucleus is mesokaryotic in nature.
5. Flagella with fibrillar hairs in one row.
6. Sexual reproduction is absent.
7. The storage product is paramylon, formed in the cytoplasm.

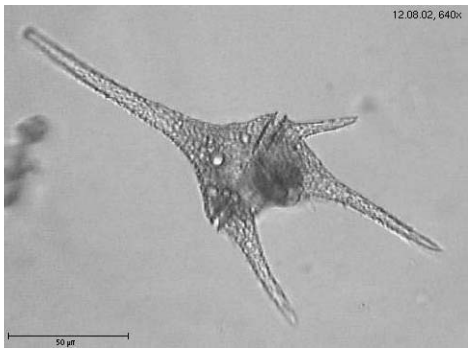
e.g., *Eutreptia, Peranema. Eutreptiella, Euglena* etc.

2. Phylum. Dinophyta (dinoflagellates)

It consists of only one Class:

Dinophyceae.

Class Dinophyceae:



1. A typical motile dinoflagellate consists of an epicone and hypocone divided by a transverse girdle or cingulum.
2. The epicone and hypocone are normal-ly divided into a number of thecal plates.
3. The cells can be photosynthetic or colourless and heterotrophic.
4. Photosynthetic organisms have chloro-plasts surrounded by one membrane of chloroplast endoplasmic reticulum, which is not continuous with the outer membrane of the nuclear envelop.
5. Chlorophyll a and c2 are present in the chloroplasts, with peridinin and neo- peridinin being the main carotenoids.
6. Presence of pyrenoid in chloroplast.
7. Storage product is starch.
8. Eye-spot is present.
9. Nucleus is dinokaryoyic or mesokaryo-tic in nature.

The class Dinophyceae is further divided into 4 orders:

e.g., *Prorocentrum, Ornithocercus, Ceratium, Gymnodinium*.

Group 4. Eukaryotic algae:

Eukaryotic algae with chloroplast surrounded by two membranes of chloroplast endo-plasmic reticulum.

1. Phylum. Cryptophyta (cryptophytes)

It consists of only one Class:

Cryptophyceae.

Class Cryptophyceae:

1. Plants are found in both marine and fresh water habitat.
2. The plant body is dorsiventral in shape, with the cells flattened in one plane.
3. Cells contain chlorophylls a and c2.
4. A few members lack chloroplast and are heterotrophic in nature, but most have a single-lobed chloroplast with a central pyrenoid.
5. Phycobiliproteins occur inside the thyla-koids of the choloroplasts.

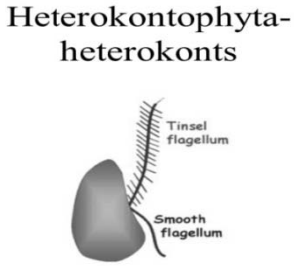
Common genera: *Cyathomonas, Chilomonas* etc.

1. Phylum. Heterokontophyta (heterokonts):

The phylum is further divided into nine (9) classes.

These are:

A. Class. Crysophyceae (golden-brown algae)





1. Presence of chlorophyll c1 and c2.
2. Flagella inserted in the cell perpendi-cular to one another.
3. One photoreceptor as a swelling on the short flagellum.
4. Eye-spot commonly present.
5. Chlooroplast endoplasmic reticulum present.
6. Radially or bilaterally symmetrical silica scales may occur.
7. Contractile vacuole anterior.

Common genera: *Ochromonas, Paraphysomonas, Dinobryon, Anthophysa* etc.

B. Class. Synurophyceae:

1. Presence of chlorophyll c1.
2. Flagella inserted into the cell approximately parallel to each other.
3. Presence of two photoreceptors, one on each flagellum.
4. Eye-spot absent.
5. Chloroplast endoplasmic reticulum usu-ally absent.
6. Plant body bilaterally symmetrical.
7. Silica scales are common.
8. Contractile vacuole posterior.

Common genera: *Sinura, Mallomonas, Tessellaria* etc.

C. Class. Dictyochophyceae (silicoflagellates):

1. These are golden-brown algae.
2. They have tentacles or rhizopodia on basically amoeboid vegetative cells.
3. Amoeboid vegetative cells are mostly restricted in this group (and Xantho-phyceae).

e.g., Rhizochromulina, Phaeaster, Chrysoamoeba, Pedinella Apedinella, Pseudopedinella, etc.

D. Class. Pelagophyceae

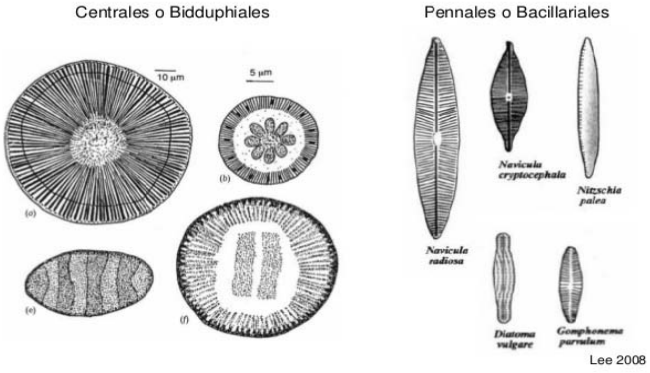
1. Basically unicellular algae.
2. The cells are very small (3-5µm) mem-bers of the ultraplankton and appear as small spheres with indistinct protoplasm under the light microscope.

Common genus: *Pelagomonas*.

E. Class. Bacillariophyceae (diatoms):

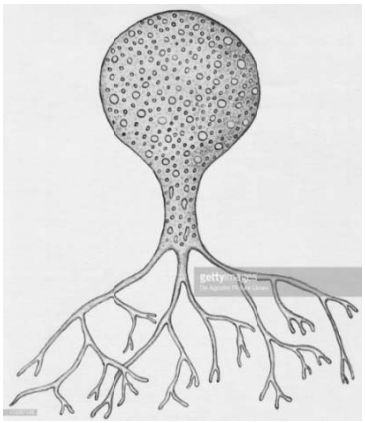
Important characteristics:

1. Plant body is unicellular and free-living. Sometimes they are present in colony.
2. Depending on the mode of nutrition, they may be photosynthetic autotrophs, photosynthetic symbionts or colourless heterotrophs.
3. Vegetative cells are diploid (2n).
4. The cells are surrounded by a rigid two- part box-like cell wall (upper part is epitheca and lower part is hypotheca), composed of silica, called the frustule.



5. The cells are bilaterally or radially symmetrical.
6. The chloroplast contains chlorophylls a, c1 and c2 with the major carotenoid being the golden-brown fucoxanthin.
7. Reserve food is fat and volutin.
8. Motile cells have single flagellum.
9. Reproduction takes place by cell divi-sion and auxospore formation.
e.g., *Amphora, Navicula, Pinnularia* etc.

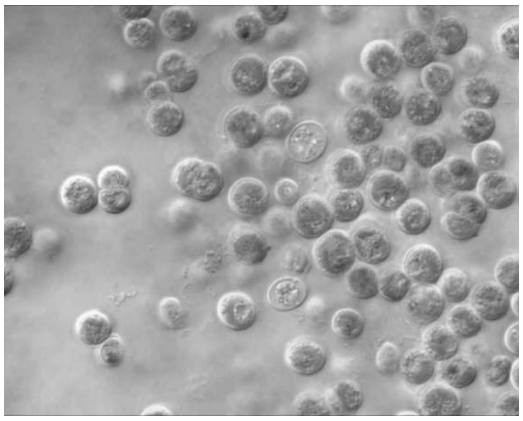
F. Class Raphidophyceae (chloromonads):



1. They are commonly marine.
2. Chloroplasts contain chlorophyll a and c.
3. Two membrane of chloroplast endo-plasmic reticulum.
4. Anterior flagellum is commonly tinsel, whereas the posterior flagellum is naked.
Common genera: *Heterosigma, Chattonella*.

G. Class. Xanthophyceae (yellow-green algae):

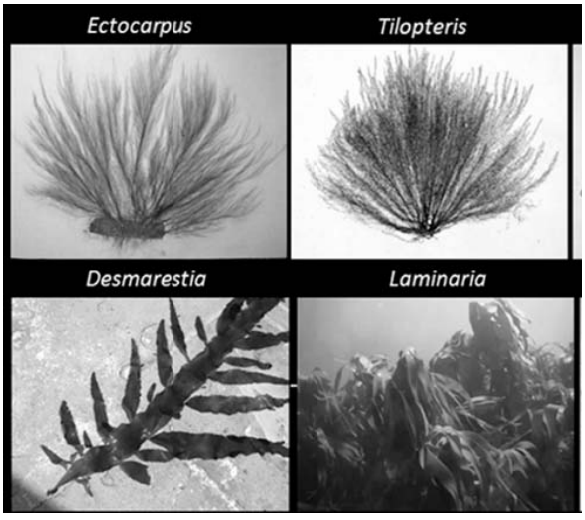
1. Cells are eukaryotic and cell wall is composed of pectic substance.
2. The main pigments are Chlorophyll a and e, β-carotene and xanthophylls.
3. Chloroplasts are without pyrenoids.
4. Reserve food is oil.
5. Flagella are two in number, unequal in length and are situated anteriorly. The longer one tinsel and shorter one whiplash type.



6. Reproduction takes place by all the three means vegetative, asexual and sexual (isogamous type).
Common genera: *Botrydium, Ophiocytium, Tribonema* etc.

H. Class. Eustigmatophyceae:

1. Plant body is unicellular and live in fresh water or in soil.
2. Chloroplasts contain chlorophylls a, β- carotene with two major xanthophylls being violaxanthin and vaucherioxanthin.
3. Vegetative cells usually have characte-ristic polygonal pyrenoids (absent in zoospores).
4. The pyrenoid projects from the inner face of the plastid on a narrow stalk and cytoplasm is surrounded by flat plates of the unidentified photosynthate.
Common genera: *Pleurochloris, Polyedriella*.



I. Class. Phaeophyceae:

1. Most of the members are marine.
2. Cell wall is composed of cellulose along with alginic and fucinic acid.
3. Pigments. Fucoxanthin is the dominant pigment, in addition to chlorophylls a, c and carotene.
4. Reserve food is laminarin (polysaccha-ride), manitol (alcohol) and fat.
5. Some lower members have naked pyrenoid like bodies.

6. Zoospores are flagellated, one of which is tinsel type.

7. Sexual reproduction ranges from iso-gamy to oogamy.

Common genera: *Ectocarpus, Laminaria, Dictyota* etc.

3. Phylum. Prymnesiophyta (haptophytes)

Class. Prymnesiophyceae:

1. It is a group of unicellular flagellates, characterized by the possession of a haptonema between two smooth flagella.

2. Chloroplasts have chlorophylls a, c1 and c2 with fucoxanthin as the major carotenoid.

3. Diadinoxanthin and β-hexanoyl fuco-xanthin also occur in the group.

4. Two membranes of the chloroplast endoplasmic reticulum are present.

5. The anterior end of the cell has a large golgi apparatus, and sometimes con-tractile vacuole.

6. The cells are commonly covered with scales.

e.g., *Prymnesium, Emiliania, Hymenomonas* etc.

Economic Importance of Algae

1. Food:

Algae have been in use as human food for centuries in various parts of the world. Algae are taken in several ways according to the choice and taste of the people. They may be taken as a salad, cooked with meat or eaten as vegetable, sprinkled with oatmeal or fried with meat.

Some are added for flavour to various dishes, while extract from others is taken as a beverage. Their nutritional value is quite high, as they contain a good amount of proteins, carbohydrates, fats and vitamins, specially A, B, C and E.

Commonly used species, are mostly marine, and they belong to Chlorophyceae [*Ulva lactuca* (Sea lettuce), *Enteromorpha compressa, Caulerpa racimosa*], Phaeophyceae (*Laminaria saccharina, Alaria esculenta, A. fistulosa, Sargassum* sp., *Durvillea* sp.), Rhodophyceae [*Porphyra tenera, P. umbilicalis, P. laciniata, Rhodymenia palmata*, (Dulse), *Chondrus crispus* (Irish moss), *Gigartina stellata, Gracilaria* sp.] and Cyanophyceae (*Nostoc* sp.).

In Japan, about 20 different kinds of algae are being harvested and eaten. '**Aonori**' is a preparation of *Monostroma,* '**Kombu**' of *Laminaria* and '**Asakusa-Nori**' of *Porphyra tenera*.

The salient feature of *Chlorella* is that the cell is rich in protein and vitamin contents (Single cell protein, SCP). It contains all the amino acids known to be essential for the nutrition of human being as well as animals.

2. Fodder:

The sea weeds as fodder have been widely used in Norway, Sweden, Denmark, Scotland, America, China and New-Zealand. In Norway, *Rhodymenia palmate* has come to be known as 'Sheep's weed' since sheep are very fond of this particular alga. *Laminaria saccharine, Ascophyllum* sp., *Sargassum* sp. and *Fucus* sp., are equally liked by the catties.

In many countries factories have been established to process the seaweed into suitable cattle-feed. Eggs, from hens fed on sea weed meal, have increased iodine content while increased butter-fat content of milk is reported from cattle whose diet is supplemented with sea-weed meal.

3. Pisciculture:

Algae, both floating and attached forms, marine as well as fresh water, provide the primary food for fish and other aquatic animals. The great fishing grounds of the seas are found where these are present in large numbers. In many countries pond culture for fishes has been taken up and they are fed with various forms of algae.

Judged by the works carried out by various investigators in India, on the food and feeding habits of fishes (Singh, 1956), it appears that the Green algae, the Diatoms and some Blue-greens are most

widely eaten up by the fishes. Fish food is mainly the planktons (the floating forms), phytoplankton's and zooplanktons. Zooplanktons develop by feeding upon the phytoplankton's.

4. Fertilizers:

The large Brown and Red algae are used as organic fertilizers, especially on land close to the sea. The weed is used either directly or as a seaweed meal. A concentrated extract of seaweed is also sold as a liquid fertilizer. Coralline algae *Lithothamnion calcareum* and L*ithophyllum* sp. are used profusely for liming the soil. Similar is the use of Chara which becomes encrusted with calcium carbonate.

However, the greatest utility of the algae, as a friend to the farmers, is seen in some common forms belonging to Cyanophyceae for their capacity to fix atmospheric nitrogen and thus enriching the soil. In the paddy fields they have been seen to produce an effect almost similar to that of manuring with 30 kg. of ammonium sulphate per acre (Watanabe, 1959).

Aulosira fertilissima, the common Blue-green algae of the Indian rice fields is found to add 47-6 lb. of nitrogen fixed /acre/crop (Singh, 1962). At the same time there is a considerable increase in the total organic matter content of the soil. In India, the nitrogen-fixing blue-green algae play a part of tremendous importance in maintaining the fertility of the rice fields.

5. Reclamation of Alkaline, 'Usar' Land:

In India, vast tracts of land cannot be cultivated for crops because of high alkalinity of the soil, commonly known as 'usar' soil. The 'usar' lands would be cultivable, if their pH could be lowered, and organic contents and the water holding capacity of the soil increased. Exactly all these functions are carried out by the blue-green algae.

During the rainy season the blue-green algae, notably species of Nostoc, Scytonema, Anabaena and Aulosira, grow in plenty. According to R. N. Singh (1950), these algae can be of use in the reclamation of the **'usar' lands**. The process involves a series of successive growth of the algal crop in a water-logged condition.

6. Binding of Soil Particles:

Algae act as an important binding agent on the surface of the soil. Disturbed or burnt soils are soon covered with a growth of green and blue-green algae thus reducing the danger of erosion. The role of Cyanophycean members as a pioneer in colony formation and thus in soil formation is well known.

7. Commercial Products:

Many forms of marine algae, Phaeophyceae and Rhodophyceae, are highly valuable for certain commercial products, chiefly agar-agar, algin or alginic acid and carrageenin.

8. Agar-Agar (Agar):

Agar-agar is obtained from various species of red algae for e.g., *Gelidium corneum, G. cartilageneum, Gracilaria lichenoides,* and species of *Chondrus, Gigartina, Furcellaria, Phyllophora, Pterocladia, Ahnfeldtia and Gampylaephora* (Round, 1966). It is a non-nitrogenous extract obtained almost in a pure mucilaginous form. The chief constituent of agar is a carbohydrate galactan.

The algae are collected, bleached and the mucilaginous matter is extracted with water under pressure. The purified agar is sold in the form of flakes, granules or strips which are brittle when dry but become tough and resistant when moist.

The important use of agar is in microbiology and tissue culture (in the preparation of culture media for growing algae, fungi and bacteria in the laboratories).

Other uses are in the cosmetics, paper and silk industries, in dentistry for making impressions, in canning fish, to prevent the soft fish from being shaken to pieces during transit, in sizing material, in clarifying liquors etc. It is also used as food and in the preparations of ice-cream, jellies, sweets and baking.

9. Carrageenin:

This is a metabolic product similar to agar, obtained from *Chondrus crispus, Gigartina stellata* and *Iridaea laminaroides*.

The mucilage has several important industrial applications for e.g., in textile industry, in paper making to give body to the paper, in the manufacture of straw and felt hats as a stiffening agent; as an ingredient in cosmetics, shoe-polishes, hand lotions, tooth paste etc., as an emulsifying and suspending agent, in the baking, dairy industries and in clarifying liquors.

10. Algin and Alginates:

Algin is a calcium magnesium salt of alginic acid present in the intercellular substance of the Phaeophyceae. Because of its special colloidal properties alginic acid and its derivatives find considerable use in industry. Its salts are used in the manufacture of variety of goods ranging from ice-cream, salad cream, custard and jams to cosmetics, films, fabrics, ceramics and textiles.

They are also used as a suspending agent in compounding drugs, lotions and emulsions; in the rubber industry in latex production; as an insulating material and as dental impression powder, as a gel in the freezing of fish and in the medicinal antibiotic capsules.

The harvesting of the weed depends upon the genera used and their habitat. Species of *Laminaria, Ascophyllum, Macrocystis, Nereocystis, Ecklonia, Durvillea* and *Sargassum* are the chief sources of commercial algin.

11. Medicinal use:

Alaria was once used for strengthening the stomach and restoring the appetite after sickness. Alginates are used for their haemostatic nature; fucoidin and sodium lamanarin sulphate are used as 'blood anticoagulant'. *Digenia simplex*, a Rhodophycean alga, provides an antihelmnitic drug. Agar-agar, for its absorptive and lubricating action, is used medicinally in the prevention of constipation.

12. Antibiotics:

The antibacterial product **chlorellin**, obtained from *Chlorella* is well known. The antibacterial effects are more pronounced against coliforms and other related intestinal bacteria.

Extracts from *Rhodomela larix* and *Ascophyllum nodosum* are effective against both gram positive and gram negative bacteria. Several algae, e.g., *Halidrys, Pelvetia, Laminaria, Polysiphonia, Nitzschia* and *Hapalosiphon*, have been reported to possess antibiotic or antibacterial properties which, however, need further confirmation.

13. Sewage Disposal:

Sewage disposal is one of our main defences against those diseases which are spread by the agency of human waste. There is only one way of sewage disposal and that is into water, streams, rivers, lakes or the sea. Sea side towns can dispose the sewage directly into the sea but in other cases the sewage should be treated before disposal.

Essentially, there are two phases of sewage treatment—the physical and biological. The most important and common physical processes are straining and tedious. The biological process i.e., sewage disposal is simple and less expensive.

Sewage disposal treatment is essentially a process of biochemical oxidation and its basic requirement is oxygen. The parts or amount of oxygen needed for the purification of 100,000 parts of sewage by weight is called the Biochemical Oxygen Demand (B. O. D.).

In other words, we may say that the ever present bacteria break down the sewage into its components complex organic compounds into such simple inorganic compounds as ammonia, carbon dioxide etc. and water with the needed amount of oxygen.

This oxygen demand may be supplied artificially which is quite expensive or through the agency of the photosynthetic algae which grow in sewage disposal ponds The purification of sewage is designed to render the organic wastes present in the sewage harmless and inoffensive.

Only after extensive research has the relationship that exists between these sewage algae and bacteria become apparent. The most common algal species present in the sewage oxidation ponds are *Chlamydomonas, Scenedesmus, Chlorella, Euglena, Eudorina* and *Pandorina*.

14. Funori:

A product similar to agar obtained from *Gloiopeltis furcata* and from species of *Chondrus*, Ahnfeldtia, Iridaea and Grateloupia, is chiefly used as a glue and as a sizing agent.

15. Diatomite (Kieselguhr):

Fossil forms of diatoms in some regions are found in large deposits which are called 'Diatomaceous earth' or 'kieselguhr'. Silica, the basic constituent of glass and granite rock, is deposited on the cell walls of the diatoms. Because the silica walls are hard and chemically inert, the sediments accumulate in marine and fresh water basins.

It is used as an industrial catalyst, as filler in paints and varnishes, in paper industry; and in insulation materials for use at extremes of temperatures (Round, 1966). It is also used widely to absorb coloured substances from oils and other liquids; as a cleaning powder in soaps and in metal polishes.

16. Other Products:

From members of Phaeophyceae, two important products mannitol and fucoidin are obtained. Mannitol is used in food and medicinal products, inks and plastics etc. and fucoidin is used as a mucilage and in medicines.

The burnt '**ash**' of larger Brown algae, specially the **Kelps** belonging to Laminariales, has been used for the extraction of minerals (sodium sulphate, potassium chloride and potassium sulphate), iodine and bromine. It is also used as a source of soda in the manufacture of soaps, glassware and alum.

Fungi:

Fungus (pl. fungi) is a Latin word which means mushrooms. Fungi are nucleated, spore bearing, achlorophyllous organisms which generally reproduce sexually and asexually, and whose usually filamentous branched somatic structures are typically surrounded by cell walls containing cellulose or chitin, or both (Alexopoulos, 1952).

In simpler words it may also be defined as "non-green, nucleated thallophytes". The common examples of fungi are the yeasts, molds, mushrooms, polypore's, puff balls, rusts and smuts. The branch of botany that deals with the study of fungi is known as mycology (Gr. mykes = mushroom + logos = discourse) and the person knowing fungi is known as mycologist.

The Italian botanist Pier' Antonio Micheli deserves the honor of being called 'Founder of the science of mycology' because he was the first person to give somatic description of fungi in his book Nova plant-arum Genera published in 1729. Anton De Bary (1831-1888) is called the 'father of modern mycology'. At present about 5100 genera and more than 50,000 species of fungi are known.

Characteristics of Fungi:

1. Fungi are cosmopolitan in distribution i.e., they can grow in any place where life is possible.

2. They are heterotrophic in nature due to the absence of chlorophyll. On the basis of their mode of nutrition, they may be parasite, saprophyte or symbionts.

3. The plant body may be unicellular (*Synchytrium, Saccharomyces*) or filamentous (*Mucor, Aspergillus*). The filament is known as hypha (plural, hyphae) and its entangled mass is known as mycelium.

4. The hypha may be aseptate i.e., coenocytic (without septa and containing many nuclei) or septate. The septate mycelium in its cell may contain only one (monokaryotic), two (dikaryotic) or more nuclei.

5. The septa between the cell may have different types of pores: micropore (*Geotrichum*), simple pore (most of the Ascomycotina and Deuteromycotina) or dolipore (Basidiomycotina, except rusts and smuts).

6. The cells are surrounded by distinct cell wall (except slime molds), composed of fungal cellulose i.e., chitin; but in some lower fungi (members of Oomycetes), the cell wall is composed of cellulose or glucan.

7. The cells generally contain colourless proto¬plasm due to absence of chlorophyll, contai¬ning nucleus, mitochondria, endoplasmic reti¬culum, ribosomes, vesicle, microbodies, etc.

8. The cells are haploid, dikaryotic or diploid. The diploid phase is ephemeral (short-lived).

9. In lower fungi like Mastigomycotina, the reproductive cells (zoospores and gametes) may be uni- or biflagellate, having whiplash and/or tinsel type of flagella. But in higher fungi like Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina, motile cells never form at any stage.

10. In response to functional need, the fungal mycelia are modified into different types such as: Plectenchyma, Stroma, Rhizo- morph, Sclerotium, Hyphal trap, Appresorium, Haustorium, etc.

11. The unicellular fungi, where entire plant body becomes converted into reproductive unit, are known as holocarpic fungi (e.g., *Synchytrium*). However, in many others, only a part of the mycelial plant body is converted into reproductive unit, thus they are called eucarpic fungi (e.g., *Pythium, Phytophthora*).

12. They reproduce by three means: Vegetative, asexual and sexual.

(a) Vegetative reproduction takes place by fragmentation (*Mucor, Penicillium, Fusarium*), budding (*Saccharomyces, Ustilago*) and fission (*Saccharomyces).*

(b) Asexual reproduction takes place by different types of spores. These are zoospores (*Synchytrium*), conidia (*Pythium, Aspergillus*), oidia (*Rhizopus*), chlamydospore (*Fusarium*), etc. The spores may be unicellular (*Aspegillus*) or multicellular (*Alternaria*).

Classification of Fungi:

Taxonomy has a dual purpose first to name an organism according to some internationally accepted system and then to indicate the relationship of the particular organism with other living organisms.

The classification of fungi is still in a state of flux. A stable or ideal scheme is yet to be proposed.

The grouping or categories used in the classification of fungi are as follows:

Kingdom
Division
Class
Order
Family
Genus
Species

The kingdom is the largest of the categories and includes many divisions: each division may include many classes and so on down to the species which is the unit of classification. Each of these categories may be divided into subgroups, subdivisions, subclasses, suborders, if necessary. Species are sometimes broke-down into varieties, biological strains and physiological or cultured races.

In accordance with the recommendations of the committee on International rules of Botanical Nomenclature:

(a) The name of divisions of fungi should end in—mycota.

(b) The name of subdivisions should end in—mycotina.

(c) The name of classes should end in—mycetes.

(d) The name of subclasses should end in—mycetideae.

(e) The name of orders should end in—ales.

(f) The name of families should end in a suffix—aceae.

Genera and species have no standard endings. The name of an organism is binomial. It is composed to parts—the first is noun designating the genus in which the organism has been classified, and the second is often an adjective describing the noun which denotes the species. The first letter of each generic name is always a capital.

Classification of Fungi by Ainsworth G. C. (1966, 71, 73):

Ainsworth G. C. (1966, 71, 73) proposed a more natural system of classification of fungi. This classification is based on morphology, especially of reproductive structure. He includes fungi along with slime molds under the kingdom Mycota.

Based on the presence or absence of Plasmodium and pseudoplasmodium; the kingdom Mycota is further divided into two divisions:

Myxomycota i.e., slime molds and Eumycota or true fungi. Divisions are subsequently divided into subdivision, class, subclass, order, family and then to genus. According to his classification, division ends in mycota, subdivision in mycotina, class in mycetes, subclass in mycetidae order in ales and family in aceae.

A schematic outline of Ainsworth's (1973) classification is given:

Kingdom: Mycota

Important features:

i. Free-living, parasitic or mutualistic symbionts, devoid of chlorophyll.

ii. Cell wall composition is very variable, majority contain chitin and glucan.

iii. Reserve food materials are oil, mannitol and glycogen.

iv. Except some unicellular members, majority are filamentous.

A. Division. Myxomycota:

Wall-less organisms possess either a Plasmodium (a mass of naked multinucleate protoplasm having amoeboid movement) or a pseudoplasmodium (an aggregation of separate amoeboid cells). Both are of slimy consistency, hence slime molds.

1. Class. Acrasiomycetes (cellular slime molds)
2. Class. Hydromyxomycetes (net slime molds)
3. Class. Myxomycetes (true slime molds)
4. Class. Plasmodiophoromycetes (endo- parasitic slime molds).

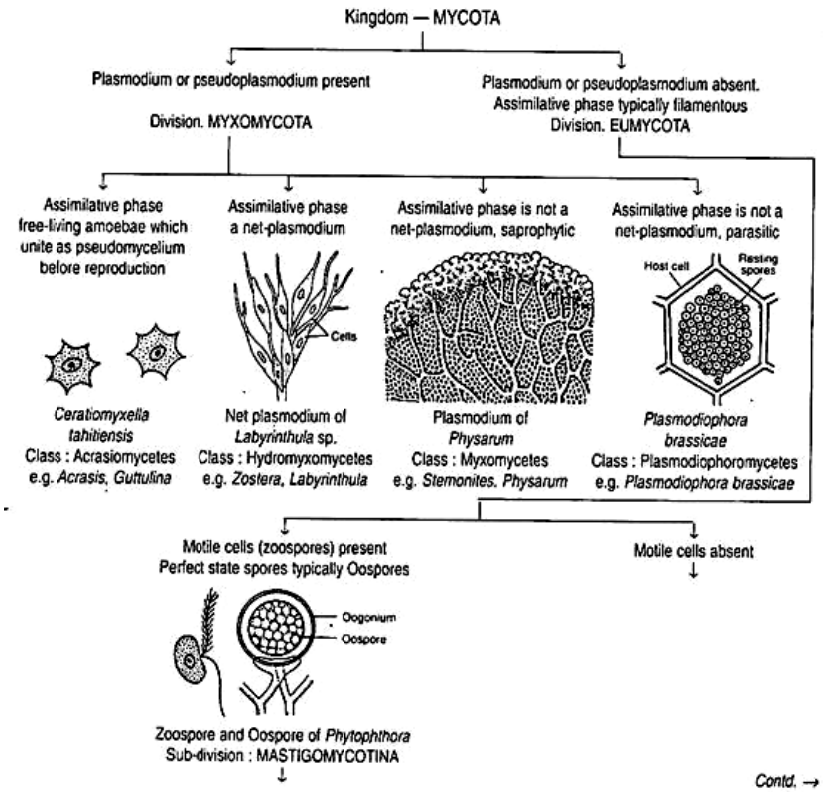
B. Division Eumycota (True fungi, all with walls):

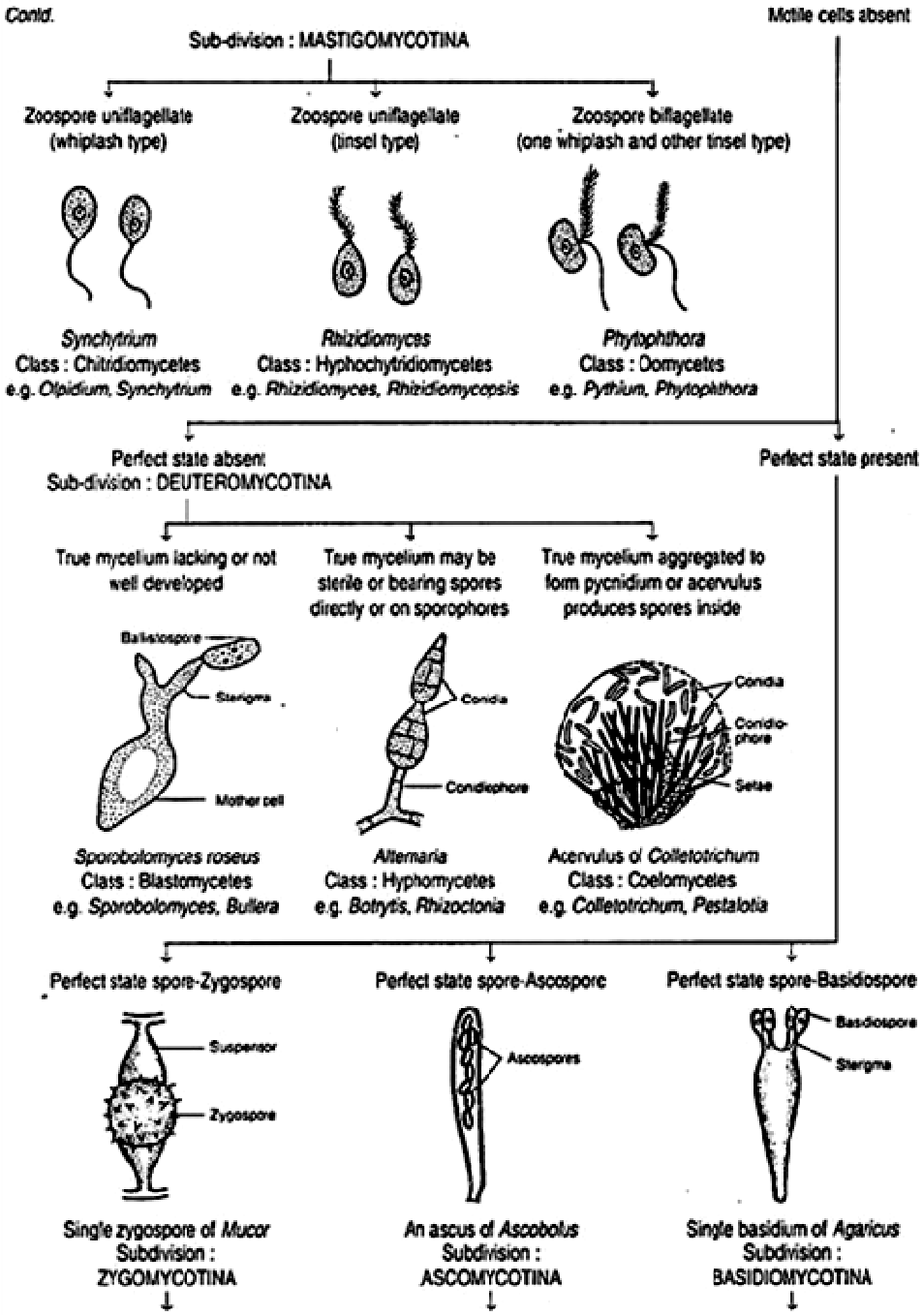
a. Subdivision Mastigomycotina (motile cells – zoospores present, perfect state spore-oospore).

1. Class. Chitridiomycetes (unicellular, zoospore with single whiplash flagellum).
2. Class. Hyphochytridiomycetes (unicellular, zoospore with single tinsel flagellum).
3. Class. Oomycetes (aseptate mycelium, zoospores with two flagella).

b. Subdivision. Zygomycotina (mycelium aseptate, perfect state spore-zygospore).
1. Class. Zygomycetes (mycelium immersed in the host tissue).
2. Class. Trichomycetes (mycelium not immersed in the host tissue).
c. Subdivision. Ascomycotina (yeasts or septate mycelium, perfect state spore- ascospores formed in ascus, usually within ascocarp).
1. Class. Hemiascomycetes (no ascocarp, asci naked).
2. Class. Loculoascomycetes (fruit body an ascostroma, asci bitunicate i.e., 2-walled).
3. Class. Plectomycetes (fruit body cleistothecium, asci unitunicate i.e., 1-walled).
4. Class. Laboulbeniomycetes (fruit body perithecium, asci unitunicate, exoparasite of arthopods).
5. Class. Pyrenomycetes (fruit body perithecium, asci unitunicate, not parasitic on arthopods.
6. Class. Discomycetes (fruit body apothecium, asci unitunicate).
d. Subdivision. Basidiomycotina (yeast or septate mycelium, perfect state spore – basidiospore formed on a basidium).
1. Class. Teliomycetes. Basidiocarp lacking, teliospores grouped in sori or scattered within the host tissue, parasitic on vascular plant.
2. Class. Hymenomycetes. Basidio- carp present. Hymenium is completely or partly exposed at maturity. Basidiospore ballistospores.
3. Class. Casteromycetes. Basidiocarp present. Hymenium enclosed in basidiocarp. Basidiospore not ballistospores.
(e) Subdivision. Deuteromycotina or Fungi imperfecti. Yeast or septate mycelium. Perfect state unknown.
1. Class. Blastomycetes. Budding (Yeast or Yeast like) cells with or without pseudomycelium. True mycelium lacking or not well-developed.
2. Class. Hyphomycetes. Mycelia sterile or bearing asexual spore directly or on conidiophore, in various aggregation.
3. Class. Coelomycetes. Mycelial; asexual spore formed in pycnidium or acervulus.

Schematic representation of the outline with figure, the classification of G.C. Ainsworth (1973) is given:





4. **Salient Features of Important Classes of Fungi:**

The important classes of true fungi are the Chytridiomycetes, Oomycetes, Zygomycetes, Ascomycei Basidiomycetes and Deuteromycetes (according to Alexopoulos, 1962).

Their respective salient features as follows:

Class—Chytridiomycetes:

1. The members of this class are usually aquatic but some live in moist soil.

2. Most of the members are parasitic on algae. However, some members have also parasitized small water animals and seed plants.

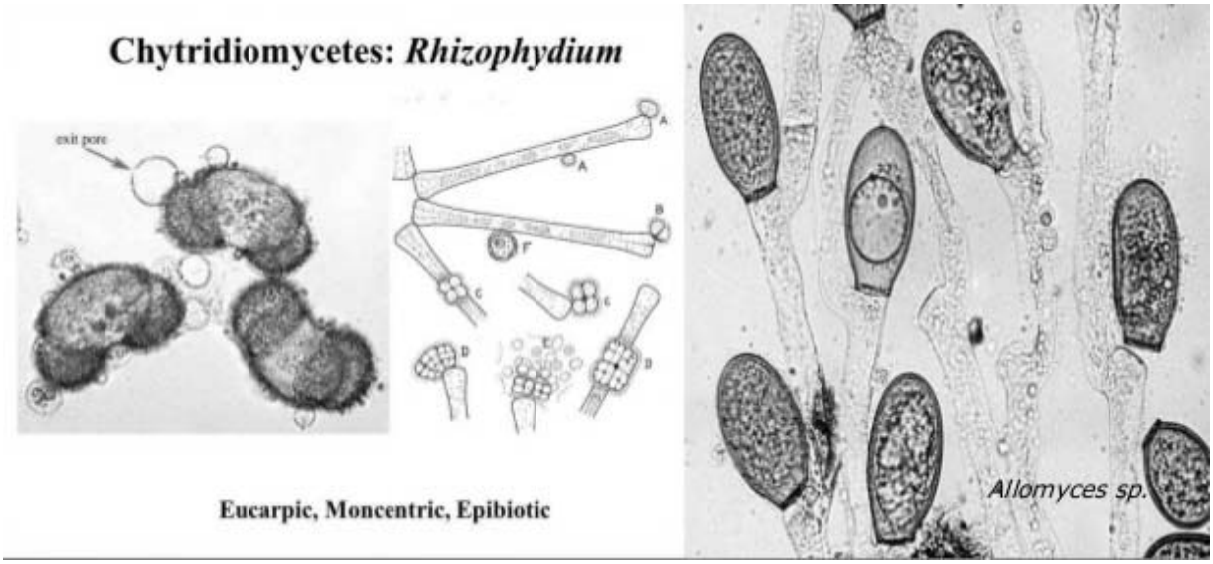
3. The thallus is coenocytic, holocarpic or eucarpic or filamentous.

4. The cell wall is made up of chitin (a polymer of N-acetyl glucosamine).

5. All the members of this class produce motile cells (zoospores or gametes), each equipped with a single posterior, whiplash flagellum.

6. Some members of this class (e.g., *Allomyces*) exhibit a true alternation of generation.

7. The important member of this class is *Synchytriun endobioticum*. It causes the important disease Black wart of potato.



Class—Oomycetes:

1. The members of this class are characterized by oogamous sexual reproduction.

2. The members of this group exhibit progressive evolution from aquatic to land habitat. Some members of this class are aquatic, others are terrestrial and still others live in association with terrestrial seed plants.

3. Biologically the members are aquatic saprophytes or parasites. Some members are terrestrial facultative to obligate parasites.

4. The thallus may be unicellular or filamentous. The filamentous forms are coenocytic.

5. The chief component of the cell wall is cellulose.

6. This class is an assemblage of unique fungi in possessing a diploid thallus and meiosis occurs before the formation of gametes.

7. Asexual reproduction takes place by bi-flagellated zoospores. The zoospores are kidney shaped or pear shaped having two flagella. One of the flagellum is of tinsel type and the other of whiplash type.

8. Important diseases caused by the members of the class are mildews, blights (late blight of potato caused by Phytophthora infestans) and white rust (white rust of crucifers caused by Albugo Candida) of crop plants.

Class—Zygomycetes:

1. This class includes about 70 genera and 450 species. The members are terrestrial in this class. They live in soil, on dung or on decaying plant and animal matter.

2. Biologically the members are saprophytic but some are parasitic on plants, insects and soil animals (e.g., Amoeba and nematodes). Some members attack the human body causing the disease mucormycosis.

3. The thallus is coencocytic and filamentous.

4. The cell walls are chiefly composed of fungal chitin, cellulose may also be present along with it.

5. The most characteristic feature of zygomycetes, is the complete absence of motile (flagellate) sexual or asexual cells.

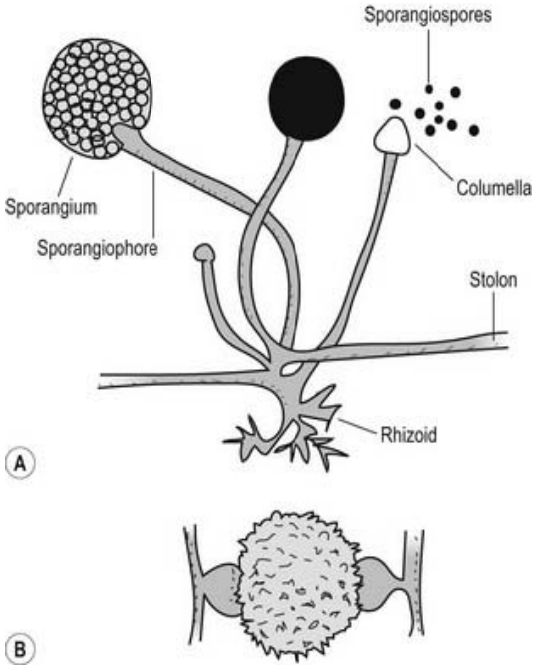
6. Asexual reproduction takes place by means of non-motile sporangiospores produced in large number within terminal sporangia.

7. Chlamydospores are present.

8. Sexual reproduction takes place by gametangial copulation. The gametangia of equal or unequal size unite to form the resting spore called zygospore.

9. Zygospore on germination produces a hypha which bears a terminal sporangium.

10. Economically the members of this class are very important. They are employed in industry to produce organic acids like oxalic acid, lactic acid and fumaric acid.



Class—Ascomycetes:

1. Generally the members of this class are terrestrial. Some, however, are marine.

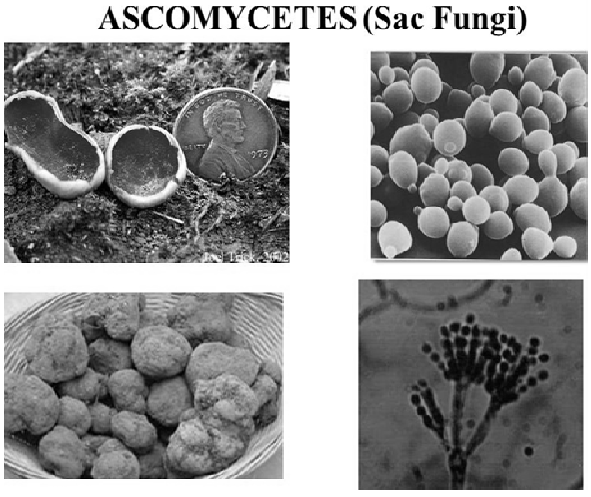
2. Terrestrial members are saprophytic as well as parasitic. The saprophytic members grow on soil rich in humus, decaying vegetable or animal matter, on dung, food stuffs and rotting logs. Parasitic members attack both plants and animals including man.

3. The thallus may be unicellular (e.g., Yeast) while others are filamentous having septate mycelium with uninucleate or multinucleate cells.

4. Component of cell wall is chitin.

5. Asexual reproduction takes place by fission, budding, fragmentation, arthrospores, chlamydospores or conidia, according to species and environmental conditions.

6. Commonly called as sac fungi on account of the presence a sac-like structure termed as the ascus. This ascus is the product of sexual reproduction.



7. The ascus contains a definite number of spores called the ascospores.

8. Motile phase is completely absent in the sexual and asexual reproduction.

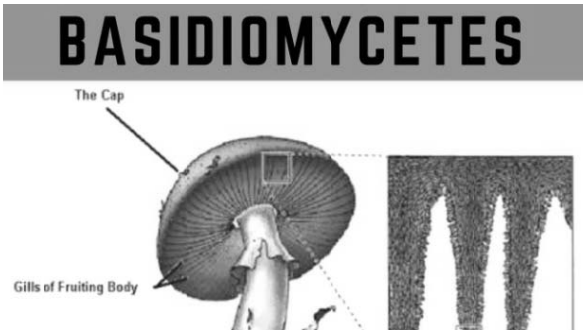
9. Origin of dikaryophase takes place in life cycle.

10. Members are economically important. They are employed in agriculture, medicines and various industries.

11. Important diseases caused by the members of this class are apple scab, powdery mildew of grapes and peaches etc.

Class—Basidiomycetes:

1. The members of this class are characterized by the exogenous production of spores termed as basidiospores.



2. Generally the members of this class are terrestrial. Some, however, grow on logs and tree stumps.

3. Biologically the members are parasites (rusts and smuts) as well as saprophytes.

4. The vegetative mycelium is well developed, septate and is of three different types—primary, secondary and tertiary.

5. Septal pore in this class is complex. It is dolipore type.

6. Clamp connections are present.

7. Motile cells are absent in life cycle.

8. Asexual reproduction is by conidia and chlamydospores.

9. Sex organs are absent in this class but monokaryotic hyphal cells or oidia etc. act as sexual cells.

10. The characteristic reproductive organ of this class is ba sidium. In it both karyogamy and meiosis take place.

11. Some members cause very serious diseases of plants like rusts and smuts (e.g., Puccinia graminis tritici causes black rust of wheat; Ustilago tritici causes loose smut of wheat).

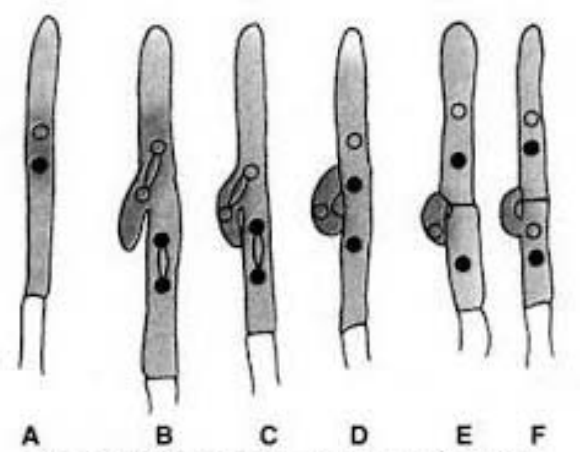
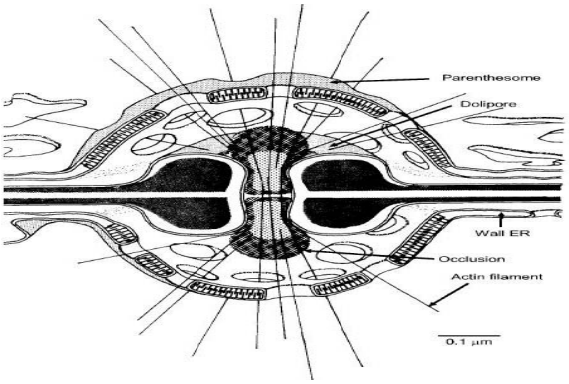


Fig. 13.3 (A-F). *Basidiomycetes.* Diagram illustrating the formation of clamp connections.

Dolipore/parenthesome septum

Perforated (homobasidiomycetes)

Imperforated (heterobasidiomycetes)



Functions:

1.To secure the integrity of hyphal cell;

2.To maintain intercellular communication and transfer of cell organelles, except nuclei;
3.Repair of hyphal damage - pore rapidly plugged by electron dense material in the compartment of a hypha adjacent to the damaged segment.

Class—Deuteromycetes:

1. This class includes only those members of fungi in which asexual or imperfect stage is known. The sexual or the perfect stage is unknown.

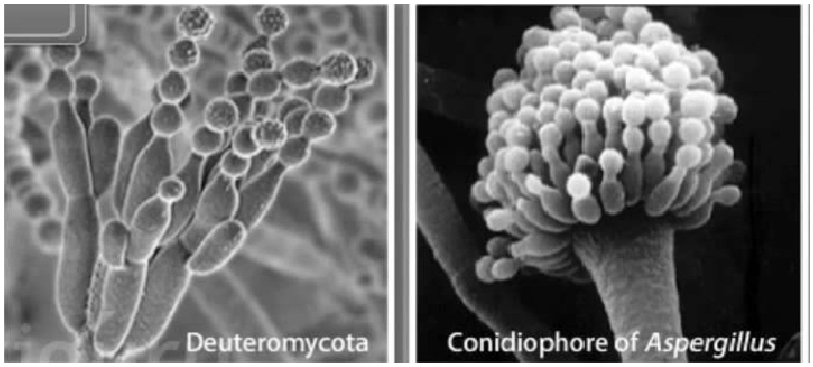
2. Biologically the members are saprophytes as well as parasites.

3. The vegetative mycelium is septate and profusely branched.

4. Some of the members of this class resemble with members of Ascomycetes and a few resemble with Basidiomycetes in structure and reproduction.

5. Parasexual cycle (sometimes nuclear fusion occurs followed by reduction division) has been observed in some members of this class.

6. Many members cause serious diseases of plants e.g., early blight of potato caused by *Alternaria solani* and red rot of sugarcane caused by *Colletotrichum falcatum*.



a) The Cell Wall of the Fungal Cell:

Except slime molds (Myxomycetes), the fungal cell consists of a rigid cell wall and cell organelles. However, composition of cell wall of different fungal groups differs. Chemical analysis of cell wall reveals that it contains 80-90% polysaccharides, and remaining proteins and lipids.

Chitin (a polymer of N-acetyl glucosamine), cellulose (a polymer of D-glucose) or other glucans are present in cell walls in the form of fibrils forming layers. In most of the fungi the cell wall lacks cellulose (except Oomycetes) usually chitin and cellulose are found together e.g. Ceratocystis and Rhizidiomyces contain a form of chitin called fungus cellulose. It is similar to the chitin of insects.

The microfibril layers run parallel to the surface. Several non-fibril materials are also associated with micro-fibrils. Though chitin is the most usual component yet cellulose is present in cell walls of Oomycetes along with glucans. An amino acid and hydroxy-protein are present in the cell wall of Oomycetes along with cellulose.

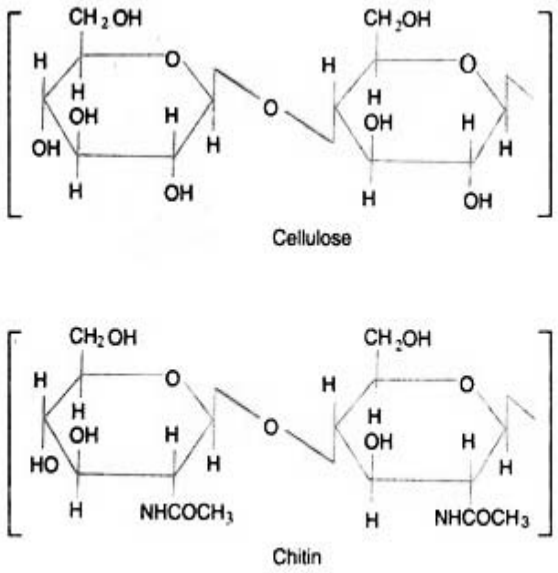


Fig. 4.36 : Structural formula representing the units of cellulose and chitin.

Several other substances have also been found to be associated together in cell walls and cell wall components such as proteins enzymes, etc. In Peronospora and Saprolegnia true cellulose is present, but in Phytophthora and Pythium cellulose is totally absent and glucans predominates in their walls. In the cell wall of some fungi the presence of chitin has been reported.

The basic constituents of cell walls of Zygomycetes, Ascomycetes and Basidiomycetes are chitin. But in yeasts and some Hemiascomycetidae chitin is absent. Micro-fibrils of mannans and β-glucan constitute their cell wall. Various chemical substances found in cell walls seem to be correlated with fungal taxonomy.

Table 4.5 : Taxonomy of fungal cell walls.

Categories	*Taxonomic groups*	*Features*
Cellulose-glycogen	Acrasiales	Pseudoplasmodia
Cellulose-glucan	Oomycetes	Biflagellate zoospores
Cellulose-chitin	Hypochytridiomycetes	Anteriorly uniflagellate zoospores
Chitosan-chitin	Zygomycetes	Zygospores
Chitin-glucan	Chytridiomycetes	Posteriorly unitlagallate zoospore
	Ascomycetes	Septate hyphae, ascospores
	Basidiomycetes	Septate hyphae, basidiospores
	Deuteromycetes	Septate hyphae
Mannan-glucan	Saccharomycetaceae	Yeast cells, ascospore
	Cryptococcaceae	Yeast cells
Mannan- chitin	Sporobolomycetaceae	Yeast cells, ballistospores
	Rhodotorulaceae	Yeast (carotenoid pigment)
Polygalacturosamine-galactan	Trichomycetes	Heterogenous group

(b) The Protoplast in the Fungal Cell:

The living substance of the cell within the cell wall is the protoplast. It lacks the chloroplasts but is differentiated into the other usual cell parts such as plasma or cell membrane, vacuolated cytoplasm, cell organelles and one or more nuclei.

Cell Membrane:

It is a delicate, extremely thin, living membrane which closely invests the protoplast. The cell or plasma membrane is pressed against the cell or hyphal wall except for occasional invaginations in some regions. The Invagination is either in the form of an infolded convoluted pocket or a pouch enclosing granular or vesicular material.

Moore and Mc Lear (1961) named it lomasome. Actually the plasma membrane is the surface layer of the protoplast altered to perform special functions. It is differentially permeable and shows a typical tripartite structure under the electron microscope. There is an electron dense layer on either side of the less dense central region.

Cytoplasm:

Within the plasma membrane is the colorless cytoplasm in which sap-filled vacuoles may occur. In young hyphae and hyphal tips, the cytoplasm appears rather uniform and homogeneous. Immersed in the cytoplasm are structures known as the organelles and inclusions.

The organelles are living structures, each with a specific function. The inclusions are dead, have no specific function and thus are not essential to cell survival.

Amongst the cell organelles are included the endoplasmic reticulum, mitochondria, ribosomes, Golgi

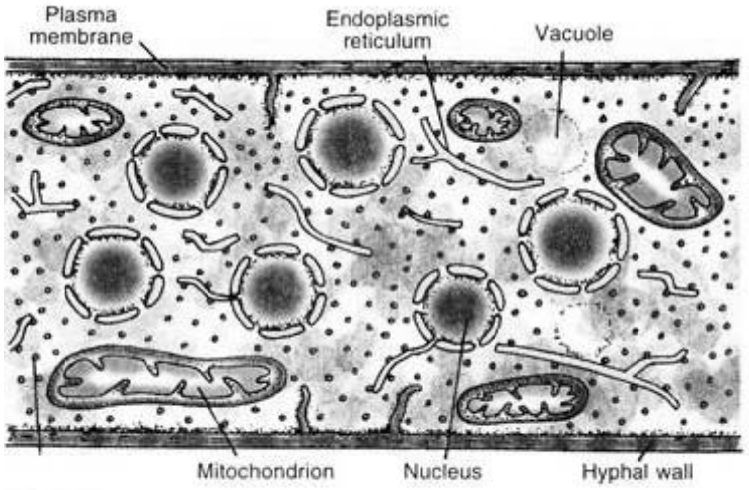


Fig. 1.9. *Fungi*. Fine structure of a hypha near the growing tip of *Mucor* based on an electron micrograph.

apparatus and vacuoles. Lomasomes which are membranous structures lying between the cell wall and plasma membrane are common. Examples of inclusions are the stored foods (glycogen, and oil drops) pigments and secretory granules.

(i) Endoplasmic Reticulum:

The presence of endoplasmic reticulum in the fungal cytoplasm has been demonstrated by the use of electron micro-scope. It is composed of a system of membranes or microtubular structures usually beset with small granules which by some scientists are likened to the ribosomes. In many fungi, the endoplasmic reticulum is highly vesicular. Usually it is loose and more irregular than in the cells of green plants.

(ii) Mitochondria:

The cytoplasm contains small, usually spherical bodies known as the mitochondria. Each mitochondrion is enveloped by a double membrane. The inner membrane is infolded to form the cristae which are in the form of parallel flat plates or irregular tubules.

The cristae contain the same fluid that fills the space between the two membranes. The mitochondria function as the power house of the cell. There is no fundamental difference between the mitochondria of fungi and those of green plants. However, Hawker (1965) holds that the cristae of fungal mitochondria are fewer, flatter and more irregular than those of the green plants.

(iii) Golgi Apparatus (Dictyosomes):

With the exception of Oomycetes there is less certainty of the occurrence of structures similar to those of the golgi apparatus (dictyosomes) m fungi. Moore and Muhlethaler (1963) reported a golgi apparatus consisting of three flattened sacs surrounded by many bubble-like structures in Saccharomyces cells.

(iv) Vacuole:

The cytoplasm of young hyphae or fungal cells and hyphal tips lacks vacuoles. They appear further back or in the old cells. With age, they enlarge and show a tendency to coalesce and ultimately reduce the cytoplasm to thin lining layer immediately within the cell wall.

(v) Inclusions:

The cytoplasm contains various kinds of inclusions. Examples of stored foods are lipid globules, granules of glycogen, oils and the carbohydrate trehalose, proteinaceous material and volutin. The glycogen may occur in vacuoles.

There are no starch grains. Of the pigments, the fungi lack chlorophyll. Carotenoids are often conspicuous by their presence and may occur throughout the cytoplasm or concentrated in the lipid granules or distributed in the cell wall. The cytoplasm, in addition, secretes several kinds .of ferments, enzymes and organic acids.

Nucleus:

The cytoplasm in the individual cells contains one, two or more globose or ellipsoid nuclei which in the somatic portion are small and usually range from 1-2 or 3µ in diameter. They cannot be seen without special techniques.

Structurally the nucleus consists of:

(i) A central, dense body with a clear area around it.

(ii) Chromatin strands, and

(iii) The whole structure surrounded by a definite nuclear, membrane.



Nucleolus.

The central body takes heavy iron haematoxylin stain and is usually Feulgen-negative. In electron micrographs, it appears as an amorphous or granular mass. Mycologists usually designate it as the nucleolus. Bakerspigel (1960) stated that it contains RNA. During nuclear division, the chromatin strands become organised into chromosomes which are extremely small and difficult to count.

Under the electron microscope, the nuclear membrane is seen to consist of inner and outer layers of electron dense material and the middle one of electron transparent substance. The nuclear membrane has pores. At certain points, the nuclear membrane is continuous with the endoplasmic reticulum.

Hyphal Forms:

In response to functional need, the fungal mycelia are modified into different types of struture:

1. Plectenchyma:

When the component hyphae completely interwoven to form a compact thick tissue, it is called plectenchyma.

It is of two types:

Prosenchyma or prosoplectenchyma, where the hyphae remain more or less parallel to each other, retain their individuality and do not fuse and pseudoparenchyma or para-plectenchyma, where the hyphae are com-pletely fused to each other, form a compact mass and lose their individuality. In cross-section the whole mass looks like paren-chyma of angiospermic plant.

2. Sclerotia (sing. Sclerotium):

It is a compact structure of different shapes and sizes, formed by the aggregation of mycelia. It may be round to elongated pod-like, very minute dots to large ball-like structure weighing approximately 14 kg (30 lbs). It may survive for many years as resting stage.

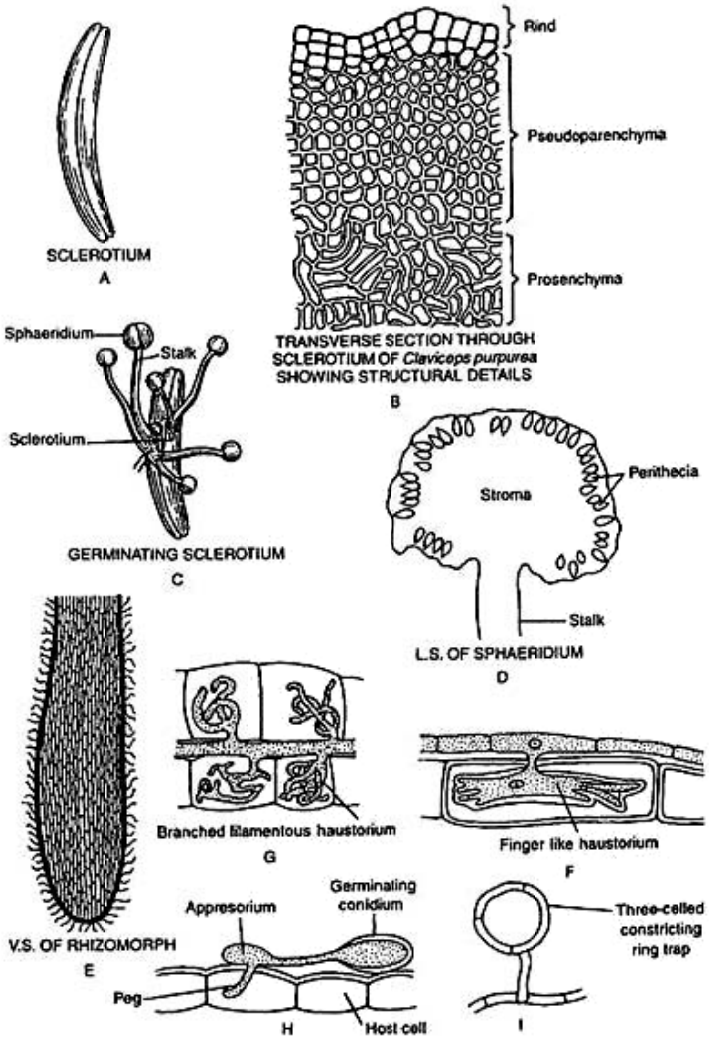


Fig. 4.6 : Different type of modifications of the hyphal structure : A-D. *Claviceps purpurea*, E. *Armillariella mellea* (= *Armillaria mellea*), F. Haustorium of *Erysiphae graminis* in epidermal cell of oat in longitudinal section, G. Haustoria of *Peronospora calotheca* in stem cells of *Asperula odorata*, H. Appresorium of *Erysiphe graminis tritici* on wheat, and I. Hyphal trap of *Monocresporium*

3. Rhizomorph:

In this case, hyphae are aggre-gated longitudinally in varying degree of complexity, where the hyphae lose their indi-viduality and the whole structure behaves as an organised unit. They can withstand adverse environmental conditions and after few years, they can start growing.

4. Haustoria (sing. Haustorium):

These are intracellular outgrowth of the mycelium that grows intercellularly for absorption of nutri-ent from host cells. They are of various shapes and sizes, like simple, knob-like, coiled, branched, etc. They penetrate the cell wall and generally do not rupture the cell membrane during absorption.

5. Appresoria (sing. Appresorium):

It is the swollen tip of germ tube or mycelium of plant pathogenic fungi which helps the myce-lium to adhere to the surface of the host and also helps in penetration.

6. Hyphal trap:

Certain fungi develop sticky hypha or hyphal loops to catch predators like nematode, protozoa, small animals etc., known as hyphal trap. The fungi of this kind is known as Predaceous fungi.

7. Stroma:

It is a solid body of various shapes and sizes, formed by the compact aggrega-tion of mycelium. Reproductive structures and fruit bodies are developed inside the stroma.

Reproduction in Fungi:

In unicellular fungi (*Synchitrium, Saccharomyces*), entire vegetative cell is transformed into a reproductive unit, called **Holocarpic**. However, in others (*Pythium, Penicillium, Helminthosporium*), only a part of the vegetative body forms reproductive unit and the rest portion remains as vegetative, called **Eucarpic**.

The fungi reproduces by all the three means:

1. Vegetative Reproduction:

It takes place by the following ways:

(a) Fragmentation:

It is common in filamen-tous fungi (*Rhizopus, Alternaria, Fusarium*) where the hyphae break up into two or more fragments due to some external force and each one develops into a new individual.

(b) Budding:

It takes place in unicellular fungi (*Saccharomyces, Schizosaccharomyces*). A small outgrowth, the bud emerges out from the parent cell. Nucleus divides into two and one passes to the bud. The bud is then separated by partition wall, but continues its growth.

(c) Fission:

Normally unicellular fungi (*Saccharomyces, Schizosaccharomyces*) reproduce by this method, where the vegetative cell elongates, and divides into two daughter cells of equal size by simple constriction in the middle with simultaneous nuclear division.

2. Asexual Reproduction:

It takes place by means of several types of spore generally form during favourable condition. The spores may be unicellular (*Penicillium, Aspergillus*) or multicellular (*Fusarium, Helminthosporium*).

Some of the spores are:

(a) Zoospore:

The zoospores may be uni- or biflagellate, generally pear-shaped, produced in sporangium, e.g., *Synchytrium, Phytophthora*.

(b) Conidia:

These are exogenously pro-duced non-motile spores develop by constriction at the end of specialised hyphal branches, called conidiophores. They may produce singly (*Phytophthora, Pythium*) or in chain (*Penicillium, Aspergillus*).

(c) Oidia:

In some fungi (*Mucor mucedo*), the hyphal tips often divide by trans-verse wall into large number of small segments, may remain in chain or becomes free from each other, these are known as oidia. The oidia on germina-tion develop into new plants.

(d) Chlamydospore:

The chlamydospores are thick walled round to oval in outline, coloured brown or black. They produce either terminally or in intercalary at some intervals throughout the length of hyphae, e.g., *Fusarium*.

(e) Sporangiospores:

These are globose, multinucleate, non-motile aplanospores, formed inside the sporangium. The sporangiospore germinates by producing germ tube. Later on, it develops pro-fusely branched mycelium.

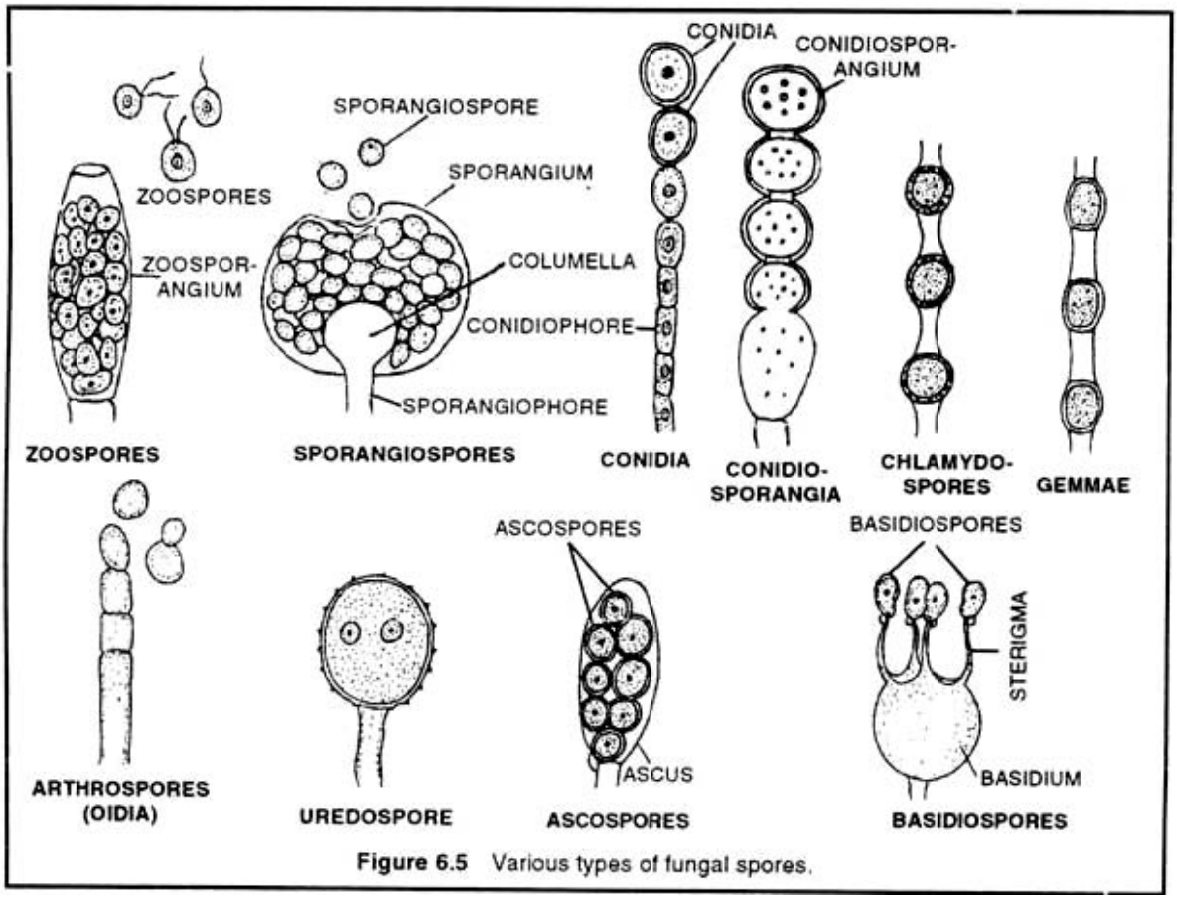


Figure 6.5 Various types of fungal spores.

Sexual Reproduction:

It is the process of union between two compatible nuclei. The nuclei in some members are contributed by two well-organized gametes.

The whole process of sexual reproduction consists of three phases, in the sequence of plasmogamy, karyogany and meosis:

(i) Plasmogamy:

It involves the union of two protoplasts, brings two haploid nuclei close together in the same cell.

(ii) Karyogamy:

It involves the fusion of two haploid nuclei brought together during plasmogamy. This results in the forma-tion of diploid nucleus i.e., zygote, which is ephemeral (short-lived).

(iii) Meiosis:

It follows karyogamy and reduce the number of chromosome from diploid zygote nucleus to original haploid number in the daughter nuclei.

The plasmogamy i.e., the first phase of sexual reproduction, differs in different fungi.

The different methods of plasmogamy are:

(a) Planogametic Copulation:

Planogametes are motile gametes. This process involves the fusion of two gametes, where either one or both are motile.

Depending on the structure and nature of gametes, it is of three types:

Isogamy, Anisogamy and Oogamy:

(i) Isogamy:

The uniting gametes are morphologically similar, but physio-logically different. This process is common in primitive unicellular fungi, e.g., *Synchytrium*.

(ii) Anisogamy:

Both the uniting gametes are morphologically simi-lar, but different physiologically and in size. The smaller one is more active, considered as male and the larger less active one as female, e.g., *Allomyces*.

(iii) Oogamy:

Both the uniting gametes are morphologically and physio-logically different. The male gamate is smaller and motile, and the female gamete is larger and non-motile, e.g., *Monoblepharis*.

(b) Gametangial Contact:

The uniting gametes are present in different gametangium, thus the male and female gametangia are known as antheridium and Oogonium (Ascogonium in Asc-mycotina), respectively. The gametes are never released from gametangium. Both the gametangia come in close contact and transfer male gamete to the egg through fertilization tube. The gametangia do not lose their identity, e.g., *Ascobolus, Pythium*.

(c) Gametangial Copulation:

The process involves the fusion of the entire content of the uniting gametangia.

Such fusion occurs in the following two ways:

Various Methods of Plasmogamy in Fungi

(i) The two gametangia fuse by the dissolution of their common wall resulting into the formation of a sin-gle cell in which content of both the gametangia mix with each other and their morphological identity are completely lost, e.g., *Rhizopus, Mucor*.

(ii) The entire thallus acts as game-tangium. Both the gametangia come in close contact and the male gametangium transfer its entire content to the female gametangium through the pore developed in con-tact area e.g., *Rhizophidium, Polyphagus*.

(d) Spermatisation:

Certain fungi produce many unicellular non-motile, male cells, the spermatia. The spermatia are brought in contact by agents like wind, water and insect either to the trichogyne of the ascogonium or to somatic hyphae or even to special receptive hyphae. The wall at the point of contact dis-solves and content of spermatia passes to the female organ, e.g., *Puccinia, Podospora*.

(e) Somatogamy:

In many higher fungi belonging to Asomycotina and Basidiomycotina, the development of gametes and gametangia are complete-ly lacking. In such fungi, somatic hyphae anastomose with each other to bring together the compatible nuclei. It is regarded as a reduced and efficient form of sexuality, designated as somatogamy, e.g., *Polyporus, Agaricus, Morchella*.

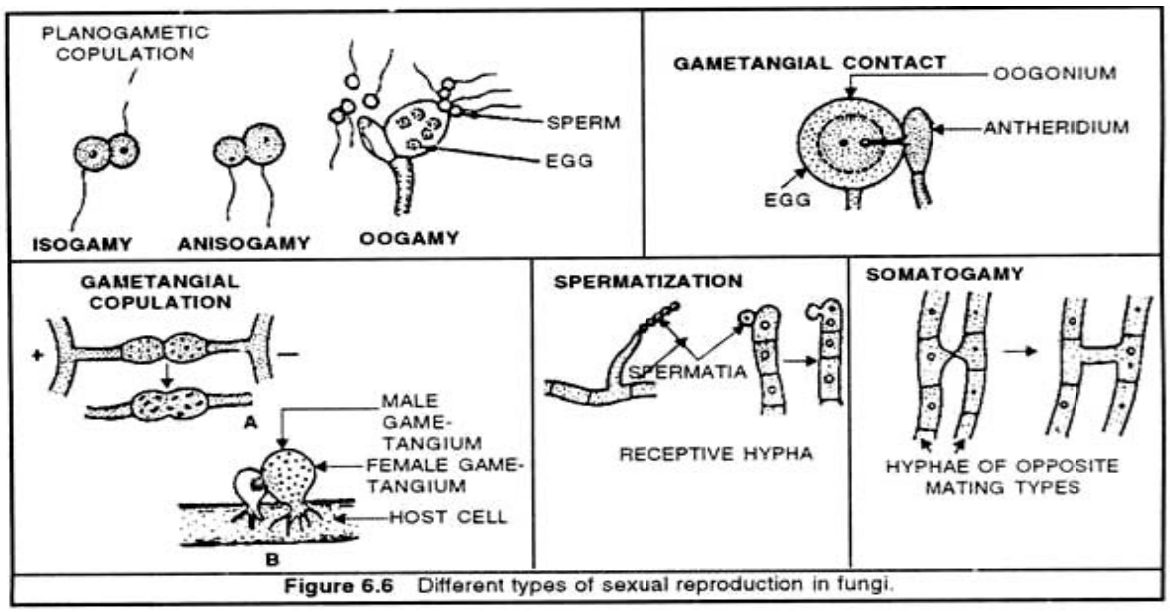


Figure 6.6 Different types of sexual reproduction in fungi.

Heterothallism

The term Heterothallism was first used by an American geneticist A.F. Blakeslee in 1904 when he observed that zygospores could develop in some spp. only when two mycelia of different strains were allowed to come in contact with each other.

According to Blakeslee (1904) Heterothallic condition is **"essentially similar to that in dioecious plants and animals and although in this case the two complimentary individuals which are**

needed for sexual reproduction are in general not so conspicuously differentiated morphologically as in higher forms, such a morphological difference is often distinctly visible."

He concluded that the Zygospore formation is a sexual process. In homothallic species, the mycelium is bisexual while the mycelium in heterothallic species is unisexual, (+) and (-) strains represent the two different sexes.

Heterothallism may therefore be defined as the condition in which Zygospore formation takes place only when mycelia arising from asexual spores of two genetically different mating types (+) and (-), are allowed to interact.

On the other hand the condition, in which one individual originating from a single asexual spore is capable of forming zygospores independently, is known as Homothallism.

.

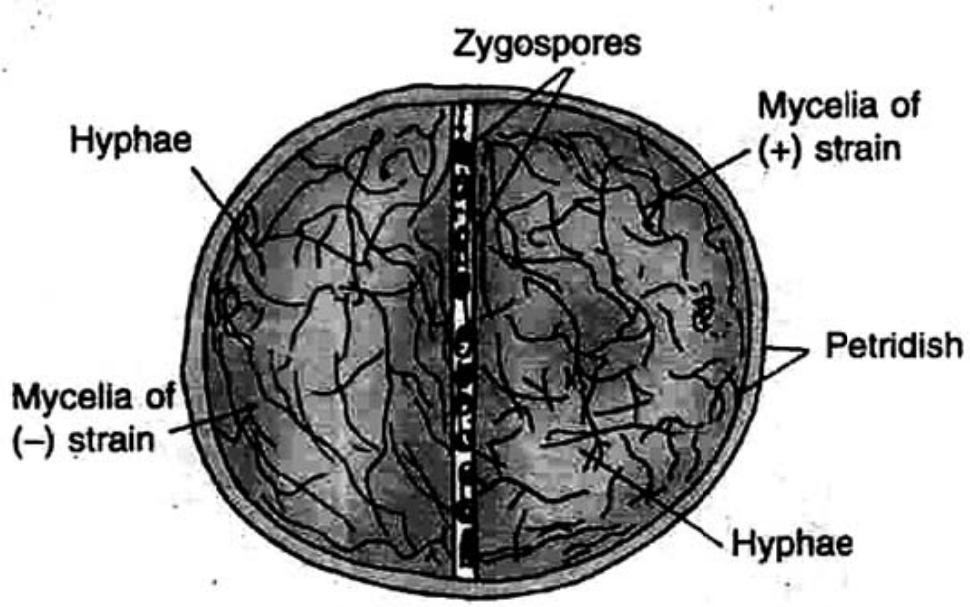


Fig. 17.2 *Mucor hiemalis*. Zygospore formation indicating Heterothallism.

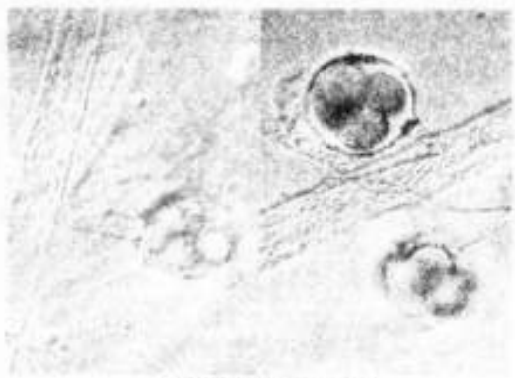
Morphological heterothallism:

Morphological heterothallism may be defined as the condition when morphologically different male and female sex organs are produced in two closely associated mycelia.

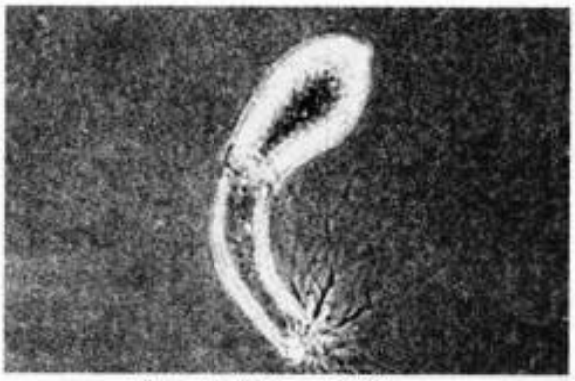
The two sex organs or gametes are so morphologically different that it is easier to term one of them as male and the other as female-examples of such type of morphological heterothallic fungi are: Achlya ambisexualis, A. bisexualis, Blastocladiella variabilis, Dictyuchus monosporus, Phytophthora palmivora and Peronospora parasitica (photographs given below).

However, in Blastocladiella variabilis the male and female gametangia are morphologically distinct, the male being smaller than the female.

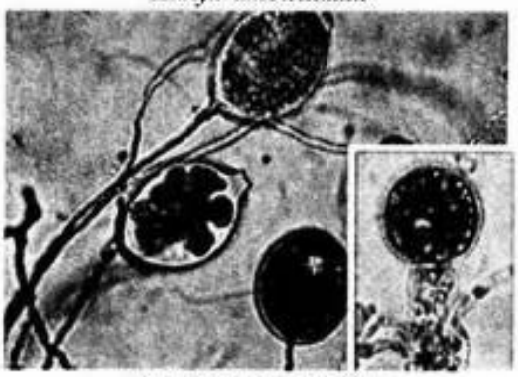
Whitehouse (1949) also used the term haplodioecious for morphologically heterothallic species of fungi.



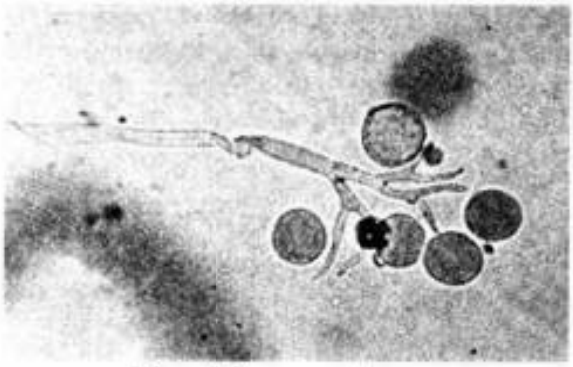
Achlya ambisexualis



Blastocladiella variabilis



Phytophthora palmivora



Phytophthora parasitica

Physiological Heterothallism:

In physiological heterothallism, the interacting thalli differ in mating type or incompatibility, irrespective of the presence or absence of the sex organs or gametes. This means that sexual reproduction takes place by two morphologically similar but physiologically different hyphae in physiological heterothallism.

The gametangia as well as gametes do not show morphological differentiation but physiologically they behave differently.

Physiological heterothallism may be of two types:

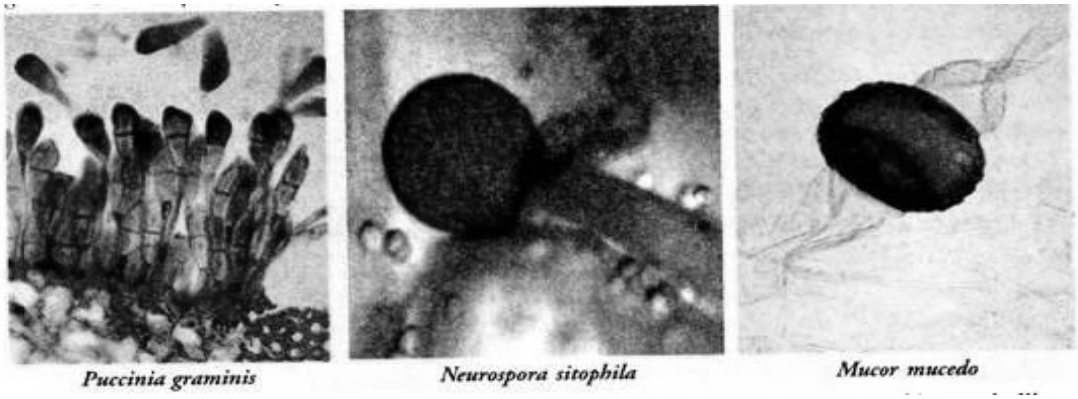
(i) Two Allelomorphs or Two-Allele Heterothallism:

When nuclei of both the mating types are different in genetic characters, this type of Heterothallism is known as Two-Allele heterothallism. In these types compatibility is governed by a pair of Alleles represented by A and a located at single same locus of the chromosome.

Due to the dominance of A over a, A is represented by (+) and a by (-). At the time of meiosis, separation of the chromatids take place. Half of the haploid spores thus have (+) and the other half (-) allele.

The spores bearing (+) allele will produce (+) mycelia and the spores with (-) allele will give rise to (-) mycelia. The mycelia of (+) and (+) and (-) and (-) are self-sterile or self-incompatible. Thus, two complimentary mating types (+) and (-) are essential for sexual reproduction.

Two-Allele heterothallism has been reported in several fungi of like Ascobolus magnificus, Puccinia graminis, Neurospora sitophila Mu mucedo, Ustilago kolleri etc. (photographs given below)



Puccinia graminis *Neurospora sitophila* *Mucor mucedo*

(ii) Multiple Allelomorph or Multiple Allele Heterothallism:

In this type of heterothallism, more than two (multiple) alleles determine the sexual compatibility. These may be located at one (bipolar) or two (tetrapolar) loci.

Because of the larger number of alleles involved in this type of heterothallism, chances of mating of compatible strains increase.

As stated above, the multiple allele heterothallism may be of two types:

(a) Bipolar Multiple-allele heterothallism

(b) Tetrapolar multiple-allele heterothallism.

(a) Bipolar Multiple-Allele Heterothallism:

This type of heterothallism is controlled by multiple alleles at a single locus, instead of a pair of Alleles. For example, if the locus is named as L, the multiples alleles will be designated as L_1, L_2, L_3, L_4—Ln and these are present on the single locus L.

The meiotic division will give rise .to thalli which may be of several mating types, generally equal to the number of alleles. The thallus containing the allele L_1 can mate with a thallus of any mating type except L_1.

Similarly L_2 can mate with any thallus except that containing L_2 allele and so on. In this type of heterothallism, incompatibility factors are more commonly involved. Bipolar multiple allele heterothallism is characteristic of Basidiomycetes except rusts and smuts

	L_1	L_2	L_3	L_4
L_1	N	C	C	C
L_2	C	N	C	C
L_3	C	C	N	C
L_4	C	C	C	N

Fig. 17.3. Bipolar multiple allele heterothallism. C-compatible, N-non compatible

(b) Tetrapolar Multiple Allele heterothallism:

This type of heterothallism is characteristic of Basidiomycetes except rusts. In this type of heterothallism, which is very similar to bipolar multiple allele heterothallism, compatibility is determined by two loci.

Multiple allele—the compatible factor is present on two loci L_1 and $L_2$of two Chromatids of a chromosome. At the time of meiotic division, both the loci are separated with chromatids.
It is estimated that at least 100 alleles are present on each locus. In Schizophyllum commune, 122, alleles of factor A and 61 of B have been identified in the laboratory.

According to rough estimates, the number of alleles may be even more, about 350-450 of L_1, and 65 of L_2. Any two mating types, which differ in allele present on L_1 and L_2 are compatible.

If the allele composition of mating type is A_1 B_1, it would be compatible with any other type of allele composition except A_1 B_1. But the mating type with allele composition is not fully compatible with allele composition A_2 B_2 or A_2 B_2.

Figure 17.4 fully explains type of heterothallism which has been reported in Ustilago maydis and Comprinus firmaterius.

	A_1B_1	A_1B_2	A_2B_1	A_2B_2
A_1B_1	N	NFC	NFC	C
A_1B_2	NFC	N	C	NFC
A_2B_2	NFC	C	N	NFC
A_2B_2	C	NFC	NFC	N

Fig. 17.4. Tetrapolar multiple allele heterothallism. N-non compatible, NFC—Not fully Compatible, C-Compatible.

This type of heterothallism encourages out-breeding. Whereas in bipolar multiple Allele heterothallism, the out-breeding is 25%, in tetrapolar, it is 100%. This may be due to enormous increase in the number of possible mating types of thalli.

According to Garrett (1963), **"heterothallism promotes the out-breeding and therefore subserves the same end as the sexual process, which it renders most efficient. Hetrothallism is not the same as sex, it is refinement super imposed upon it."**

Parasexual Cycle:

In some fungi, true sexual cycle comprising of nuclear fusion and meiosis is absent. These fungi derive the benefits of sexuality through a cycle known as Parasexual Cycle.

The Parasexual Cycle is defined as a cycle in which plasmogamy, karyogamy and meiosis (haploidisation) take place but not at a specified time or at specified points in the life-cycle of an organism.

Generally parasexual cycle occurs in those fungi in which true sexual cycle does not take place. The members of class Deuteromycetes (Deuteromycotina) in which sexual cycle does not occur, exhibit parasexual cycle generally.

Parasexual cycle was first discovered by Pontecarvo and Roper of University of Glasgow in 1952 in Aspergillus nidulans, the imperfect stage of Emericella nidulans.

Since then parasexual cycle has been discovered not only in several members of Deuteromycetes but also in fungi belonging to Ascomycetes and Basidiomycetes.

Steps Involved in Parasexual Cycle:

According to Pontecarvo (1958), parasexual cycle in A. nidulans involves the following steps:

(i) Formation of heterokaryotic mycelium

(ii) Fusion between two nuclei (Karyogamy)

(a) Fusion between like nuclei

(b) Fusion between unlike nuclei

(iii) Multiplication of diploid nuclei

(iv) Occasional Mitotic crossing over.

(v) Sorting out of diploid nuclei

(v) Occasional haploidisation of diploid nuclei, and

(vii) Sorting of new haploid strains.

A brief account of these steps are being presented below:

Thus, after the parasexual cycle has operated for some time, the mycelium may contain the following types of nuclei:

(a) Haploid nuclei like those of both the parents,

(b) Haploid nuclei with various new genetic recombinations,

(c) Several types of diploid homozygous nuclei,and

(d) Several types of diploid heterozygous nuclei.

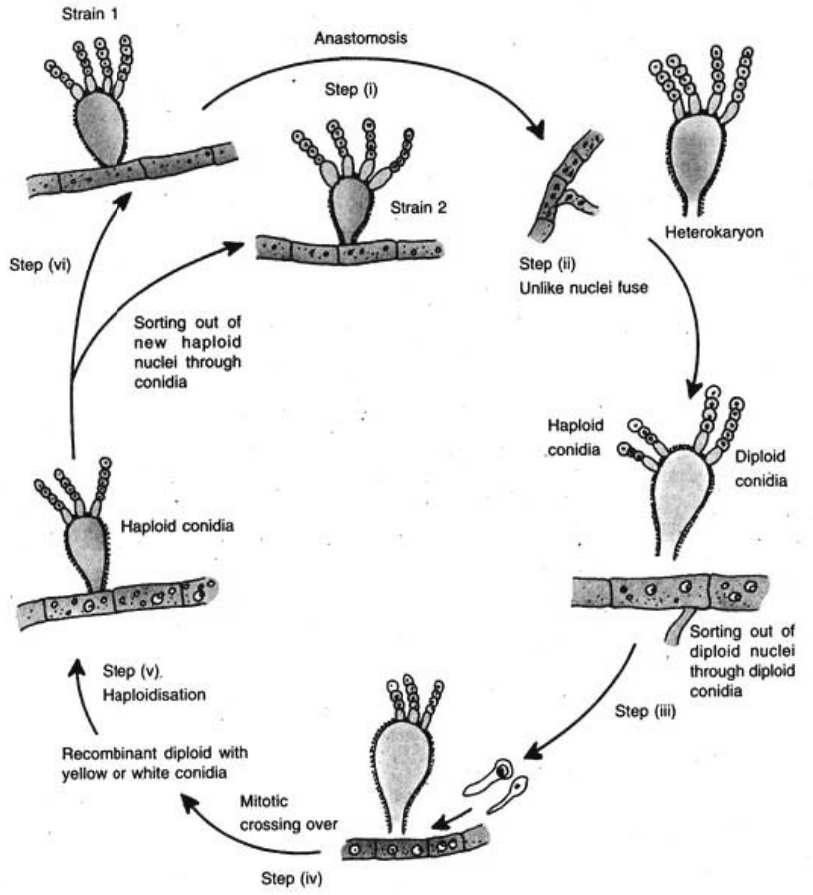


Fig. 17.10. Pontecarvo's (1958) idea of Parasexual cycle

Significance of Parasexual Cycle:

Parasexual cycle is of importance in industrial processes. Several fungi which are used in various industrial processes belong to fungi imperfecti or Deuteromycetes and in these fungi only parasexual cycle operates.

New and better strains of these fungi are obtained by mutation through parasexual cycle. The strains of desirable characters can be developed through mitotic recombinations.

Parasexuality can also be applied in the analysis of genetic and physiological processes of perfect and imperfect fungi. Parasexual cycle has also been successfully employed in genetic control of pathogenicity and host-range in several species of Fusarium.

Economic importance of fungi:-

1. Role of Fungi in Medicine:

Some fungi produce substances which help to cure diseases caused by the pathogenic microorganisms. These substances are called the **antibiotics**.

The term antibiotic, therefore, denotes an organic substance, produced by a microorganism, which inhibits the growth of certain other microorganisms. The most important antibiotics are produced by the moulds, actinomycetes or bacteria.

They are used to combat the evil effects of pathogenic bacteria and viruses. The use of antibiotics is not limited to disease treatment.

The discovery of antibiotic agents as drugs is comparatively a recent history. The role of fungi m producing antibiotic substances was first established by Sir Alexander Fleming in 1929.

He extracted the great antibiotic drug **Penicillin** from *Penicillium notatum*. It was the first antibiotic to be widely used. Penicillin is an organic substance lethal to microbes. It is far more effective than ordinary drugs and germicides.

It has no adverse effect on human protoplasm but kills bacteria especially gram-positive type. Penicillin is now produced on a commercial scale all over the world including India from the improved strains of *P. notatum and P. chrysogenum*.

Streptomycin is obtained from *Streptomyces griseus*. It is of great value in medicine. It destroys many organisms which are not killed by penicillin particularly the gram-negative organisms. A numbers of antibiotics have also been extracted from *Aspergillus* cultures.

However, these have not been proved so effective as penicillin. Some of the actinomycetes which are not considered to be true filamentous bacteria are the sources of many antibiotics such as chloromycetin, aureomycin, terramycin, etc.

They inhibit the growth of many pathogenic bacteria and are also used successfully in the treatment of various virus diseases. Many animal and human diseases which do not respond readily to other antibiotics are effectively cured by aureomycin.

Griseofulvin which is recovered from mycelium of *Penicillium griseofulvum* and many other species has antifungal properties. It acts on the hyphae by interfering with wall formation Consequently the hyphal tips curl and cease to grow.

Claviceps purpurea produces sclerotia in the ovaries of the flowers of grasses such as rye The sclerotium is called the ergot of rye. Ergot is used in veterinary and human medicine.

It contains a mixture of alkaloids which cause rapid and powerful contractions of the uterus. The medicine is thus used to control bleeding during child birth. Ergot is highly poisonous. A derivative of ergot known by the name of **lysergic acid (LSD)** is used in experimental psychiatry.

2. Role of Fungi in Industry:

The industrial uses of fungi are many and varied. In fact the fungi form the basis of many important industries. There are a number of industrial processes in which the biochemical activities of certain fungi are harnessed to good account.

A brief sketch of some of the most important of these processes is given below:

(i) Alcoholic fermentation:

In brewing or wine making industry alcohol is the important product. The other by-product which is carbon dioxide was formerly allowed to escape as a useless thing.

Now carbon dioxide is also considered a valuable by-product. It is collected, solidified and sold as “dry ice”. In the baking or bread- making industry CO2 is the useful product.

It serves two purposes:

(i) Causes the dough to rise.

(ii) Makes the bread light.

The other by-product, which is alcohol, is incidental. The yeasts secrete the enzyme complex called zymase which brings about conversion of sugar into alcohol. Many excellent yeast strains are now available.

The yeasts lack diastase. So they cannot break starch into sugar. There are a number of fungi popularly known as the moulds. They secrete a whole range of enzymes and thus bring about fermentation of complex carbohydrates.

In producing industrial alcohol moulds are employed as starters to bring about scarification of the starch. At the second stage yeast is employed to act on the sugar.

M, rouxii and some species of *Rhizopus*. *Aspergillus flavus* is used in the production of African native beer.

(ii) Enzyme preparations:

Takamine on the basis of his intensive study of the enzymes produced by *Aspergillus flavus-oryzae* series has introduced in the market a few products of high enzymic activity. These are Digestin, Polyzime, Taka diastase, etc. They are used for dextrinization of starch and desiring of textiles.

Cultures of Aspergillus niger and A. oryzae on trays of moist, sterile bran yield a well-known amylase which contains two starch splitting components.

Invertase is extracted from Saccharoymces cerevisiae. It has many industrial uses. It hydrolyses sucrose to a mixture of glucose and fructose.

(iii) Preparation of organic acids:

Oxalic acid is the fermentation product of *Aspergillus niger*. Citric acid is made by mould fermentation. Many species of *Penicillium* are used for the purpose. The acid is produced on a commercial scale and is cheaper than the acid made from the citrus fruits.

The gluconic acid is prepared from sugars. The moulds chiefly employed for this purpose are some species of Penicillium and Aspergillus.

The gallic acid as the fermentation product of an extract of tannin by Aspergillus gallomyces.

(iv) Gibberellins:

These are plant hormones produced by the fungus *Gibberella fujikuroi* which cause a disease of rice accompanied by abnormal elongation. Gibberellin is used to accelerate growth of several horticultural crops.

(v) Cheese Industry:

Certain fungi popularly known as the cheese moulds play an important role in the refining of cheese. They give cheese a characteristic texture and flavour.

The two chief kinds of mould refined cheese are:

(a) Camembert and Brie types. They are soft.

(b) Roquefort Gorgonzola and Stilton types. They are green or blue veined cheese. The moulds concerned are *Penicillium camemberti* and *P. caseicolum* in the first type and P. *roqueforti* in the second type.

(vi) Manufacture of Proteins:

As a supplement to the normal diet, some fungi particularly the yeasts are employed to synthesize proteins. The yeast (*Saccharomyces cerevisiae* and *Candida utilis*) contain high percentage of protein of great nutritive value.

(vii) Vitamins:

The yeasts, are the best source of vitamin B complex. A number of preparations of high potency have been made from the dried yeast or yeast extracts and sold in the market.

A number of moulds and yeasts are utilised in the synthesis of Ergosterol which contains Vitamin D. Riboflavin—another vitamin useful both in human and animal food—is obtained from a filamentous yeast, *Ashby gossypii*.

3. Role of Fungi in Agriculture:

The fungi play both a negative and a positive role in agriculture.

A. Negative Role:

They have a negative value because they are the causative agents of different diseases of our crop, fruit and other economic plants. These fungal diseases take a heavy toll and cause tremendous economic losses.

The modest estimate is that about 30 thousand different diseases (including bacterial and virus) attack the economic plants grown for food or commercial purposes.

The more important of these diseases are:

(i) Damping off disease:

The seedlings of almost every type of plant grown as a commercial crop such as tomatoes, com, cotton, mustard, peas, beans, tobacco, spinach, etc., are prone to this disease. It is caused by a species of *Pythium*.

(ii) The potato blight:

(Late blight of potatoes) is another destructive crop disease. It does a great damage to the potato tubers. A heavy attack of this disease in Ireland in 1845 destroyed the entire potato crop and caused so severe a famine that over a million Irish people migrated to U.S.A. Besides potatoes, it infects egg plants, tomatoes, etc.

(iii) Downy mildews of grapes:

It ruins the vine yards and thus causes heavy losses to the crop. When the disease was first introduced into France from U.S.A, it caused a havoc to the vine yards.

(iv) Ergot disease of rye:

It is an important disease of a cereal crop—rye. It results in the formation of poisonous sclerotia in the rye kernel. It is called ergot of rye. Ergot is highly poisonous to man. Ergot poisoning causes hallucinations, insanity and finally death.

(v) Apple scab:

It is a serious disease of the apple crop. It lowers the quality as well as quantity of the fruit.

(viii) Red rot disease of sugarcane:

It is a serious disease of sugarcane whose incidence has increased during the last few years, particularly in the northern parts of the country.

(ix) Rust diseases:

They attack our cereal crops and forest timber. Some of them such as black stem rust, yellow rust and orange rust are a serious threat to our wheat crop.

They affect the agricultural economy of our country seriously. The farmer and the Agriculture department must wage a constant war against them.

TABLE II

List of some common disease of human beings caused by fungi

	Name of the disease	Pathogen
1.	Aspergillosis	*Apsergillus flavus, A. niger*
2.	Blastomycosis	*Blastomyces dermatidis*
3.	Otomycosis	*Aspergillus fumigatus*
4.	Neuritis	*Mucor pusillus*
5.	Onychomycosis	*Trichophyton purpureum*
6.	Candidiasis	*Candida albicans*
7.	Histoplasmosis	*Histoplasma capsulatum*
8.	Geotrichosis	*Geotrichum candidum*
9.	Chromomycosis	*Cladosporium immitis*
10.	Allergy	Spores of *Aspergillus, Chaeotomium etc*
11.	Dermatomycosis	*Trichoderma viride*

TABLE I

List of some important plant diseases caused by fungi

	Name of the disease	Pathogen
1.	Club root of Crucifers	*Plasmodiophora brassicae*
2.	Wart disease of potato	*Synchytrium endobioticum*
3.	Stem Rot of Papaya	*Pythium aphanidermatum*
4.	Damping off of seedlings	*Pythium sp.*
5.	Late blight of potato	*Phytophthora infestans*
6.	White rust of crucifers	*Albugo candida*
7.	Downy mildew of peas	*Peronospora pisi*
8.	Green ear disease of Bajra	*Sclerospora graminicola*
9.	Powdery mildew of peas	*Erysiphe polygoni*
10.	Powdery mildew of wheat	*Erysiphe graminis*
11.	Leaf curl of peaches	*Taphrina deformans*
12.	Stem gall of coriander	*Protomyces macrosporous*
13.	Ergot disease of rye	*Claviceps purpurea*
14.	Rust of wheat	*Puccinia graminis*
15.	Rust of pea	*Uromyces pisi*
16.	Rust of gram	*Uromyces ciceris-arieteni*
17.	Rust of Linseed	*Melampsora lini*
18.	Covered smut of barley	*Ustilago hordei*
19.	Loose smut of wheat	*Ustilago nuda*
20.	Bajra smut	*Tolyposporium penicillariae*
21.	Grain smut of Jowar	*Sphacelotheca sorghi*
22.	Bunt of wheat	*Tilletia tritici*
23.	Early blight of potato	*Alternaria solani*
24.	Wilt of pigeon pea	*Fusarium oxysporum*
25.	Red rot of sugarcane	*Colletotrichum falcatum*
26.	Tikka disease of groundnuts	*Cercospora personata*
27.	Stripe disease of barley	*Helminthosporium graminieum*

(a) Destruction of timber:

Several fungi such as *Polyporus, Serpula lacrymans, Fusarium negundi, Coniophora cerebella, Lentinus lapidens and Penicillium divaricatum* cause destruction of valuable timbers by reducing the mechanical strength of the wood.

(b) Destruction of textiles:

Several fungi are able to grow on cotton and woolen textiles causing their destruction. These include spp. of *Alternaria, Penicillum, Aspergillus, Mucor and Fusarium*. Spp. of Stachybotrys causes destruction of cotton in storage. *Chaetomium globosum* is reported to cause greatest damage to textiles.

(c) Destruction of Paper:

Paper pulp wood is destroyed by the growth of Polyporus adustus, Polystictus hirsutus etc. several fungi such as species of *Chaetomium, Aspergillus, Stachybotrys, Alternaria, Fusarium, Dematium, Mucor, Cladosporium* etc. cause extensive damage to paper of books, newspapers and paper industry.

B. Positive Role of Fungi:

Some soil fungi are beneficial to agriculture because they maintain the fertility of the soil. Some saprophytic fungi particularly in acid soils where bacterial activity is at its minimum cause decay and decomposition of dead bodies of plants and their wastes taking up the complex organic compounds (cellulose and lignin) by secreting enzymes.

The enzymes convert the fatty carbohydrate and nitrogenous constituents into simpler compounds such as carbon dioxide, water, ammonia, hydrogen sulphide, etc.

Some of these return to the soil to form humus and the rest of the air from where they can again be used as raw material for food synthesis. There are fungi in the soil which produce more ammonia from proteins than the ammonifying bacteria.

Besides, many saprophytic fungi of decay maintain the never ending cycle of carbon dioxide which is a most important raw material for plant photosynthesis in nature.

They also bring about rot, decay and decomposition of animal and plant remains releasing plant nutrients in a form available to green plants as food. The soil fungi utilize many inorganic salts.

These are prevented from being lost from the soil by leaching. Some fungi form mycorrhizal association with the roots of certain plants and help them in their nutrition.

Such plants will grow satisfactorily only when the mycelium of the appropriate fungal partner is present in the soil. The fertile soil contains twice as much living fungus cell material as the material from bacteria and other soil microorganisms.

Giberrrelin produced by *Gibberella fujikuroi* is used as growth hormone accelerating plant growth.

Many insect pests can be controlled by the growth of fungi such as *Empusa sepulchrasis, Metarrhizium anisopliae, Cordyceps melothac* etc.

Some common fungal inhabitants of the soil help to combat diseases caused by soil borne fungi. *Trichoderma lignorum* and *Gliocladium fimbriatum* are found in damp soils. They have an inhibitory effect on the growth of the mycelium of *Pythium.*

They serve to suppress fungi causing the damping off disease of the seedlings and thereby influence favourably the growth of crops.

Drechsler (1937) reported that there are some predacious fungi in the soil. They trap and destroy the nematodes (eel worms). Some species of these predatory soil fungi form loops on the mycelium (A). These loops act as nooses.

They tighten and strangle the nematodes as they try to pass through (B). The mycelium later sends out special hyphae (C) to absorb nutrition from the captive. Some

predatory soil fungi produce conidia which are sticky.

As the nematodes pass through the soil the stickly conidia stick to their bodies. There the conidia germinate to produce hyphae which penetrate into the tissues of the host and absorb nourishment.

At the National Botanical Research Institute, Lucknow and at several other national institutes, fungi are being tested as biopesticides especially as nematicides and as fungicides.

An important fungus being used as nematicide is Beauveria bassiana against borers, thrips, and aphids. *Trichoderma viride* and *T. harzianum* are other examples which are used against a large number of soil-borne pathogens.

4. Role of Fungi as Food and as Food Producers:

Many species of fungi are edible, about 2000 species of them have been reported from all over the world. Of these, about 200 are said to occur in the Western Himalayas.

Many edible fungi are of great economic value as food. They are regarded as delicacies of the table. There are said to be over 200 species of edible fungi.

The fructifications of some fungi such as the field mushroom *Agaricus campestris* (dhingri), *Podaxon podaxis* (Khumb), the honey coloured mushrooms, the fairy ring mushrooms, the puff balls (*Lycoperdon* and *Clavatia*), morels (*Morchella, guchhi*), and truffles are edible.

The content of available food in them is not high but they supply vitamins and are valuable as appetisers. Yeasts and some filamentous fungi are valuable sources of vitamins of the B-complex.

A few of the mushrooms are fatally poisonous, some cause only discomfort. To the former category belong *Amanita*.

The fungi are also important as producers of foodstuffs. Certain species of *Penicillium* are active in the refining of certain kind of cheeses. Some fungi, such as red bread mold, *Neurospora sitophila* and others, complete their sexual life cycle in a few days and thus make ideal organisms for the study of the laws of heredity.

The slime molds (*Physarum polycephalum*) are now widely used in research. P. polycephalum has proved an excellent experimental organism for the study of DNA synthesis, meiotic cycle and the mechanism of protoplasmic streaming.

In tropical conditions, many fungi such as *Mucor sp., Penicillium, Neurospora, Fusarium, Aspergillus* etc., grow on meat causing sufficient spoilage.

Aflatoxins the most potent carcinogenic agent-are produced by Aspergillus flavus, A. fumigatus, A. parasiticus and Penicillium islandicum on dried foods and groundnut meal.

Aflatoxins are reported to bind with DNA and prevent its transcription arresting protein synthesis. These are responsible for liver cancer in animals and human beings.

Mushroom toxins are produced by several poisonous mushrooms. These cause diarrhoea vomiting, liver damage, complete unconsciousness etc. Mushroom toxins are commonly produced by *Amanita phalloides*, spp. of Helvella and some species of Inocybe.

Ergot toxins produced by *Claviceps purpurea* contain poisonous alkaloids like ergotamine, ergometrimine, ergocrystinine, ergocrystinine and ergonovin. These cause diarrhoea, abdominal pain, vomiting and psychiatric disturbances.

Fungi as Test Organism:

Fungi form very good research material for genetical studies and other biological processes Genus *Neurospora* has become very good material for genetic studies while Physarum polycephalum is used to study steps in DNA synthesis, morphogenesis and mitotic cycle.

To detect the presence and quantity of vitamin B in given sample, Neurospora crassa is commonly used. Similarly *Aspergillus niger* is used for detection of trace elements like zinc, nickel and copper even when they are present in very minute quantities.

Bryophytes:

Bryophyta (Gr. Bryon = mass; phyton = plant), a division of kingdom Plantae comprises of mosses, Hornworts and Liverworts. They are groups of green plants which occupy a position between the thallophytes (Algae) and the vascular cryptogams (Pteridophytes).

Bryophytes produce embryos but lack seeds and vascular tissues. They are the most simple and primitive group of Embryophyta. They are said to be the first land plants or non-vascular land plants (Atracheata). Presence of swimming antherozoids is an evidence of their aquatic ancestory.

General Characteristics of Bryophytes:

1. The life cycle of bryophytes is distinctly differentiated into gametophytic and sporophytic phases (heteromorphic).

2. The gametophytic phase is predominant and ecologically persistent, i.e., green, indepen-dent and long-lived.

3. The sporophytic phase is very short-lived, and completely dependent upon the gametophyte.

4. Unlike most of the higher plants, bryophytes are not found as single individuals but in groups of individuals which have charac-teristic features depending on their family, genus or species.

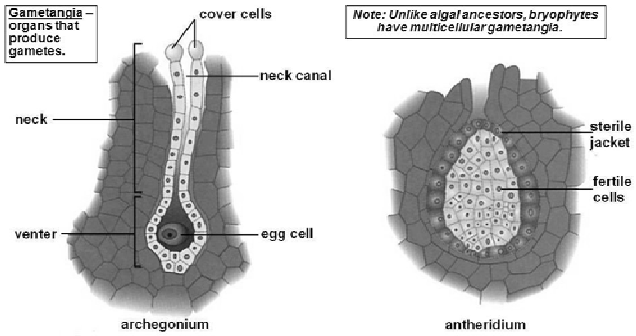
5. The gametophytic plant body is either thalloid or differentiated into the erect axis (stem) and lateral appendages (leaves).

6. Roots are absent in bryophytes. The rhizoids perform the function of roots. They are either unicellular and unbranched or multicellular and branched.

7. They never form xylem tissue, the special lignin-containing water-conducting tissue that is found in the sporophytes of all vas-cular plants. However, the plant body is made up of parenchymatous cells only.

8. They reproduce by vegetative and sexual methods. Asexual reproduction is comple-tely absent in bryophytes.

9. Vegetative propagation takes place by some special structures, like gemmae, tubers, protonema, cladia, innovation, etc.



10. Sexual reproduction is only of oogamous type. They produce large, multicellular sex organs for reproduction. Bryophytes are unisexual, either homothallic (monoecious) or heterothallic (dioecious).

11. The male sex organs, called antheridia, are stalked, globose or ovoid with one celled thick jacket surrounding androgonial mother cells.

12. The female sex organs, called archegonia, are vase-shaped or flask-shaped structure having the basal swollen venter containing a ventral canal cell and an egg, and the upper elongated neck containing neck canal cells. Both the venter and neck are surrounded by the sterile jacket.

13. The sperms are motile and biflagellate having two whiplash flagellae.

14. Bryophytes require water for sperm dispersal and subsequent fertilisation.

15. The sperms move short distances in the water film and ultimately reach the open necks of the archegonia. The slimy mucilage secretions in the archegonial neck help to pull the sperm downward to the egg.

16. The zygote does not pass any resting phase. Embryonic growth of the sporophyte begins within the venter of the archegonium soon after fertilisation.

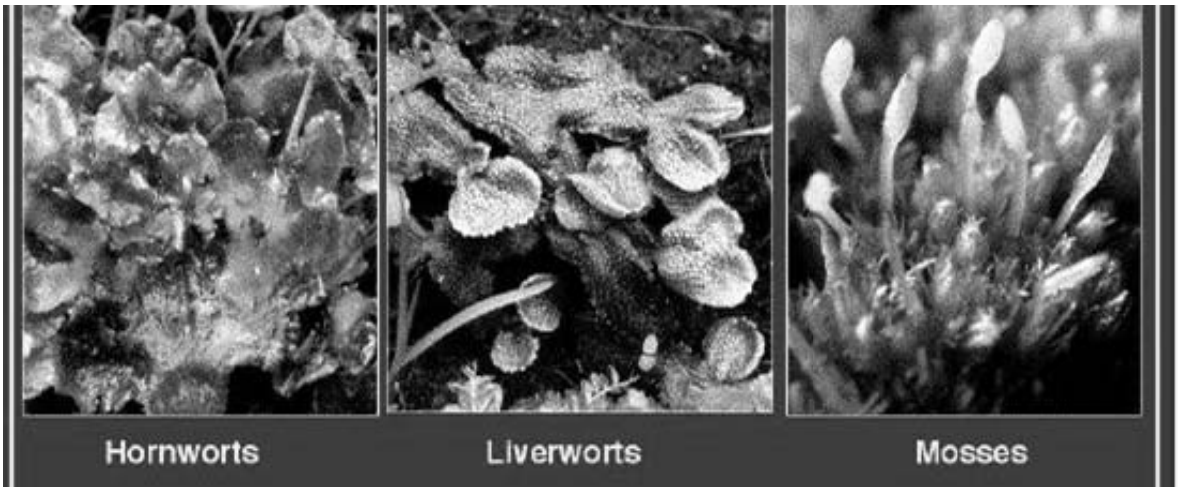
17. The embryo follows exoscopic mode of development. In this development, the zygote first divides transversely to form an outer epibasal cell and an inner hypobasal cell and the embryo develops from the epibasal cell. Thus, the shoot forming apical cell is directed outwards, i.e. towards the neck of the archegonium.

18. The sporophyte consists of only bulbous foot, with or without an unbranched seta and a single terminal sporangium Sometimes the sporophyte is represented only by a capsule (*Riccia*). The capsule has a protective cove-ring called calyptra which is a part of gametophyte.

As the sporophyte of bryophytes is monosporangiate (containing a single sporan-gium), they are called sporogonium.

19. Bryophytes are homosporous — isospores (spores are identical morphologically and physiologically) are produced from the sporogenous cells of the capsule.

20. The spore after germination either produces a filamentous germ tube that gives rise to a young gametophyte (*Riccia, Marchantia*) or produces a protonema which bears leafy buds that will ultimately form the adult gametophytic shoot.



Bryophyte classification:

The term Bryophyta was first introduced by **Braun (1864)**, however, he included algae, fungi, lichens and mosses in this group.

The rank of division Bryophyta to this well-defined group of plants was first given by Schimper (1879).

Eichler (1883) was the first to divide Bryophyta into two groups:

Group I. Hepaticae

Group II. Musci.

Proskauer (1957) however changed the name Anthoceropsida to Anthoerotopsida and thus, the Bryophyta may be classified as follows:

Division

Bryophyta

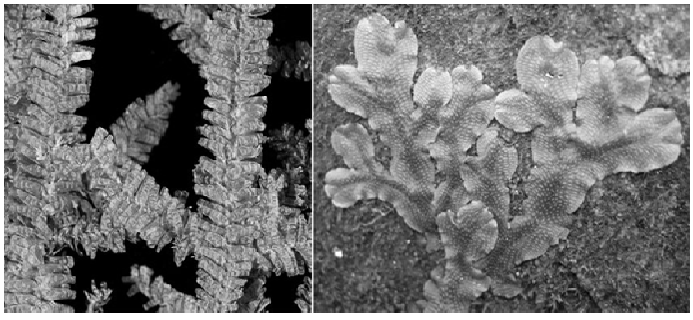
Class 1.
Hepaticopsida (Hepaticae)
Class 2.
Anthocerotopsida (Anthocerotae)
Class 3.
Bryopsida (Musci)

Hepaticopsida:

1. This class includes about 280 genera and 9500 species.

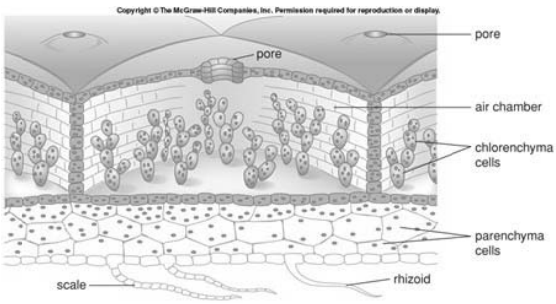
2. The name of this class is derived from a latin word Hepatica which means liver. Hence members of this class are commonly known as liverworts.

3. Plant body is gametophytic and the gametophyte is either thalloid or foliose.



4. Thalloid forms are prostrate, lobed, dorsiventral and dichotomously branched.

5. In foliose forms, 'leaves' are entire, lobed or divided and without 'midrib'. 'Leaves arranged in two to three rows on the axis.



6. Rhizoids are unicellular and branched.

7. Photosynthetic cells contain many chloroplasts.

8. Pyrenoids are absent.

9. Sex organs are borne dorsally or apically, superficial or embedded in gametophytic tissue

10. Members may be monoecious or dioecious.

11. Sporophyte is either simple or represented by capsule o nly (e.g., *Riccia*) or may differentiated into foot, seta and capsule (e.g., *Marchantia*).

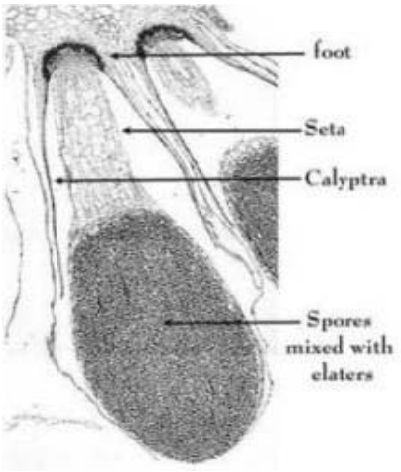




12. Archesporiuin is endothecial in origin.

13. Sporogenous tissue either for ms only spores (e.g., *Riccia*) or is differentiated into sterile elater mother cells and fertile spore mother cells.

14. Columella is absent in the capsule.



15. Elaters are unicellular, hygroscopic with spiral thickenings.

16. Capsule wall is one to several layers thick and without stomata.

17. Dehiscence of the capsule is irregular or in definite number of valves.

18. Spores on germination form the gametophytic plant body.

19. Plants show heteroinorphic alternation of generation.

Anthocerotopsida:

1. This class is represented by about 6 genera and 300 species.

2. Plant body is flat, dorsiventral, thalloid, gametophytic and variously lobed.



3. Smooth walled rhizoids are present.

4. Tuberculated rhizoids and scales are absent.

5. Internally the thallus is not differentiated into zones.

6. All cells are alike.

7. Air chambers or air pores are absent.

8. Each cell has a single chloroplast and each chloroplast contains a single pyrenoid.

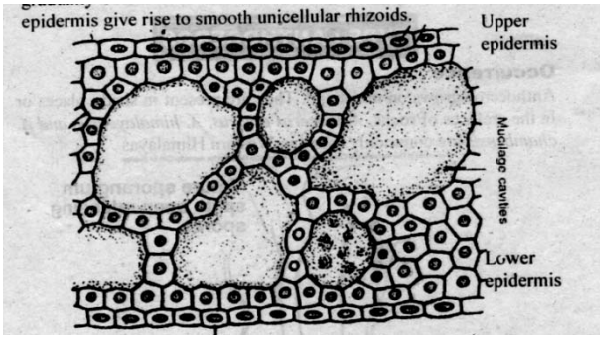
9. Mucilage cavities open on the ventral surface by slime pores.

10. Sex organs are embedded in the thallus.

11. Antheridia develop either singly or in groups in closed cavities called antheridial chambers.

12. The sporophyte is differentiated into foot, an intermediate zone or meristematic zone and capsule.

13. Due to the presence of the meristematic zone, the sporophyte shows indeterminate growth i.e., it continues to grow indefinitely.



14. Archesporium is amphithecial in origin.

15. Sporogenous tissue forms the fertile spores and sterile elaters. Elaters do not have spiral thickenings and are known as pseudo elaters.

16. Capsule wall is four to six layered thick and epidermis has the stomata.

17. Capsule matures from apex to base and usually dehisce by two valves.

Musci or Bryopsida:

1. It is the largest class in Bryophyta and includes about 700 genera and 14,000 species.



2. The main plant body is gametophytic and can be differentiated into two stages-juvenile stage and leafy stage or gametophore.

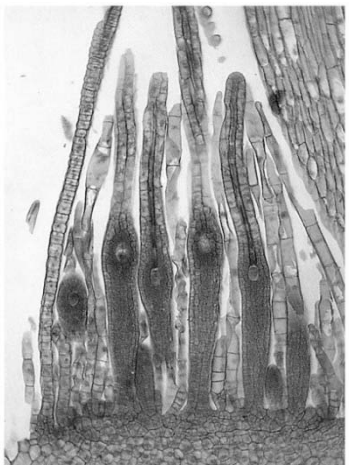
3. Juvenile stage is represented by green, filamentous branched structures called protonema. It develops from the germination of the spore.

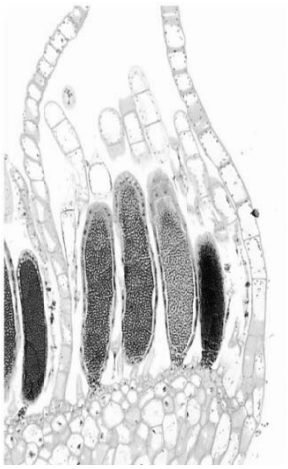
4. Gametophores are erect leafy branches which develop on the protonema.

5. Gametophores can be branched or un-branched and can be differentiated into three parts-rhizoids, ‘stem’ and ‘leaves’.

6. Branches arise below the ‘leaves’.

7. ‘Leaves’ are with midrib, un-lobed and arranged spirally in three to eight rows on the axis or





8. Rhizoids are multicellular, filamentous, branched with oblique septa.

9. The axis is differentiated into central conducting strand enclosed by cortex.

10. Sex organs borne apically in the groups on main ‘stem’ or a branch.

11. The sporophyte is green in early stages and can be differentiated into foot, seta and capsule.

12. The seta is usually elongated and rigid.

13. Columella is usually present and endothecial in origin.

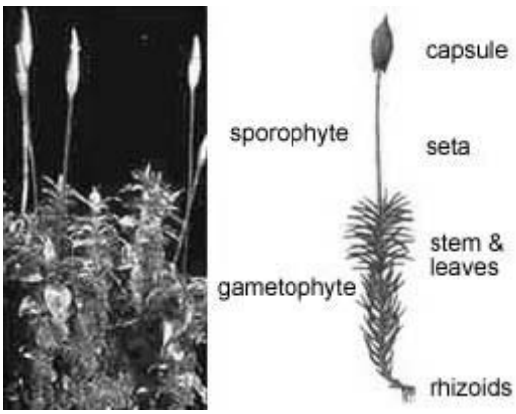
14. Archesporiurn (spore forming tissue) is differentiated only in spores.

15. Elaters are absent.

16. Dehiscence of capsule takes place by separation of lid or operculum.

17. Peristome helps in the dispersal of spores.

18. Spores on germination produce the protonema.



Vegetative Reproduction in Bryophytes:

The following are the common methods of vegetative propagation noted among the bryophytes:

1. Fragmentation or Progressive Death and Decay of the Older Parts:

Propagation through this process involves progressive death and decay of the older parts of the thallus and consequent separation of the younger parts at the point of dichotomy of the thallus.

These separated parts, called fragments, develop to form new mature thallus. This is the common method of vegetative reproduction found in liver-worts (e.g. *Riccia, Marchantia, Plagiochasma*), hornworts (e.g. *Anthoceros*) and in some mosses (e.g. *Sphagnum*).

2. By Tubers:

The tubers are round struc-tures formed on the thaiIi under unfavourable conditions like drought. They remain unaffected and lie dormant while the rest of the thallus gets dried. On the return of favourable environmental conditions, the tubers resume growth and develop into new thalli.

Tubers are frequently produced in species of Riccia (*R. discolor, R. vesicata, R. billardieri*), Anthoceros (*Anthoceros pearsoni, A. himalayensis, A. laevis*), *Asterella angustata*, and *Fossombronia himalayensis*.

3. By Gemmae:

Gemmae are special propagative organ with definite form. They are quite abundant in the members of liverworts, but less common in hornworts and mosses. They are of different shapes, stalked or sessile and may develop on different parts of the parent plant.

4. By the Formation of Adventitious Branches:

In some thailoid liverworts and horn-worts adventitious branches develop usually from the underside of the midrib of the thallus. These adventitious branches on detachment from the parent plants develop into independent plants (e.g., *Riccia fluitans, Marchantia, Targionia, Reboulia, Asterella, Sphaerocarpos, Anthoceros,* etc.). This process, in fact, achieves the luxuriant growth of Riccia fluitans rapidly covering the whole pond surface.

5. By Cladia:

Cladia are small detachable branches which serve the purpose of vegetative reproduction. These may develop either from the individual cells of leaves or stems and are known as leaf cladia (Bryopteris fruticulosa, Frullania fragilifolia) or stem cladia (e.g., Leptolejeunia sp., Drepanolejeunea sp., etc.) as per their mode of origin.

6. By the Formation of Innovations:

The formation of innovations is frequently noticed in Sphagnum (Fig. 6.69). In this case, one of the divergent branches in each node develops more strongly and becomes dominant and erect like the main axis and is called innovation.

During humification process innovation is separated from the main axis due to the progressive death and decay of the lower basal parts of the main axis and eventually establishes as an indepen-dent plant body.

7. By Protonema:

The green, filamentous protonema which develops from the germination of spore is known as primary protonema. The primary protonema may break into small pieces — either accidentally or due to the death of some cells in between. Each of these fragments bearing buds is capable of forming a new plant (e.g., *Funaria*).

The protonema that develops from any part of the plant other than the spores are called secondary protonema. They may develop from the rhizoids of gametophore (e.g., *Funaria* sp.), from leafy gametophore (e.g., *Funaria, Sphagnum*), from primary protonema (Sphagnum), from wounded portion of the leafy shoot (e.g., *Funaria*).

Additional buds develop via formation of secondary protonema, thus increasing the number of buds derived from a single spore.

8. Persistent Apices:

In many thalloid bryophytes (e.g., *Anthoceros, Cyathodium, Athalamia* etc.) and in some creeping mosses, all parts of the thallus except the growing apex becomes dry during the dry summer season. These apical parts remain dormant during dry season. These apices, on return of favourable environmental condition (rainy season), become active and form new plants.

Ecological Importance of Bryophytes:

Bryophytes are important constituents of the ecosystem in temperate and tropical forests that have significant ecological importance. Bryo-phytes are important stabiliser of substrata that later become suitable for higher plants colonisa-tion. Extensive bryophyte mats are significant in the water balance of the forest.

They are capable of absorbing water and nutrients directly through the surface. They prevent soil erosion as they have trample-resistant structure and high regenerative capacity. Some bryophytes provide suitable substrata for the biological fixation of N2 in association with blue green algae.

The recent increase in atmospheric pollution has revealed the bryophytes as “bioindicators” of pollution and accumulators of heavy metals.

The ability of bryophytes to grow on open and nutrient poor areas and their tolerance to desic-cation can be exploited in successful stabilisa-tion of soil on road sides and open areas. Bryophytes also harbour a number of inverte-brates and provide them shelter, food and a place for deposition of eggs.

The details are as:

1. Bryophytes and Plant Succession:

Among the bryophytes, the mosses are consi-dered to be the most potent forms in successional process. They colonise over the nutrient-poor sites where no other plant can survive. After death and decay, they form humus, in other way increasing soil fertility.

Thus, the accumulated organic matters become suitable for the micro-organisms. The microorganism increases the nutrient availability and makes the site suitable for growth of higher plants. The important species under this category are Cephalozia media, Isopterygium elegans, Lepidozia septans, Pellia epiphylia and Tetrapis pellucida.

2. Bryophytes and Animal Association:

Bryophytes possess several attributes viz., incon-spicuous forms, relative abundance in the com-munity, ability to survive in extreme environ-mental conditions and water absorbing and retention capacity, which affect the distribution and abundance of dependent animals and microorganisms.

(i) Bryophytes and Animal Succession:

While bryophytes participate in the early stages of plant succession,' their associated animals form similar stages of faunal succession. For example, moss cushions developing on rock faces are first colonised by rhizopods, rotifers, nematodes and ciliates.

As dead material form under the cushions, rotifers and tardigrades become abundant and arthropods begin to appear. As a thicker decomposition layer is formed, the composition of the fauna becomes similar to that of the soil fauna.

(ii) Shelter:

Water retention is a unique feature that makes bryophyte community an attractive habitat for many invertebrates. Bryophytes provide food and nesting materials for small mammals and invertebrates. Indirectly, they serve as a matrix for a variety of interactions between organisms. Insects are the most richly represented on bryophytes.

Many protozoa, rotifera, nematodes, earthworms, molluscs, arthropods like spiders, millipeds, centipeds and various crustaceans are found in bryophyte com-munities. Large pores of Sphagnum leaves facili-tate the entrance of water and allow unicellular animals to enter the leaf cells and live inside them.

(iii) Food:

Many invertebrates feed on bryophytes. Orthopterans, beetles, moth and caterpillars bite and chew whereas bugs, aphids and mites suck out the contents of moss cells.

(iv) Ovipositor and Pupation:

The animals which feed on bryophytes also ovideposit their eggs there. Snails and slugs are frequently depositing their eggs upon the gametophores. Water beetles appear to live preferentially among mosses and spend their dormancy period.

Many insects associated with bryophytes deposit their eggs there, and -the larval stages often browse on the gametophores. Pupation of the water beetle takes place within a small cell.

(v) Camouflage:

Some insects have morphologies, surface patterns or appendages that permit them to blend in with their bryophyte habitat. A few insects paste the parts of game-tophores on their wings and thus camouflage themselves against predation.

Camouflage may be used by larvae that construct their cases from blades of *Fontinalis, Hygrophynum, Anomobryu*m and *Plagiochila*.

3. Bryophytes and Cyanobacteria:

In natural association, cyanobacteria typically grow in association with bryophytes. Nitrogen is often a limiting nutrient for plant growth. Even small contributions from biological nitrogen fixation may, therefore, be important to the ecosystem.

Some mosses, hornworts and liverworts provide suitable habitats for the biological fixation of nitrogen in association with cyanobacteria (e.g., *Nostoc*). The ability of cyanobacteria to fix atmospheric nitrogen allow a few bryophytes to grow in areas that are naturally low in nitrogen and serve as fertiliser to soil.

4. Bryophytes as Ion-Exchanger:

The cell walls of *Sphagnum* function as ion-exchanger. They rapidly absorb cations, such as calcium and magnesium, supplied by rain water, and in exchange^ release hydrogen ions into the water. Rydrogeri ions make the soil acidic (pH 3-4). Therefore, Sphagnum creates as well as main-tains a nutrient-poor, acidic environment that fosters their own growth, but is mostly intolerable to other plants.

5. Bryophytes Maintain Water Balance in the Forests:

In forests, especially in the montane tropics, bryophytes (especially *Sphagnum*) absorb huge quantities of water and maintain humidity over dry periods, thus preventing rapid run-off and flooding. Without bryophytes, rain-forest would be merely wet and mountain rocks would be barren.

The huge bryophyte mats in the forest floor slow down and delay run-off during rain. It has been predicted that the excessive flooding in India is at least partly due to loss of bryophyte covers.

6. Bryophytes Conserve Soil and Prevent Soil Erosion:

On bare and disturbed soil bryophytes are primary pioneers and they have the ability to stabilise soils. The soils in semi-arid regions are held in place by crusts predominant-ly composed of bryophytes, thus preventing the soil from blowing away. They also prevent soil erosion by slowing down and delaying run-off during rain.

When clay-rich soil has been laid bare due to landslides or road making, the first colonisation and subsequent stabilisation are substantially by bryophytes. The soil surface rapidly becomes bound together by rhizoid pro-duction followed by rapid branching of prostrate stems, thus preventing further soil erosion.

7. Bryophytes as Pollution Indicator:

The investigations with bryophytes in relation to different pollutants prove their potential as bioindicators of pollution. Due to their habitat diversity, structural simplicity, totipotency and rapid rate of multiplication bryophytes appear to be ideal organisms for pollution studies both under field and laboratory conditions.

Phytosociological and eco-physiological studies indi-cate that the decline and absence of mosses — especially epiphytic ones — in urban-industrial areas is a phenomenon primarily induced by air pollution caused by different gaseous and parti-culate pollutants. These plants can be reliable indicators and also monitor the air pollution.

Some bryophytes are very sensitive to pollution and show visible symptoms of injury even in the presence of minute quantities of pollutants. Such plants serve as good bio-indicators of the nature and degree of pollution. Some bryophytes have the capacity to absorb and retain pollutants in quantities much higher than those absorbed by other plant groups present in the same habitat.

Their efficient absorbing capacity is due to the absence of cuticle, presence of single cell thick lamina and larger surface area as compared to the volume. These plants, therefore, act as effective sink of pollutants and prevent their recycling for a considerable period of time.

Economic importance of Bryophytes:

1. Protection from soil erosion:

Bryophytes, especially mosses, form dense mats over the soil and prevent soil erosion by running water.

2. Soil formation:

Mosses are an important link in plant succession on rocky areas. They take part in binding soil in rock crevices formed by lichens. Growth of Sphagnum ultimately fills ponds and lakes with soil.

3. Water retention:

Sphagnum can retain 18-26 times more water than its weight. Hence, used by gardeners to protect desiccation of the seedling during transportation and used as nursery beds.

4. Peat:

It is a dark spongy fossilized matter of Sphagnum. Peat is dried and cut as cakes for use as fuel. Peat used as good manure. It overcomes soil alkalinity and increases its water retention as well as aeration. On distillation and fermentation yield many chemicals.

5. As food:

Mosses are good source of animal food in rocky and snowclad areas.

6. Medicinal uses:

Decoction of Polytrichum commune is used to remove kidney and gall bladder stones. Decoction prepared by boiling Sphagnum in water for treatment of eye diseases. Marchantia polymorpha has been used to cure pulmonary tuberculosis.

7. Other uses:

Bryophytes arc used as packing material for fragile goods, glass wares etc. Some bryophytes act as indicator plants. For example, Tortell tortusa grow well on soil rich in lime.

Pteridophyta:

Pteridophytes are considered as first land vascular plants on Earth which appeared in the Late Silurian (~400 million years ago) as evinced by the discovery of fossil *Cooksonia*, an earliest plant with conducting system. They flourished during Devonian to Carboniferous (Late Palaeozoic) with varied forms like arborescent lycopods, giant horsetails, tree ferns and thus the Late Palaeozoic age is straightforwardly considered as “Age of Pteridophyta”.

Today, this plant group is represented approximately by 13,600 named species of ferns and lycophytes, mostly dominated by ferns (more than 10,000 species) and reached at second highest position among land plant groups after angiosperm. However, the statures of most of the present-day forms are herbaceous instead of arborescent as was in ancient ages.

Pteridophytes, especially the ferns, are not only worthy in forming a balanced ecosystem, but they attract common people for their exquisite lacy foliage and so are valued as horticultural plants since long. The plants are also explored worldwide as natural resources for food, fibre, handicraft, construction material, bio-fertilizer, medicine etc. for human benefit. Ferns and lycophytes have even been used by tribal people in many countries since ancient ages as ethno-medicine and in traditional rituals and ceremonies for spiritual purposes. Recently researchers are paying attention to utilize various terrestrial and aquatic ferns for heavy metal accumulation from the environment by proving their potential in phytoremediation. The existing knowledge from many places across the world gradually enriches the understanding that easy growing common ferns have abilities to absorb unamenable soil pollutants particularly heavy metals/metalloids. As such, the multifaceted usages of ferns and lycophytes have bound people for considering this group of plants as unequivocal component in their life.

Habitat

- Generally, grow in tropic, mesic forest
- Also occur in temperate region

- Even they grow in alpine region (*Lycopodium*)
- May grow along the mangrove region at sea side (*Acrostichumaureum*)
- Some ferns grow in desert region
- Some grow at road side in polluted area

Habit

- Generally, herb and shruby (e.g. *Oleandra*)
- Tree fern (e.g. *Cyathea*, *Dicksonia*)
- Climbers (e.g. *Lygodium*)

General Characters

- The pteridophytes are vascular plants (plants with xylem and phloem) that produce neither flower nor seeds. Instead, they reproduce and disperse only via spores.
- Plants characterised by a very distinct alteration of generations with independent inconspicuous and sort-lived gametophyte (sexual) and conspicuous and dominant sporophyte (asexual) stages (Fig. 1).

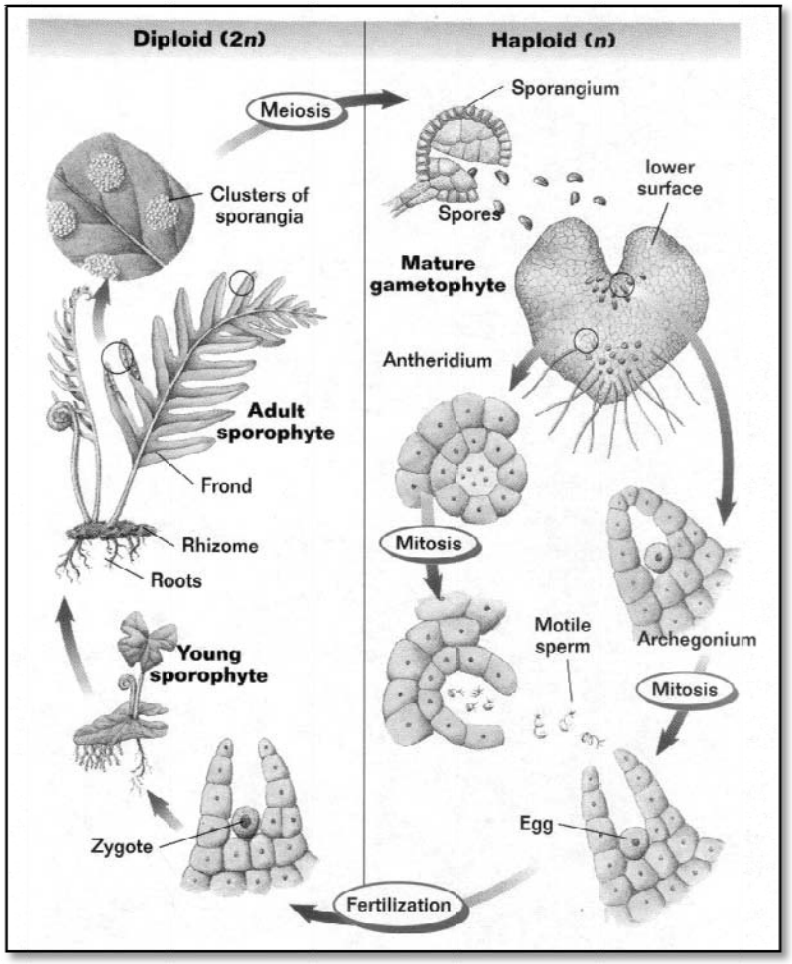


Fig. 1: Alteration of generations with sexual and asexual stages
(Source: https://www.bioexplorer.net/plant-life-cycle.html/)

- Sporophyte vascular herbs, shrubs (*Oleandra*), some with limited secondary thickenings (*Botrychium*, *Isoetes* root), mostly terrestrial, rupestral (lithophytes) or epiphytic, climbers (*Lygodium*), sometime floating, submerged or emergent aquatic; mostly soft or delicate, sometimes harsh or stout; rarely arborescent (*Cyathea*, *Dicksonia*) (Fig. 2).

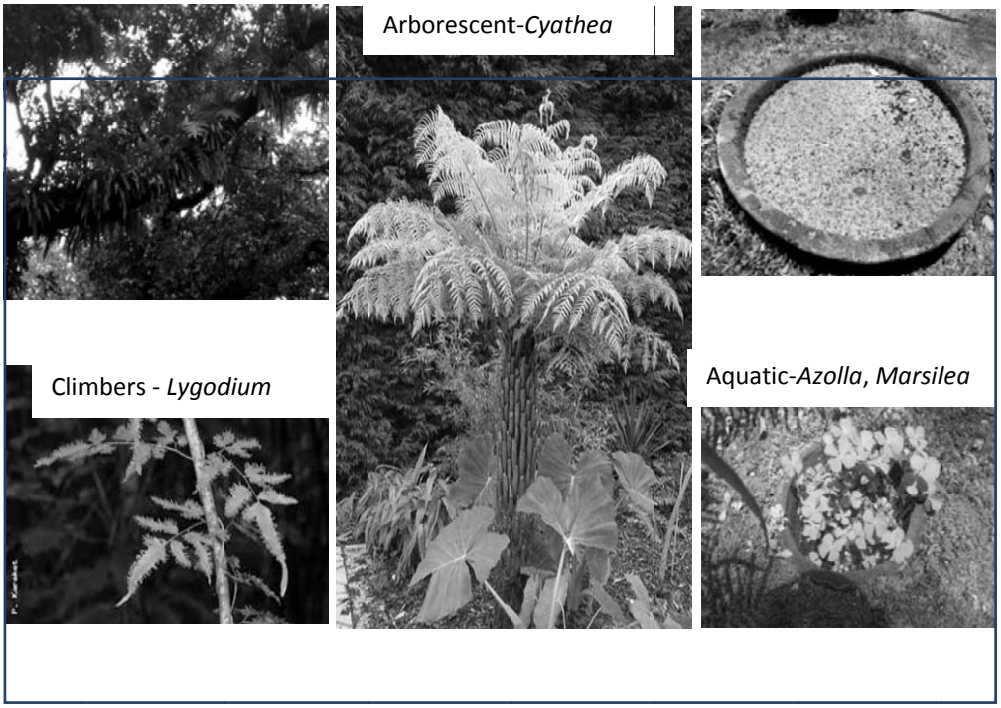


Fig. 2: Diversity of Pteridophytes

- Sporophytic plants with leaves, axes and roots (the latter missing in Psilotaceae) and well developed conducting tissue.
- Stem often rhizomatous, radial or dorsiventral, erect, prostrate, climbing, or subterranean; often with scales and/or hairs; stele simple to complex i.e. protostelic, solenostelic, dictyostelic and polystelic.
- Leaves either small, simple and bract-like or linear with a simple vein, straight in bud, or a broad frond with branched or divided veins (Fig. 3a), simple to several times pinnately divided, conduplicate or mostly circinate in bud (Fig. 3b); bearing sporangia (Fig. 3).

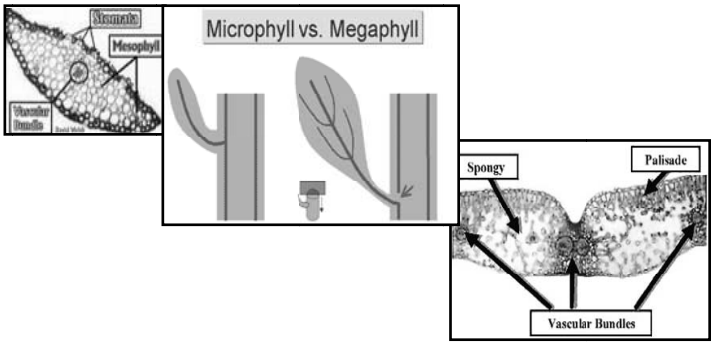




Fig. 3a: Microphyll and megaphyll **Fig.3b:Circinate vernation**

- Leaf stalk or stipe is mostly present; stipules are mostly lacking.
- Sporangia thick or thin walled, homosporous or heterosporous, mostly grouped in sori or fused in synangia, or present as special structures viz., spike or sporocarp or solitary in axils or sporophylls which may be grouped into strobili (Fig. 4); sori naked or often protected by an indusium.
- Spores monolete or trilete; germinating to form an avascular autotrophic or mycotrophic prothallus (gametophyte stage) bearing flagellated male gamates (antherozoids) in antheridia and/or female gamates (egg cells) in flask shaped archegonia
- Gamete transfer and fertilization by water, producing a new plant (sporophyte stage) which obliterates the prothallus.

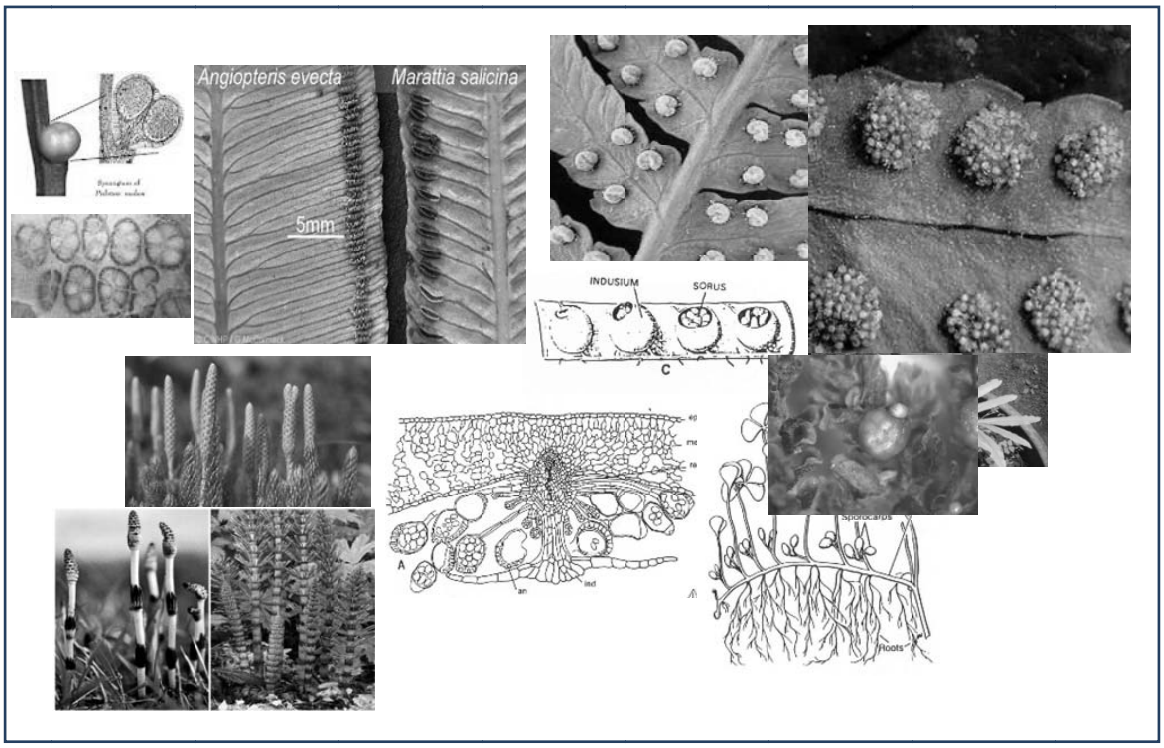


Fig 4: Diversity in reproductive structures

Fern ally

Fern ally is a general term covering a somewhat diverse group of vascular plants that are not flowering plants and not true ferns. Like ferns, these plants disperse by shedding spores to initiate an alternation of generations (*Lycopodium, Selaginella*)

True Ferns

True Ferns are vascular plants differing from lycophytes by having true leaves (megaphylls). They differ from seed plants (gymnosperms and angiosperms) in their mode of reproduction—lacking flowers and seeds.

Classification:

Based on morpho-anatomical characters, numerous classification schemes *(Bower 1928, Eames 1936, Tippo 1942, Holtumm 1949, Smith 1955, Bold 1957, Zimmermann 1959, Mehra 1961, Sporne 1962, Nayar 1970, Pichi Sermolli 1970, Kato 1983, Gifford & Foster 1989 etc.)* have been proposed for pteridophytes

PTERIDOPHYTES [based on Sporne 1962]				
CLASS	**ORDER**	**CLASS**	**SUB CLASS**	**ORDER**
PSILOPSIDA	Rhyniales	PTEROPSIDA	Primofilices	Cladoxylales
	Zosterophyllales			Coenopteridales
	Trimerophytales		Eusporangiatae	Marattiales
PSILOTOPSIDA	Psilotales			Ophioglossales
LYCOPSIDA	Protolepidodendrales		Osmundidae	Osmundales
	Lycopodiales		Leptosporangiatae	Filicales
	Lepidodendrales			Marsileales
	Isoetales			Salviniales
	Selaginellales	PROGYMNOSPERMOPSIDA		Aneurophytales
SPHENOPSIDA	Hyeniales			Protopityales
	Sphenophyllales			Archaeopteridales
	Calamitales			
	Equisetales			

Bower (1928) recognized 12 families, and divided Polypodiaceae into 15 subfamilies. His classification was based on the structure and development of the sorus. He recognized 3 groups – Simplices. Gradatae, Mixtae

Simplices	***Gradatae***	***Mixtae***
Coenopteridaceae	Hymenophyllaceae	Polypodiaceae (divided into seven tribes)
Marattiaceae	Cyatheceae	
Osmundaceae	Protocyatheaceae	
Gleicheniaceae	Plagiogyriaceae	
Schizaeaceae	Dixoniaceae	
Matoniaceae	Loxosoniaceae	

Ching (1940) divided Polypodiaceae into 33 families

Earlier classifications were based primarily on:

- External morphology of the shoot
- Apical meristem and its further segmentation
- Architecture and venation
- Vascular anatomy of stem and leaf
- Dermal appendages
- Position and structure of the sorus
- Indusial protection
- Characters of the sporangium
- Nature and form of sporangia
- Spore output per sporangium
- Mode of spore germination & type of prothallus
- Features of gametophyte
- Position and structure of sex organs
- Cytology
- Palynology

Cytological parameter is based on chromosome base numbers

- Chromosome base numbers have been used for classification purposes
- Commonly the base number is uniform for a genus or family, or it ranges around a given number
- More rarely, the number varies drastically in some genera: as in *Thelypteris*, which has x numbers ranging from 27 to 36

Recent Classification is based on molecular systematic studies in addition to morphological data.

- Smith *et al.* (2006)
- Rothfels *et al.* (2012)
- Christenhusz and Chase (2014)

Classification of Smith *et al.* 2006

This classification was based on both morphological and molecular data that was mainly obtained from

- Six chloroplast markers *(rbcl, atpA, atpB, accl, 16S rDNA, ITS)*
- One nuclear gene *(18S rDNA)*
- Three mitochondrial genes *(atp1, nad2, nad5)*

This classification divides ferns into four Monophyletic classes; 11 monophyletic orders, 37 families of which 32 are monophyletic.

The classes are- Psilotopsida, Equisetopsida, Marattiopsida ,Polypodiopsida

The complete classification scheme proposed by Smith et al. (2006)-Table 1a, b

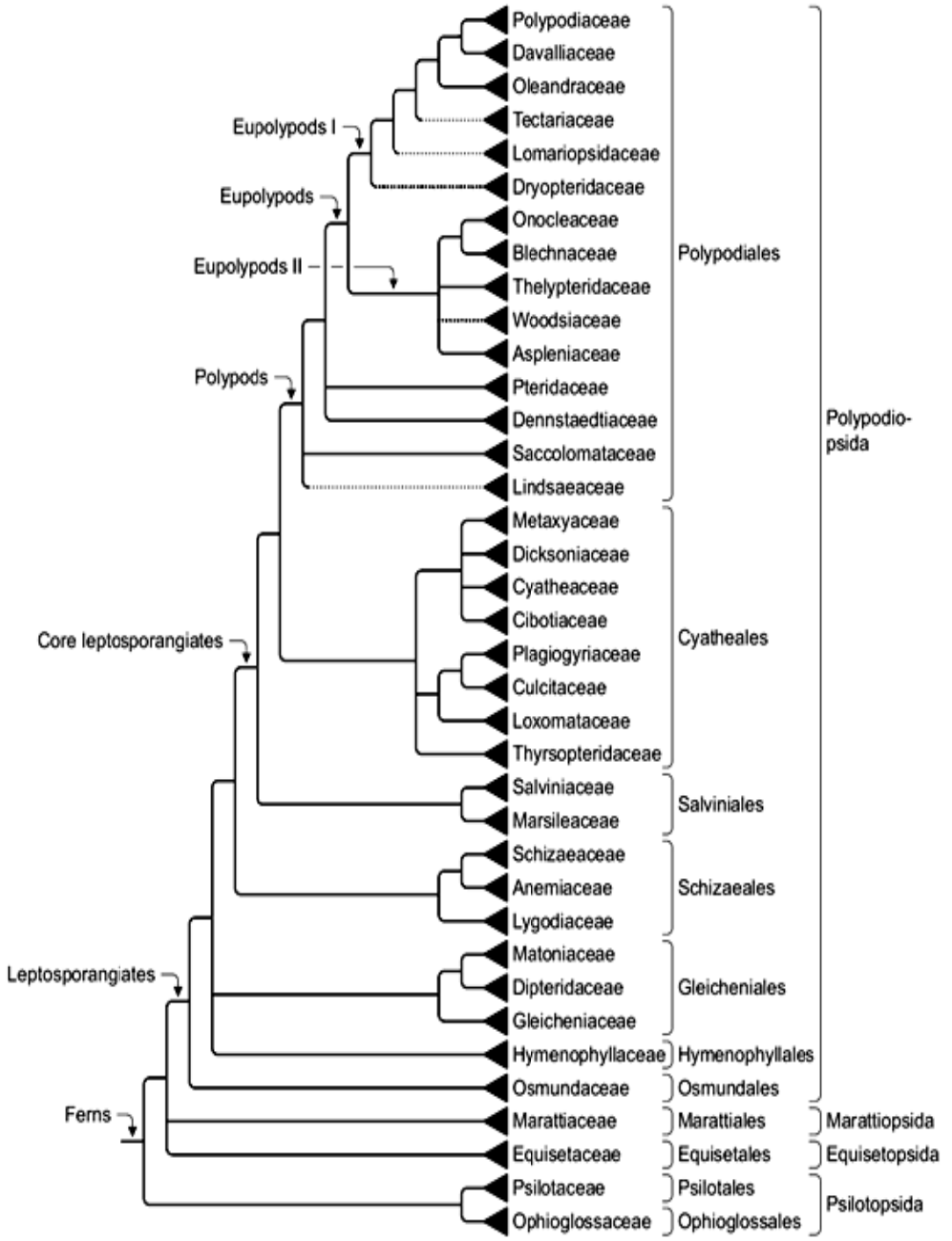


Fig. 2. Consensus phylogeny depicting fern relationships upon which our classification is based. Topology summarizes the results of previously published and ongoing phylogenetic studies (e.g., Hasebe & al., 1995; Pryer & al., 2004b; Schneider & al., 2004b; Korall & al., 2006; Schuettpelz & al., in press; Schuettpelz, unpubl.; see main text for others). Resolved nodes have received bootstrap support ≥ 70 except those drawn with dotted lines. Family, order, and class names that correspond to our classification are indicated to the right. Common names for some larger clades are indicated to the left.

Table 1a: Complete classification scheme proposed by Smith et al. (2006)

CLASS	ORDERS
Polypodiopsida	Polypodiales
	Cyatheales
	Salviniales
	Schizaeales
	Gleicheniales
	Hymenophyllales
	Osmundales
Marattiopsida	Marattiales
Equisetopsida	Equisetales
Psilotopsida	Psilotales
	Ophioglossales

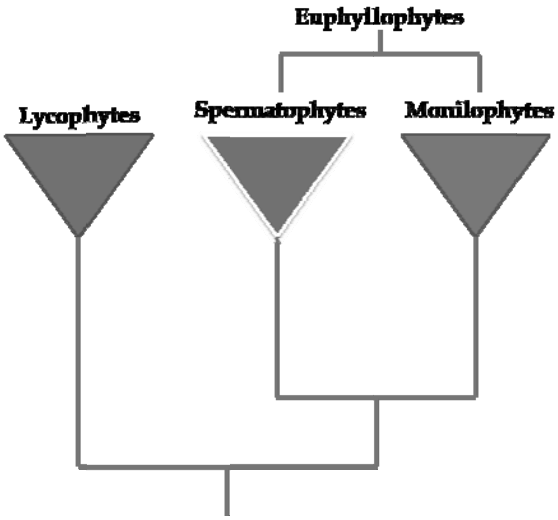


Table 1b: Classification scheme proposed by Smith et al. (2006) showing the orders

Christenhusz and Chase (2014) proposed classification based on molecular analysis

- They have aimed for a stable family and generic classification of ferns, and the current understanding of relationship of ferns and Lycopod clades.
- They are of the view that due to its vague circumscription and now evident non-monophyly, the term fern ally should be avoided.
- They also recommend that the term eusporangiate should be dispensed with because this includes ferns that are not leptosporangiate, and just describes the pleisomorphic state and the taxa included do not form a clade.
- The term ‘moniliophytes’ is unclear and has never been published as a formal taxon and more over its etymology is obscure.
- The leptospoangiate ferns are a natural group forming the majority of extant ferns

A comparison in two systems that are based on molecular data & morphology

	Smith et al. 2006	Christenhusz & Chase 2014
Families	37	38
Sub families	0	16

Terms to be dispensed with: Eusporangiate and fernally

- Although Sphenophytes share a common ancestors with fern there exact placement within fern is uncertain
- Ferns in the past include Equisetaceae & Psilotaceae
- Sphenophytes, Psilotophytes, Marattiophytes could be treated as independent lineages

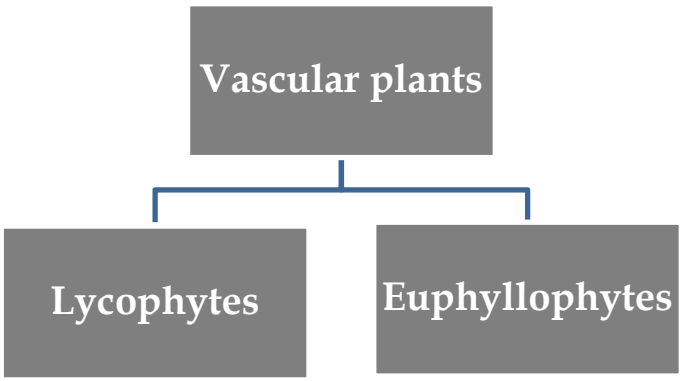
A revised family level classification for Eupolypod II Ferns (a clade of ferns in the order Polypodiales, under the class Polypodiopsida-Polypodiidae: Polypodiales) by Rothfels et al. 2012

- A family level classification for the Eupolypod II clade of leptosporangiate ferns (comprising over 2500 species), has been given on the plea that these were not well understood at the time of publication of the fern classification by Smith et al, 2006
- The composition and particularly the relationship among the major clades of this group have historically been controversial and challenged phylogenetic resolution until very recently
- Their classification reflects the current available data, largely derived from published molecular phylogenetic studies
- They provide circumscription for each family, which summarize their morphological, geographical, and ecological characters, as well as a dichotomous key to the eupolypod II families

By the pteridophytic classification it is assumed that vascular plans have a basal dichotomy separating:

Lycophytes: vascular plants having lycophylls or leaves without leaf gaps (microphylls), vein single medium, meristem intercalary

Euphyllophytes: vascular plants having euphylls or leaves with an associated leaf gap in the vascular stele (megaphylls), veins branched, and with marginal or apical meristem



This dichotomy occurred in the early – mid Devonian ca. 400 million years ago

Monilophytes (ferns) are characterized by-

- Lateral origin of the root in the endodermis
- mesarh protoxylem in shoot
- A pseudo- endospore
- Plasmodial tapetum
- Sperm cell with 30-1000 flagella

Euphyllophytes (or "Eu-phyllo-phyte" – true leaved plant): it includes most living plants except bryophytes and lycophytes
Features: Plant with upright, 3-dimensional branching
Stems: Upright growth of stems with lateral branches (pseudo-monopodial growth)
Branching in 360^0 (spiral branching off a main axis)
Leaves: True leaves in most derived forms ancestral members are leafless
Roots: True roots
Reproductive structures: Ancestral member spore-bearing; derived members are seed bearing
Geologic age: 420 my (Silurian Period) to Present
Lycophytes: include *Selaginella, Lycopodium, Isoetes*

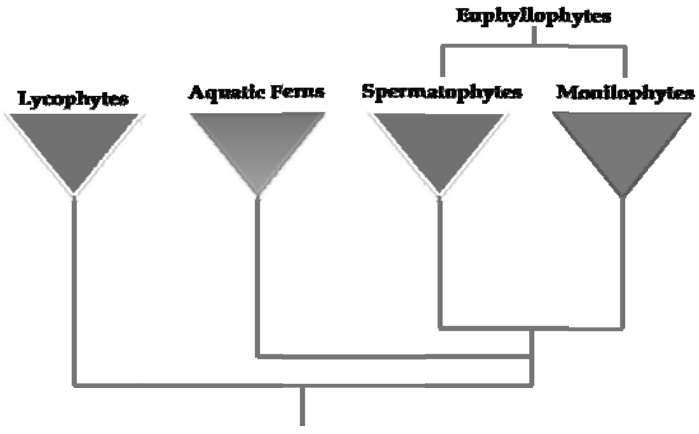
- They are the oldest living vascular plants
- Reproduce through spores
- Have a single vascular vein through their leaves

There are about 1,000 species of Lycophytes worldwide
Evolution form homospory to heterospory

- The evolution of homospory to heterospory – in which a megaspore develops into a female gametophyte that includes one or more egg cells
- and a microspore develops into a male gametophyte that includes sperm – is among the most important transitions in the evolution of plants, with profound effects on plant reproduction and the life cycle.
- However, the nuclear genome of a homosporous vascular plant has yet to be sequenced

Divorce the heterosporous ferns from the homosporous extant ferns:
The aquatic ferns at first sight do not physically resemble other homosporous ferns

- They are heterosporous; remaining extant ferns are homosporous
- Their gametophytes are endosporic and entirely different
- Morphology of the sporophyte is different
- Habit and habitat are different
- Aquatic ferns are quite different and should be treated as separate from the rest of the ferns and perhaps be treated on an equal par as the Psilotaceae, Equisetaceae etc.
- Chromosome number in Marsileaceae is rather low compared to the homosporous ferns



A suggestion given by Khullar 2015

ORDINAL FEATURES
<u>Lycopodiales</u>

- Living and extinct plants
- Sporophyte with primary growth only, no vascular cambium
- Leaves eligulate
- Majority have definite strobili

Selaginellales

- Living & extinct plants with primary growth only, no vascular cambium
- Microphylls with ligule
- Definite strobili found
- Heterosporous
- Gametophytes endosporic
- Sperms biflagellate in living members

Isoetales

- Living and extinct plants
- Sporophyte with cormlike stems
- Sc. Growth present in root
- Ligulate microphylls
- Heterosporous
- Endosporic gametophytes
- Sperms multiflagellate in living members

Ophioglossales

- Species mostly terrestrial (a few epiphytic), temperate and boreal, but a few pantropical.
- Vernation nodding (not circinate)
- Rhizomes and petioles fleshy
- Root hairs lacking
- Aerophores absent
- Fertile leaves each with a single sporophore arising at the base of, or along, the trophophore stalk, or at the base of the trophophore blade (several sporophores per blade in *Cheiroglossa*)
- Sporangia large, with walls two cells thick, lacking an annulus
- Spores globose-tetrahedral, trilete, many (>1000) per sporangium
- Gametophytes subterranean, non-photosynthetic, mycorrhizal; $x = 45$ (46)

Psilotales

- Roots absent
- Stems bearing reduced, un-veined or single veined
- Euphylls
- Sporangia large, with walls two cells thick, lacking an annulus;
- Two or three sporangia fused to form a synangium, seemingly borne on the adaxial side of a forked leaf
- Spores reniform, monolete, many (>1000) per sporangium;
- Gametophytes subterranean (*Psilotum*), non-photosynthetic, mycorrhizal; $x = 52$.

Equisetales

The spermatozoids of *Equisetum* share several important features with other ferns that support their inclusion in this clade (Renzaglia & al., 2000). Kato (1983) adduced additional morphological characters, including root characters, supporting a relationship between horsetails and ferns.

- Stems whorled, lacunate
- Leaves whorled, connate
- Sporangia with helical secondary wall thickenings (Bateman, 1991), borne on peltate sporangiophores that collectively comprise strobili
- Sporangia large, lacking an annulus, many (>1000) per sporangium
- Spores green, with circular aperture and four paddle-like, coiled elaters
- Gametophytes green, surficial; $x = 108$.

Marattiales

- Roots large, fleshy, with polyarch xylem
- Root hairs septate
- Roots, stems, and leaves with mucilage canals
- Rhizomes fleshy, short, upright or creeping, with a polycyclic dictyostele
- Vernation circinate
- Leaves large, fleshy, 1–3-pinnate (rarely simple in *Danaea*, or 3–5-foliate in *Christensenia*) with enlarged, fleshy, starchy stipules at the base and swollen pulvinae along petioles and rachises (and sometimes other axes)
- Petiole and stem xylem polycyclic
- Stems and blades bearing scales pneumathodes (lenticels) scattered all around petioles and/or rachises
- Sporangia free or in round or elongate synangia (fused sporangia), lacking an annulus, enclosing 1000–7000 spores
- Spores usually bilateral or ellipsoid, monolete
- Gametophytes green, surficial; $x = 40$ (39)

Osmundales

- Temperate and tropical
- Stem anatomy distinctive, an ectophloic siphonostele, with a ring of discrete xylem strands, these often conduplicate or twice conduplicate in cross-section
- Stipules at bases of petioles
- Leaves dimorphic or with fertile portions dissimilar to sterile
- Sporangia large, with 128–512 spores, opening by an apical slit, annulus lateral
- Spores green, sub-globose, trilete
- Gametophytes large, green, cordate, surficial; $x = 22$.

Hymenophyllales

- Terrestrial and epiphytic; pantropical and south-temperate, but gametophytes survive in north-temperate regions as far north as Alaska.
- Rhizomes slender, creeping, wiry, or sometimes erect and stouter, protostelic
- Vernation circinate
- Blades one cell thick between veins (a few exceptions)
- Stomata lacking
- Cuticles lacking or highly reduced
- Scales usually lacking on blades, indument sometimes of hairs
- Sori marginal, indusia conical (campanulate), tubular, or clam-shaped (bivalvate), with receptacles, usually elongate, protruding from the involucres
- Sporangia maturing graduately in basipetal fashion, each with an uninterrupted, oblique annulus;
- Spores green, globose, trilete;
- Gametophytes filamentous or ribbon-like, often reproducing by fragmentation or production of gemmae; $x = 11, 12, 18, 28, 32, 33, 34, 36$, and perhaps others.

Gliecheniales

- Root steles with 3–5 protoxylem poles
- Antheridia with 6–12 narrow, twisted or curved cells in walls

Schizaeales

- Fertile-sterile leaf blade differentiation
- Absence of well-defined sori
- Sporangia each with a transverse, subapical, continuous annulus

Salviniales

- Fertile-sterile leaf blade differentiation
- Veins anastomosing
- Aerenchyma tissue often present in roots, shoots, and petioles
- Annulus absent
- Plants heterosporous
- Spores with endosporous germination
- Monomegaspory
- Gametophytes reduced.

Cyatheales

- Some of the species have trunk-like stems but others have creeping rhizomes
- Some have only hairs on the stems and blades, others have scales
- Sori are abaxial or marginal, either indusiate or exindusiate
- Spores are globose or tetrahedral-globose, with a trilete scar
- Gametophytes green, cordate

Polypodiales

- Indusia laterally or centrally attached (indusia lost in many lineages)
- Sporangial stalks 1–3 cells thick, often long
- Sporangial maturation mixed
- Sporangia each with a vertical annulus interrupted by the stalk and stomium
- Gametophytes green, usually cordate (sometimes ribbon shaped in some epiphytes), surficial

Reproduction in Pteridophytes:

(i) Reproduction takes place by means of spores which are produced inside sporangia.

(ii) The development of the sporangium may be leptosporangiate (sporangium originates from a single cell) or eusporangiate (sporangium develops from a group of cells).

(iii) Sporangia may be borne either on stem or leaves. On the stem they may be terminal (e.g., *Rhynia*) or lateral (e.g., *Lycopodium*). On the leaves (sporophylls) they may be ventral, marginal (*Pteris*, *Adiantum*) or dorsal (e.g., Polypodiceae). In *Equisetum* the sporangia are borne on special structures called sporangiophores which constitute a cone. In *Marsilea*, *Azolla*, *Salvinia* sporangia are produced in sporocarps.

(iv) Spores on germination give rise to multicellular gametophytic bodies called prothalli (sing. prothallus).

(v) In homosporous Pteridophytes prothalli are monoecious (antheridia and archegonia develop on the same prothallus). In heterosporous species prothalli are always dioecious. Microspores on germination give rise to male prothalli and megaspores to the female prothalli.

(vi) Antheridia and archegonia are developed on prothalli.

(vii) Antheridium is surrounded by a single layered sterile jacket.

(viii) Archegonium consists of four vertical rows of neck cells, 1-2 neck canal cells, ventral canal cell and egg.

(ix) Antherozoids are unicellular, biflagellate (e.g., *Selaginella*) or multiflagellate (e.g., *Equisetum* and ferns) and motile.

(x) Antherozoids are attracted towards the neck of the archegonium chemotactically by certain substances like malic acid) present in the mucilaginous substance formed by the degeneration of neck canal cells and venter canal cell.

(xi) Water is essential for fertilization (zooidogamous). Therefore, Pteridophytes are also known as amphibians of the plant kingdom.

(xii) Fertilization results in the formation of zygote or oospore, which ultimately develops into well-developed sporophyte.

(xiii) The fertilized egg divides transversely or vertically. Another cross wall forms a quadrant stage producing stem, leaf, foot and root.

(xiv) Plants show heteromorphic alternation of generation. The main plant body is sporophytic and forms a dominant phase in the life cycle.

Economic Importance of Pteridophytes:

The economic importance of pteridophytes is not well-documented, because due attention has not been given towards their use in human welfare. However, there are many reports on their uses, specially as food plants, medicinal plants and horticultural plants.

Some of the aspects of economic importance of pteridophytes are given:

i. Pteridophytes Used as Food:

The young leaf tips of ferns, the circinate ptyxis or the chroziers are used as vegetable. The young fronds of Ampelopteris prolifera are sold in the market as ‘dheki shaak’ in India and Bangladesh. The croziers of Matteuccia struthiopters as canned or frozen are served as spring vegetable in USA and Canada. Leaves of *Marsilea*, commonly called ‘shushni’, are used as vegetable.

The rhizome of many ferns such as *Pteris*, rich in starch, is used as food.

The corm (modified stem) of Isoetes is used as food by pigs, ducks and other animals.

ii. Pteridophytes Used as Fodder:

Dry fronds of many ferns form the livestock for catties. The quadrifid lamina of *Marsilea* resembles a clover (*Trifolium*) has been used as fodder for animals as a substitute for clover.

iii. Pteridophytes Used as Medicine:

The spores of *Lycopodium* have been wide-ly used in pharmacy as protective dusting pow-der for tender skin and also as water-repellants. The foliages of Lycopodium are used as tincture, powder, ointment and cream as a stomachic and diuretic. The foliage decoction is used in home-opathy to treat diarrhoea, bladder irritability, eczema, rheumatism, constipation and inflammation of liver.

Equisetum is rich in silicic acid and silicates. Potassium, aluminium and manganese, along with fifteen types of flavonoid compounds, have been reported from Equisetum. The flavonoids and saponins are assumed to cause the diuretic effect. The silicon is believed to exert connective tissue-strengthening and anti-arthritic action.

Several ferns have been used as herbal medicine. An oil (5% Filmaron and 5-8% Filicic acid) extracted from the rhizome of Aspidium is used as a vermifuge, especially against tape-worm. The decoction of Asplenium is used for cough and a good hair wash. The expectorant of Polypodium is used as a mild laxative, while the tonic is used for dyspepsia, loss of appetite and hepatic problem.

The root decoction of *Osmunda regalis* is used for treatment of jaun-dice. The ointment made from its root is used for application to wound. The extraction of Osmanda vulgaris, commonly known as ‘Green oil charity’, is used as remedy for wounds. The chemically active principal ‘Marsiline’ isolated from Marsilea is found to be very effective against sedative and anti-convulsant principal.

The rhizome and frond bases of *Dryopteris* have been used to determine the origin and pathways of dispersed pathogenic insects like corn ear- worm. The preparation of Ophioglossum vulga-tum as ‘Green oil charity’ is also used as remedy for wounds.

iv. Pteridophytes Used as Horticultural Plants:

Many species of pteridophytes are cultivated for their aesthetic value. Many variants and cultivars of Psilotum have been brought in culti-vation in nurseries and greenhouses in the nick-name of 'whisk fern'.

Some epiphytic species of Lycopodium (e.g., *L. phlegmaria, L. lucidulum*) are aesthetically more valued and can be grown on hanging baskets.

Several species of Selaginella are used as a ground cover in an undisturbed area because of their decent foliage and colour. *Selaginella willdenovii, S. uncinata,* etc., are grown in gar-dens for their decent blue colour. 5. lepidophyl-la, S. bryopteris, etc., are sold as dried under the name 'resurrection plants' which rejuvenate on contact with water.

Several ferns such as *Angiopteris, Asplenium, Marattia, Microsorium, Nephrolepis, Phymatodes,* etc., have aesthetic values for their beautiful habit, graceful shape of the leaves, and beautiful soral arrangement. Thus, these characte-ristics make them horticulturally important plants.

v. Pteridophytes Used as Biofertiliser:

Azolla is a free-floating water fern which can multiply very quickly through vegetative propagation. There are hundreds of moss-like leaves harbouring live colonies of dinitrogen fixer Cyanobacterium — *Anabaena azollae*.

The relationship between the alga and Azolla is sym-biotic where the alga provides nitrogen to the plant. Thus, Azolla in full bloom in the water-logged rice fields may serve as a green manure. Rice farmers of our country are using Azolla as biofertiliser for the better production of their crops.

vi. Pteridophytes Used as Indicator Plants:

Like angiosperms, pteridophytes are being used as indicator plants.

Equisetum accumulates minerals, especially gold, in their stem. The rate of accumulation even reaches up to 4.5 ounce per ton. Equi-setum may be referred to as gold indicator plants which help in searching a region for gold ore deposits. Similarly, Asplenium adulterinum is an indicator of nickel and Actinopteris australis is a cobalt indicator plant. Thus, these plants are found to be valuable in prospecting for new ore deposits.

vii. Pteridophytes Used for Various Purposes:

There are various applications of pterido-phytes:

The stem of *Equisetum* was used for polishing wood in ancient times and to clean utensils.

The roots and stems of *Osmunda* are used to make beds for growing orchids. Water boiled with Lycopodium clavatum is used for dyeing the woollen clothes which becomes blue when dipped in a bath of Brazil wood.

The powder of *Lycopodium* is highly inflammable and is used in pyrotechny and for artificial lighting. Thus, *Lycopodium* powder finds its wide use in demonstration of artificial lighting on the stage, because it disperses easily in the air and only a small quantity is needed to produce an explosion.

Some of the pteridophyte members are con-sidered to be the obnoxious weeds. *Pteridium aquilinum* is a carcinogenic plant which can rapidly invade the open forest lands, thus elimi-nating the other plants of the forest floor. The free-floating water fern, Salvinia, quickly propa-gates vegetatively, and thus occupy the entire water surface of lakes, ponds and irrigation reser-voirs preventing free flow of water.

Gymnosperms:

The term gymnosperms (gymnos = naked; sperma = seed) was introduced by Theophrastus in 300 BC to describe plants with unprotected seeds. According to Goebal gymnosperms are phanerogams without ovary.

The phanerogams or Spermatophyta (sperm = seed; phyton = plant) or seed plants are those plants which reproduce by means of seeds, not spores. Gymnosperms are the vascular plants where seeds are not enclosed within an ovary (opposite to an angiosperm or flowering plants where seeds are enclosed by mature ovaries or fruits).

In these plants the ovules are borne naked or the surface of the megasporophylls, which are often arranged in the cones. Fossil records indicate that the gymnosperms must have evolved approximately 300 million years ago from non-seed producing ancestors of the extinct division of Progymnospermophyta which were fern like in appearance (form a bridge between pteridophytes and angiosperms).

Gymnosperms were dominant plants over the earth's surface during the jurassic and cretaceous periods of mesozoic era. At present about 83 genera and approximately 790 species of living gymnosperms are distributed throughout temperate, tropical and arctic regions of the world.

External Features of Gymnosperms:

1. Gymnosperms are predominantly woody plants, represented by trees, shrubs or rarely climbers.

2. They are usually xerophytic, some of them are deciduous while others are evergreen. *Sequoia sempervirens* (California or Coast red wood) is probably the tallest living tree reaching a height nearly 112 m and attaining a growth of 15 m. Smallest gymnosperm is *Zamia pygmaea*.

It is 25 cm tall. *Taxodium maxicanum* has a trunk with the enormous diameter of 17 meter. The bristlecone pines (three species of pines i.e., *P. aristata*, *P. longaeva* and *P. balfouriana*) are thought to reach an age greater than that of any other single organism known, upto nearly 5000 years.

3. Plant body is sporophytic and can be differentiated into root, stem and leaves.

4. Generally the plants possess well developed tap root system. In some gymnosperms the roots show symbiotic relationship e.g., coralloid roots of *Cycas* with algae and mycorrhizal roots of *Pinus* with fungi.

5. Stem is erect, woody and branched (unbranched in *Cycas* and tuberous in *Zamia*). Presence of leaf scars on the stem is the characteristic feature of gymnosperms.

6. The arrangement of the leaves on the stem may be spiral or cyclic. They may be of one kind (monomorphic) or two kinds (dimorphic, foliage leaves and scale leaves). Foliage leaves are green, simple, may be small (microphyllous e.g., *Pinus*) or large (megaphyllous e.g., *Cycas*). Their main function is photosynthesis. Scale leaves are present around the reproductive structures and apex. They are mainly protective in nature.

Classification of Gymnosperms:

In older times gymnosperms were kept among angiosperms. It was Robert Brown (1827) who first of all recognised these plants due to presence of naked ovules and placed them in a distinct group called gymnosperms. Bentham and Hooker (1862-83) in their 'Genera Planterum' placed this group in between dicotyledonae and monocotyledonae.

The classification of gymnosperms is quite controversial because several genera and a few orders like the cordiatales and cycadeoidales are known only in fossil state. Attempts have, however, been made from time to time to classify them. Some of the important classifications are as follows:

Van Tieghem (1898) treated gymnosperms as one of the two divisions of Spermatophyta and further divided it as follows:

Classification Proposed by Sporne (1974):

Sporne (1974) adopted classification of Pilger and Melchior (1954) and recognized following three classes and nine orders:

1. Cycadopsida: Pteridospermales, Bennettitales, Pentoxylales and Cycadales
2. Coniferopsida: Cordaitales, Coniferales, Taxales and Ginkgoales
3. Gnetopsida: Gnetales

Cycadales:

(i) Cycadales include 11 living genera and more than 100 species of cycads.

(ii) Cycads resembles with the pteridophytes.

(iii) The members are woody sporophytes which appear palm-like.

(iv) All are dioecious,

(v) Young leaves show circinate vernation.

Examples: *Cycas*, *Microcycas*, *Zamia pygmaea* (smallest gymnosperm), *Chigua*, *Stangeria* etc.

Ginkgoales:

(i) Ginkgoales include only one living member i.e. *Ginkgo biloba* (Maiden hair tree) and is the oldest living seed plants persisted with little change till now.

(ii) Leaves are leathery, fan- shaped and deciduous

(iii) Male strobili catkin-like,

(iv) Wood pycnoxylic.

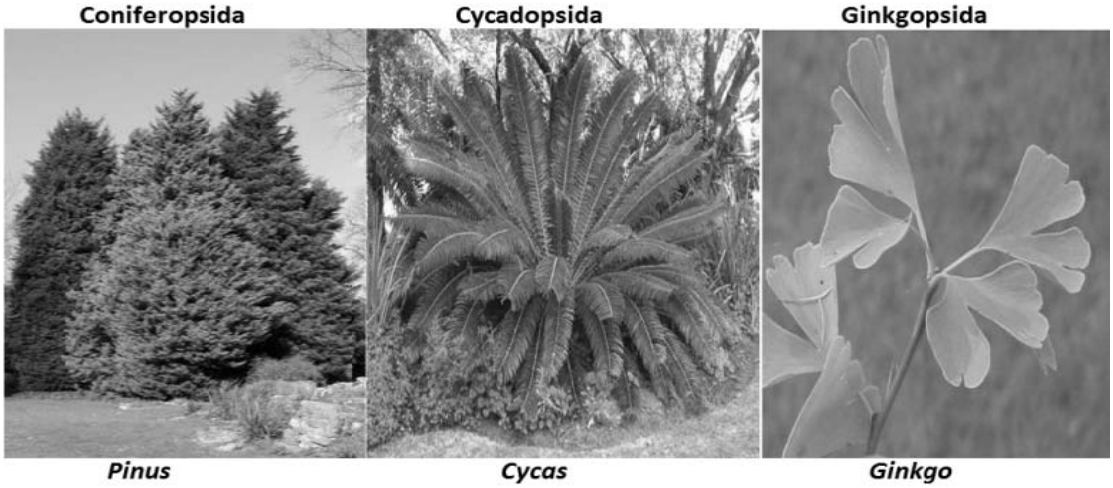
(v) Ovules arise in groups (2-10).

(vi) Endosperm has beak like protuberance called tent pole.

Coniferales:

(i) It is the largest order of living gymnosperm.

(ii) All members are perennial with ex-current (conical) appearance,

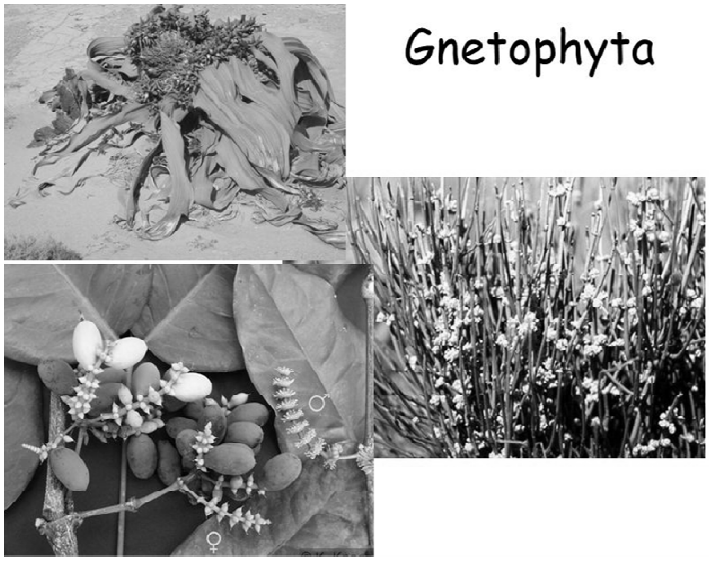


(iii) Leaves dimorphic i.e. foliage and scale leaves present,

(iv) Wood is pycnoxylic.

(v) Pollens and seeds are winged,

(vi) They are dominant forest-makers in the colder region of earth due to – xerophytic nature, evergreen nature, presence of mycorrhizas, scale leaves, resins to plug injury, enzymes activity even at – 35° C.



Examples:

Pinus, Sequoiea (Red wood tree, the largest gymnosperm, 366 ft.), *Taxodium*,*Juniperus, Saxegothea, Araucaria* (commonly called Monkey Puzzle)

Gnetales:

(i) A small but highly evolved group of gymnosperms represented by 3 genera i.e. *Ephedra*, *Gnetum* and *Welwitschia*.

(ii) They are the ancestors of Angiosperms (flowering plants),

(iii) Unlike other members, gnetales have vessels in xylem.

(iv) Flowers arranged in compound strobili or inflorescence,

(v) Embryo with 2 Cotyledons.

Economic Importance of Gymnosperms:

1. Ornamental value:

A number of gymnosperms are grown as ornamental plants, e.g., *Cycas, Araucaria, Thuja* etc.

2. Food Value:

i. 'Sago' starch obtained from pith and cortex of stem of *C. revolute, C. rumphi* etc.

ii. 'Seed starch' obtained from seeds of *Cycas rumphii,* Dioon edule etc. It is prepared into flour and cooked before eating.

iii. Seeds of *Pinus gerardiana* (chilgoza) are edible.

iv. 'Kaffir bread' prepared from the stem pith of Encephalartos.

v. Young leaves of Cycas cooked as vegetables.

3. Medicinal value:

i. Ephedrine (alkaloid) extracted from *Ephedra* used in treating asthma, cough, cold, bronchitis etc.

ii. Tincture of *Ephedra* is a cardiac stimulant.

iii. The juice extracted from young leaves of *Cycas revoluta* is used for curing blood vomiting and flatulence.

4. Industrial Use:

i. Gum-*Cycas* gum used as adhesive, antidote for snake bites and using malignant ulcers.

ii. Tannins – Tannins extracted from bark of *Araucaria, Pinus, Sequoia* etc. used in leather industry.

iii. Canada balsam – It is turpentine obtained from *Abies balsamea* and used as a mounting medium in biological preparations.

iv. Amber (fossil resin) – obtained from *Pinus succinifera*. Wood of Pinus is used for doors, poles, beams, railway wagon flooring etc.

v. Plywood prepared from *Podocarpus*.

vi. Papers like newsprints, writing and printing papers are being prepared from the wood pulp of *Pinus, Picea,Abeis, Gnetum* etc.

vii. The leaves of cycads are used for preparing baskets, mats, hats, brooms etc.

viii. The fibres obtained from the leaves of *Cycas* and Macrozamia are used for stuffing pillows and making mattresses.

5. Source of oils:

i. Oils extracted from seeds of *C. revoluta, Macrozamia reidlei, Pinus cembra* and *Cephalotaxus drupacea* are used as edible oils.

ii. Red cedar wood oil extracted from the heart wood of *Juniperus virginiana* is used for cleaning microscopic preparations and for oil immersion lenses.

iii. Oils obtained from *Cedrus deodara, Ciyptomeria japonica* and *Cupressus serm-perivirens* are used in preparations of perfumes.

Angiosperms – The Flowering Plants.

Angiosperms are those seed plants in which seeds are formed inside fruits and the sporophylls are organised into flowers.

1. Flowering plants or angiosperms are the most recently and highly evolved plants. They appeared on earth about 130 million years ago. First angiosperm fossil recorded from mid cretaceous period is Archaefructus from China and Archaeanthus from Canada.

2. They are the most abundant and conspicuous plants with about 270,000 (220,000 dicots and 50,000 monocots) species.

3. Flowering plants occur in most environments on the earth. Sea is the only exception. Here only two angiosperms are found. They are Zostera and Thalassia. Wolffia is smallest (0.1 cm) while Eucalyptus regnans is largest (114 m height). Angiosperms are most wide-spread in plains as well as hills.

They have been recorded at an altitude of 6000 m in the Himalayas or permanently frozen Antarctica. Even deserts possess flowering plants. Some of these desert plants are able to grow soon after good shower, flower and shed seeds in a few weeks of wetness available in the area. One species is reported to occur in hot springs at 60°C.

A small orchid lives underground. It has mycorrhizal association which helps it obtain nourishment from decaying organic matter. In moist areas some plants grow perched on other plants. They are dependent upon the larger plant for space only (neither food nor water). Such plants are called epiphytes. Epiphytes are popularly known as space hosts, e.g., Vanda.

4. Sporophylls are aggregated to form flowers. Therefore, angiosperms are also called flowering plants.



5. Both microsporophyIIs and megasporophylls are specialised. A microsporophyll or stamen consists of a filament and an anther. A megasporophyll or carpel is rolled and partly sterilized to produce a stigma, style and ovary containing ovules. Within ovules are present highly reduced female gametophytes known as embryo sacs. Embryo sac formation is preceded by meiosis. So all the cells of embryo sac are haploid.

6. Pollination is through several agencies but most prominent amongst them is by ani-mals, especially insects. The flowers possess showy petals, edible pollen or nectar for this.

7. Pollen grains or microspores reach stigmatic surface found at the tip of carpel or megasporophyll.

8. Female gametophyte or embryo sac develops up to 8-nucleate state prior to fertilisation. There is a three cell egg apparatus (one egg cell or oosphere and two synergids), three antipodal cells and two polar nuclei. The two polar nuclei fuse to form a diploid secondary nucleus.



9. Archegonia are absent. Instead, there is one oosphere surrounded by two specialised synergid cells that attract the pollen tube. The latter brings two naked non-flagellate male gametes.
10. There is double fertilisation. One male gamete fuses with oosphere or egg cell to form zygote. It is called generative fertilization or syngamy. The second male gamete fuses with diploid secondary nucleus to form triploid primary endosperm nucleus (PEN). It rep-resents vegetative fertilization. The latter is also called triple fusion since three nuclei are involved in its formation. Total 5 nuclei take part in double fertilization.
11. Zygote develops into embryo. Central cell containing triploid primary endosperm forms triploid endosperm. Endosperm provides nourishment to developing embryo. Syner-gids and antipodals degenerate after fertilization.
12. Fertilized ovules ripen into seeds. The seeds are covered by fruits. A fruit is technically a ripened ovary. Fruits not only protect the seeds but also help in their dispersal.
13. Xylem contains vessels.
14. Phloem possesses sieve tubes and companion cells.
15. Secondary growth occurs in stem and root of some angiosperms, placed in group dicots.
Angiosperms or Flowering Plants:

Alternation of Generations: Life Cycle of Angiosperms:

In the life-cycle of angiosperms, there is alternation of nutritionally independent and more complex sporophyte with the inconspicuous, reduced and parasitic gametophytes. The sporophyte, which may be a herb, shrub or a tree is differentiated into roots, stem and leaves each with a vascular tissue with the highest degree of perfection.

In addition, it bears floral leaves or sporophylls organised into a structure called the flower. The sporophylls are of two kinds microsporophylls and megasporophylls. Each microsporophyll bears four microsporangia. The latter contains microspores which are differentiated by meiosis from the diploid microspore mother cells.

The megasporophyll in a flower part, which is called the ovary, contains one or more ovules. The ovule consists of one or two integuments enclosing the nucellus or megasporangium.

Towards the micropylar end of the megasporangium is differentiated, by meiosis, a single functional megaspore

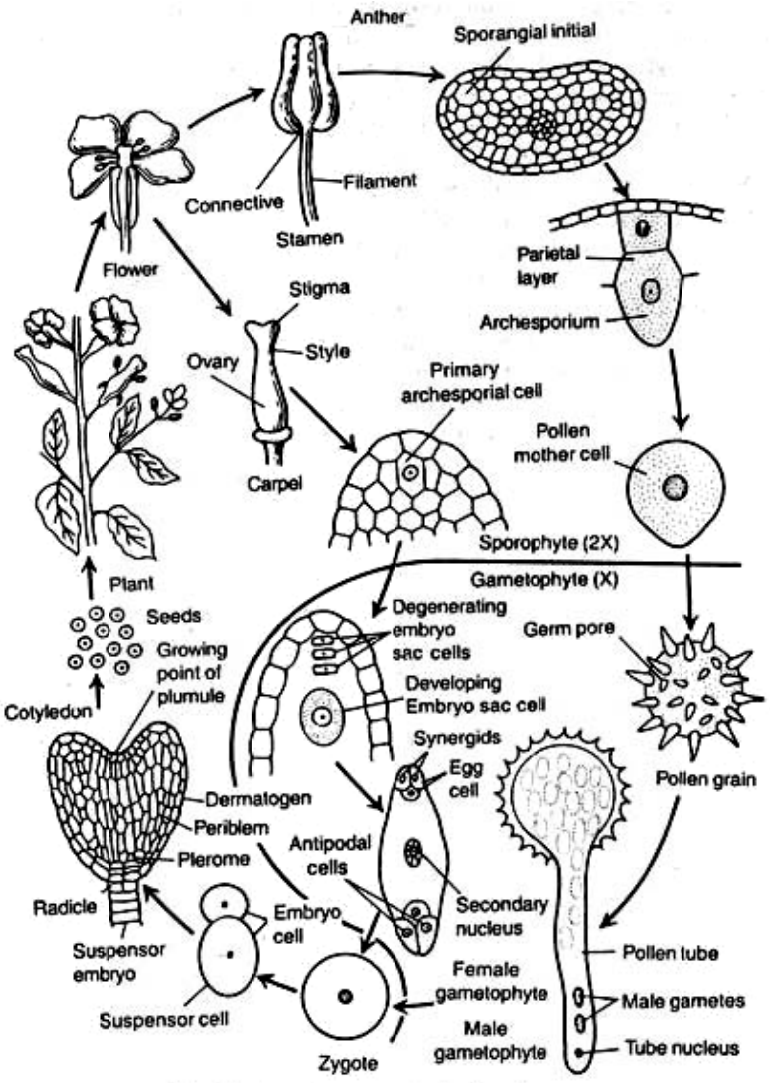


Fig. 6.1. Life-history of a typical angiosperm

which is haploid. The microspores and megaspores are the first structures of the gametophyte generation.

The sporophyte generation in the angiosperms, thus consists of the sporophyte plant, flowers, microsporophylls and megasporophylls, microsporangia, megasporangia, microspore mother cells and the megaspore mother cells.

With meiosis the sporophyte phase switches on the gametophyte phase. The first structures of the gametophyte phase are the microspores and the megaspores. On germination, they produce the alternate structures in the life-cycle which are the male and the female gametophytes and not the sporophyte plant. The male and the female gametophytes are extremely reduced and parasitic.

The female gametophyte consists only of six cells and two nuclei. Three cells at the micropylar end form the egg apparatus. The other three at the chalazal end form the antipodal group. There are two polar nuclei in the centre. The male gametophyte consists of a pollen tube containing the tube nucleus and two male nuclei.

The eggs and the male nuclei represent the gametes which are the last structures of the gametophyte phase. With the fusion of one of the male nuclei with the egg, the gametophyte phase ends. Fertilization thus is the second critical point in the life cycle.

With it the gametophyte generation switches on to the sporophyte generation. The fertilized egg or oospore is the first structure of the next sporophyte. By segmentation it produces the embryo (i.e., the baby plant in the seed); the ovule to the seed and the ovary as a whole to the fruit.

The embryo lies dormant in the seed and the latter lies embedded in the fruit. The seed and the fruit give adequate protection to the embryo, store up food material for it, and are often well adapted for dispersal. Sooner or later as the seed germinates the embryo grows into a seedling which gradually grows into a mature plant.

Classification:

Many systems of classification of angiosperms have been proposed by many taxonomists from time to time.

It can be divided into three broad categories:

i. Artificial Systems based on superficial features.

ii. Natural systems based on form relationships.

iii. Phylogenetic systems based on evolutionary and genetic relationships.

[I] Artificial systems:

These systems of classification were based on one or few morphological characters.

In some systems habit and habitat have been considered for this purpose:

(i) Theophrastus (370 – 285 BC), a Greek philosopher, in his book Historia Plantarum classified about 480 plants into four groups on the basis of their habit-herbs, undershrub’s, shrubs and trees.

(iii) Andrea Caesalpino (1519-1603), an Italian botanist and Physician, in his book De Plant is classified about 1500 plants on the basis of habit (herbs and trees) and then subdivided them on the basis of fruits and seeds which they produced.

(v) John Ray (1627-1705), an English naturalist, in his book Methods Plantarum Nova (1682) for the first time divided herbs, shrubs and trees into dicotyledons and monocotyledons on the basis of two or one cotyledons. Broadly he divided the plants as under:

(vi) Carolus Linnaeus (also called Carl Linnaeus) (1707-1778), a Swedish naturalist in his book Species Plantarum (1753) classified 7300 species of plants into 24 classes, mainly on the basis of number, union and length of stamens. For example, he described the classes as Monandria (1 Stamen), Diandria (with 2 stamens), Triandria (with 3 stamens) and so on Polyandria (with 20 or more stamens). This system is commonly known as sexual system of classification.

[II] Natural Systems:

In these systems the organisms are classified on the basis of their natural affinities (i.e. the basic similarities in the morphology) rather than on a single character for determining the affinities.

(i) A.L. de Jussieu (1748-1836) published a natural system of classification of plants in his book Genera Plantarum secundus ordines Naturales Disposita. He grouped all plants into 15 classes which were further divided into 100 orders (now called families).

He divided the plants into three main groups, i.e. Acotyledones (plants without cotyledons, e.g., algae, fungi, mossesetc.) Monocotyledones (plants with one cotyledon) and Dicotyledones (plants with two Cotyledons). He mainly emphasized on the number of cotyledons and their presence or absence, number of petals and their presence or absence, and position of stamens.

(ii) A.P. de Candolie (1778-1841) a French botanist published Theorie elementaire de la Boanique in which he classified about 58,000 species into 161 families. He divided plants into two major groups i.e. cellulares (non-vascular plants) and vasculares (vascular plants).

(iii) Bentham and Hooker's Classification:

The most important and the last of the natural systems of classification of seed plants was proposed by two British taxonomists George Bentham (1800-1884), a self trained botanist, and Joseph Dalton Hooker (1817-1911), the first director of the Royal Botanical Garden, Kew (England).

They recorded precise description of most of the plants known at that time. Their monumental work which took about quarter of a century for completion was described in three volumes of Genera Plantarum, published in Latin during July 1862 and April 1883. Bentham and Hooker's system of classification is still used and followed in several herbaria of the world. It is supposed to be the best system for the students to identify plants in the laboratory.

Salient Features of Bentham and Hooker's system:

1. It is a classification of only the "seed plants" or phanerogams.

2. They described 97,205 species of seed plants belonging to 7,569 genera of202 families starting from Ranunculaceae up to Gramineae.

3. They classified all the seed plants into 3 groups or classes i.e. Dicotyledons (165 families), gymnosperms (3 families) and monocotyledons (34 families).

4. They included disputed orders among Ordines Anomali which they could not place satisfactorily.

5. Monocotyledons were described after the dicotyledones.

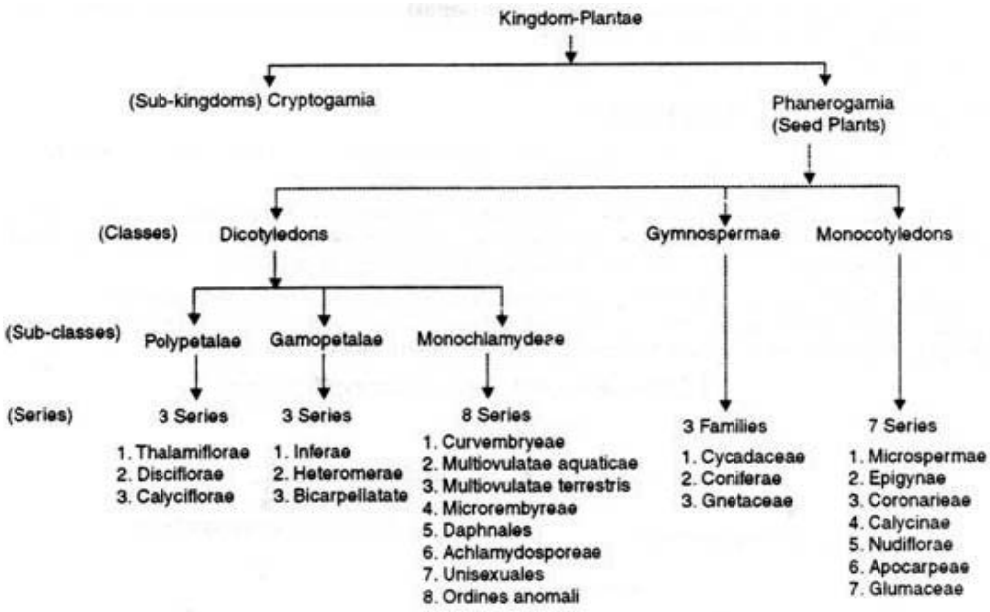
6. The dicotyledons were divided into 3 Divisions (Polypetalae, Gamopetalae and Monochlamydeae) and 14 series. Each series again divided into cohorts (modern orders) and cohorts into orders (modern families).

7. The authors did not mention anything about the origin of the angiosperms.

8. Creation of the Disciflorae, a taxon not described by the earlier taxonomists.

9. Among the Monochlamydeae, major taxa, like the series, were divided on the basis of terrestrial and aquatic habits.

10. Polypetalae carries 82 families, 2610 genera & 31,874 species. Gamopetalae carries 45 families 2619 genera & 34,556 species. Monochlamydae includes 36 families, 801 genera & 11,784 species. Similarly Monocotyledons consist 34 families, 1495 genera and 18,576 species.



Phylogenetic Systems:

Classification based on evolutionary features is known as phylogenetic system.

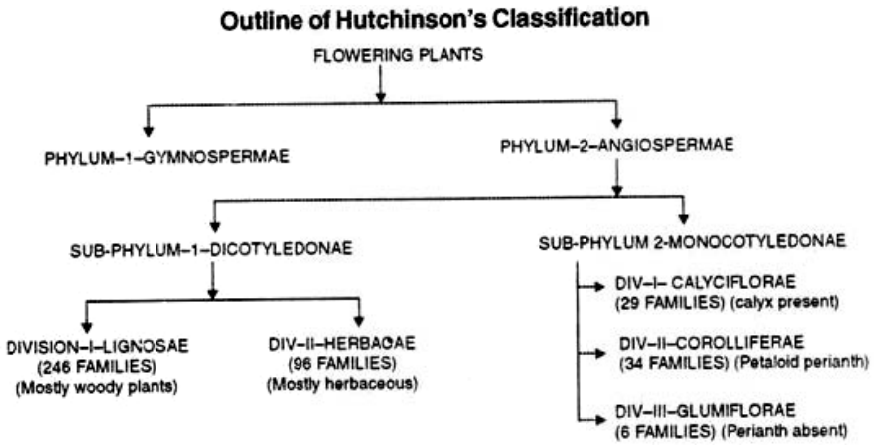
Important phylogenetic systems are:

Engler and Prantl (1884-1930):

They published detailed classification in 23 volumes of "Die Naturlichen Pflanzenfamilien". They arranged flowering plants according to increasing complexity of their floral morphology. They considered monocot' primitive than dicots.

John Hutchinson (1884-1972):

He was author of "Families of flowering plants" (in 2 volumes) and director of Royal Botanic Garden at Kew, England. Classification proposed by him was based on 24 principles. This system is mostly followed. They placed monocots after dictos.



4. Exploitation of Microbes

Microbiology may be described as one of the younger sciences with its history, as a precise subject, only dating as far back as Pasteur in the mid 1800s and his revelation both of the role of microorganisms in nature and their importance to human welfare. Medical scientists rapidly took up

the challenge, with their area of microbiology flourishing and expanding almost in complete isolation from the rest of biology.

We now know, of course, that microorganisms have always played an important, if not essential role, in the biosphere with fermented foods and beverages, plant and animal diseases and nutrient cycling foremost in their sphere of activities. Within the last twenty years, microbiology has received two enormous boosts with the developments in microbial genetics and genetic engineering probably being the most influential, and the greater awareness of pollution and environmental sustainability following a close second.

There are multiple ways to manipulate a microorganism in order to increase maximum product yields. Introduction of mutations into an organism may be accomplished by introducing them to mutagens. Another way to increase production is by gene amplification, this is done by the use of plasmids, and vectors. The plasmids and/ or vectors are used to incorporate multiple copies of a specific gene that would allow more enzymes to be produced that eventually cause more product yield.The manipulation of organisms in order to yield a specific product has many applications to the real world like the production of some antibiotics, vitamins, enzymes, amino acids, solvents, alcohol and daily products. They can also be used in an agricultural application and use them as a biopesticede instead of using dangerous chemicals or as inoculants and help plant proliferation.

Contents:

A) **Medical application**
B) **Food industry application**
C) **Agriculture application**
D) **Chemical application**

A) Medical application:

The medical application to industrial microbiology is the production of new drugs synthesized in a specific organism for medical purposes. Production of antibiotics is necessary for the treatment of many bacterial

infections. Some natural occurring antibiotics and precursors are produced through a process called fermentation. The microorganisms grow in a liquid media where the population size is controlled in order to yield the greatest amount of product. In this environment nutrient, pH, temperature, and oxygen are controlled also in order to maximize the amount of cells and cause them not to die before the production of the antibiotic of interest. Once the antibiotic is produced it must be extracted in order to yield an income.

Vitamins also get produced in massive quantities either by fermentation or biotransformation. Vitamin B2 (riboflavin) for example is produced both ways. Biotransformation is mostly used for the production of

riboflavin, and the carbon source starting material for this reaction is glucose. There are a few strains of microorganisms that were engineered to increase the yield of riboflavin produced. The most common organism used for this reaction is *Ashbya gossypii*. The fermentation process is another common way to produce riboflavin. The most common organism used for production of riboflavin through fermentation is *Eremothecium ashbyii*. Once riboflavin is produced it must be extracted from the broth, this is done by heating the cells for a certain amount of time, and then the cells can be filtered out of solution. Riboflavin is later purified and released as final product.

Enzymes can be produced through fermentation either by submerged fermentation and/ or by solid state fermentation. Submerged fermentation is referred to when the microorganisms are in contact with media. In this process the contact with oxygen is essential. The bioreactors/fermentors that are used to do these mass production of product can store up to 500 cubic meters in volume. Solid state fermentation is less common than submerged fermentation, but has many benefits. There is less need for the environment to be sterile since there is less water, there is a higher stability and concentration

for the end product. Insulin synthesis is done through the fermentation process and the use of recombinant *E.coli* or yeast in order to make human insulin also called Humulin.

B) Food industry application:

Fermentation is a reaction where sugar can be converted into a gas, alcohols or acids. Microorganisms like yeast and bacteria are used to massively produce the many things. Drinking alcohol also known as ethanol is produced by yeast and bacteria. Ethanol can also be used as a fuel source. The drinking alcohol is produced from natural sugars like glucose. Carbon dioxide is produced as a side product in this reaction and can be used to make bread, and can also be used to carbonate beverages. FermentationWine : Alcoholic beverages like beer and wine are fermented by microorganisms when there is no oxygen present.

The production of yogurt starts from the pasteurization of milk, where undesired microbes are reduced or eliminated. Once the milk is pasteurized the milk is ready to be processed to reduce fat and liquid content, so what remains is mostly solid content. This can be done by drying the milk so that the liquid evaporates or by adding concentrated milk. Increasing the solid content of the milk also increases the nutritional value since the nutrients are more concentrated. After this step is accomplished, the milk is ready for fermentation where the milk gets inoculated with bacteria in hygienic stainless steel containers and then gets carefully monitored for lactic acid production, temperature and pH.

C) Agriculture application:

Biopesticide is a pesticide derivatized from a living organism or natural occurring substances. Biochemical pesticides can also be produced from naturally occurring substances that can control pest populations in a non-toxic matter. An example of a biochemical pesticide is garlic and pepper based insecticides, these work by repelling insects from the desired location. Microbial pesticides, usually a virus, bacterium, or fungus are used to control pest populations in a more specific manner. The most commonly used microbe for the production of microbial bio-pesticides is *Bacillus thuringiensis*, also known as Bt. This spore forming bacterium produces a delta-endotoxins in which it causes the insect or pest to stop feeding on the crop or plant because the endotoxin destroys the lining of the digestive system.

Another mechanism that is used to reduce plant pathogens is by introducing other microbes that are non-pathogenic but compete for the rhizosphere, and succeed by producing anti fungal chemicals yielding plant growth. Microbial inoculants are addition of microbes into a plant that would essentially help the plant grow by introducing nutrients, and stimulating plant growth. The preparation in mass quantities of any inoculum is performed by a process called fermentation. The first step to making it is by selecting a microbial strain, and letting it grow and increase in bacterial concentration. The greater the bacterial concentration the greater the fermentation yield would be (fermentation yield is ratio of bacterial concentration to mass of substrate). The bacterial concentration can be measured by monitoring the turbidity, wet or dry weight, or residual nutrient concentration. After the inoculant is ready then it gets transferred in a fermentor where oxygen and temperature most be highly monitored for the survival of the microbes and it can vary depending on the microbe that is being used. After enough time has passed for the microbes to be properly incubated the product is ready to be extracted purified and packaged.

D) Chemical application:

Synthesis of amino acids and organic solvents can also be made using microbes. The synthesis of essential amino acids such as are L-Methionine, L-Lysine, L-Tryptophan and the non-essential amino acid L-Glutamic acid are used today mainly for feed, food, and pharmaceutical industries. The production of these amino acids is due to *Corynebacterium glutamicum* and fermentation. *C.glutamicum* was engineered to be able to produce L-lysine and L-Glutamic acid is large quantities. L-Glutamic acid had a high demand for production because this amino acid is used to produce Monosodium glutamate (MSG) a food flavoring agent. In 2012 the total production of L-Glutamic

acid was 2.2 million tons and is produced using a submerged fermentation technique inoculated with *C.glutamicum.* L-Lysine was originally produced from diaminopimelic acid (DAP) by *E.coli,* but once the *C.glutamicum* was discovered for the production of L-Glutamic acid. This organism and other autotrophs were later modified to yield other amino acids such as lysine, aspartate, methionine, isoleucine and threonine. L-Lysine is used for the feeding of pigs and chicken, as well as to treat nutrient deficiency, increase energy in a patient, and sometimes used to treat viral infections. LTryptophan is also produced through fermentation and by *Corynebacterium* and *E.coli,* though the production is not as large as the rest of the amino acids it is still produced for pharmaceutical purposes since it can be converted and used to produce neurotransmitters.

The production of organic solvents like acetone, butanol, and isopropanol through fermentation was one of the very first things to be produced by using bacteria, since achieving the necessary chirality of the products is easily achieved by using living systems. Solvent fermentation uses a series of *Clostridia* bacterial species. Solvent fermentation at first was not as productive as it is used today. The amount of bacteria required to yield a product was high, and the actual yield of product was low. Later technological advances were discovered that allowed scientist to genetically alter these strains to achieve a higher yield for these solvents. These Clostridial strains were transformed to have extra gene copies of enzymes necessary for solvent production, as well as being more tolerant to higher concentrations of the solvent being produced, since these bacteria have a range of product in which they can survive in before the environment becomes toxic. Yielding more strains that can use other subtrates was also another way to increase the productivity of these bacteria.

E) Microorganisms and detoxification of industrial waste

The problems caused by the increases in the total volumes of wastes generated globally are compounded by the presence of an expanding array of pollutants. As a consequence, all waste streams are being viewed as potential threats to the environment, thus necessitating the development of innovative waste disposal strategies.

The role of microorganisms in maintaining the carbon cycle at balance is evident from the lack of major accumulations of biological products in aerated environments. The absence of any vast build-up of naturally occurring compounds is testimony to the catabolic versatility of microbial

communities. On the other hand, the persistence in nature of some chemicals, synthesized by man, their apparent recalcitrance to biodegradation, and the frequent inability to show biological decomposition in the laboratory argue for the innate stability of many synthetic products.

The principal bacterial genera responsible for oil degradation in both soils and aquatic environments comprise mainly *Pseudomonas, Achromobacter, Arthrobacter, Flavobacterium,Micrococcus,* and *Nocardia.* Prolonged exposure of such heavy metals can have deleterious health effects on human life. Bioremediation of these heavy metals like As, Cd, Cr, Hg, Ni, Hg, and Zn can be done by either plants or microorganisms or by the combination of two. In this chapter emphasis has been given to its microbial methods. There are certain disadvantages associated with physicochemical methods of remediation; thus bioremediation is arising as alternative to these methods. It is an environment friendly approach because it is achieved via natural processes.

5. Mushrooms – their uses and cultivation

Mushrooms are the fruit bodies of edible fungi, commonly belonging to Basidiomycotina (*Agaricus campestris, A. brunnescens, Pleurotus sajor-caju, Volvariella volvacea* etc.) and rarely to Ascomycotina (*Morchella conica, M. esculenta*).

The mushrooms were used as food since long back, probably from 3000 B.C. as per ancient Indian literature. Since that time, the mushrooms are being consumed in different countries like Greece,

Egypt, France etc. The Greeks and Romans described mushrooms as "**food for the god**". During that period, people consumed the mush-rooms after collecting them from their natural habitat.

The cultivation was started in the early part of 18th century in France, but it became a thriving industry only by 1850 in Paris. In India, the first successful experimental cultivation of mushroom (*A. bisporus*) was initiated at Solan (Himachal Pradesh) in 1961. Later on, the cultiva-tion of mushrooms gradually becomes popular with edible mushrooms in different parts of our country.

Assessment of nutritional value of mushrooms has been demonstrated that they complement and supplement the human diet and these are considered as "delight of the diabetic". Mushrooms have rightly been referred to as the "ultimate health food".

But there is no definite test to confirm whether a mushroom is poisonous or edible. Alexopoulos, Mims and Blackwell (1996) expressed the warning in their book "Introductory Mycology" to the Mushroom-consumers that "you can commit many mistakes in your life, but of consuming a poisonous mushroom only once".

Value of Mushrooms:

1. Nutritive Value:

Mushrooms became popular for their food value. The food values of mushrooms are as follows:

i. Mushrooms are the richest source of vegetable protein.

ii. The protein content varies from 1.1-4.98% in common cultivable mushroom (much higher than pulses, vegetables and fruits).

iii. All the essential amino acids including **lysine** (550 mg/gm) are present in much higher amount than even egg.

iv. Mushrooms contain sufficient quantities of mineral elements such as Na, K, Ca, P and Fe.

v. Mushrooms contain folic acid.

vi. Mushrooms contain vitamins like B, C, D and K.

vii. They contain little amount of fat (0.35- 0.65% dry wt.) and starch (0.02% dry wt.).

Protein Content and Energy Value of Mushrooms and Other Vegetables

2. Medicinal Value:

Most of the mushrooms have high medicinal value to reduce blood pressure, obesity (to be fatty), constipation, atherosclerosis (fat deposition inside blood vessel) etc. Medicinal values of some mushrooms are given in Table.

Table 4.19 : Role of some edible fungi in controlling human diseases

	Name of Mushroom	Role in controlling diseases
1.	*Agaricus brunnescens* (syn. *A. bisporus*)	Stimulating digestion, Curing hypertension, Hyperacidity, Obesity, Constipation and Athero-sclerosis (fat deposition inside the blood vessel).
2.	*Auricularia polytricha* (wood ear mushroom)	Strengthening health, helping blood circulation, using for the treatment of stomach ailments (sufferings), Piles, Atherosclerosis.
3.	*Pleurotus ostreatus*	Relaxing muscle, Joint pains, Antitumorous activity.
4.	*Pleurotus sajor-caju*	Reducing the rate of nephron detoriation (renal failure).
5.	*Volvariella volvacea*	Reducing blood pressure.

3. Biological Value:

Biologically mushrooms are very much important. The biological value includes nutri-tive value, medicinal value and their efficiency in degradation of substrate.

Cultivation Procedure of Mushrooms:

It is the technique to develop the fruit bodies of edible fungi. About a dozen fungi are culti-vated in 100 countries with an annual produc-tion of 2.2 million tonnes. The common four genera are *Agaricus, Lentinus, Volvariella* and *Pleurotus*.

Together, they yield a major share of the total production. Out of many, *Agaricus brunnescens* (syn. *A. bisporus*), white button yields 56%; *Lentinus edodes*, shiitake yields 14%; *Volvariella volvacea*, paddy straw yields 8% and *Pleurotus* spp., oyster 7.7%.

The cultivation procedure of some of the mushrooms is given:

A. Cultivation of *Agaricus brunnescens* (Syn. *A. bisporus*):

The Agaricus brunnescens (syn. A. bisporus) is commonly known as white button mushroom (Fig. 4.107, 4.108). It contributes a major share in the mushroom production of the world. It is a temperate mushroom and can grow well in temperate conditions. Optimum temperature, optimum moisture, proper ventilation and good quality of spawn are very essential prerequisites for mushroom growth.

These are:

a. The optimum temperature for the mycelial growth is 24°C, while it is 14-18°C for the formation and develop-ment of fruit body.

b. Optimum moisture requires nearly at the saturation point. However, direct application of excess water in bed is harmful for the growing crop.

c. Proper ventilation is essential to remove toxic gases by the introduction of ade-quate fresh air.

d. Good quality of spawn i.e., the spawn should be prepared from the tissue of single fruit body and its productive capacity should be good enough.

The cultivation procedure is:

1. Production of spawn,
2. Preparation of compost,
3. Filling of trays with compost,
4. Spawning i.e., inoculation of compost,
5. Watering of inoculated compost filled trays,
6. Casing,
7. Harvesting of mushrooms (fruit bodies), and
8. Storage of mushrooms.

1. Production of Spawn:

The spawn (seed of mushroom) is a pure cul-ture of the mycelia grown on a special medium. The medium is prepared by the grains of wheat, rye, sorghum or bajra along with some ingredients.

The preparation of spawn mainly consists of three steps:

a. Preparation of substrate,

b. Inoculation of substrate, and

c. Incubation of inoculated substrate for spawn production.

Preparation of Substrate

Take 900 gms of grains (wheat or sorghum) in 600-900 ml of water in a container and boil for 15-20 minutes, After boiling, decant the excess water and allow the grains to surface drying by spreading on polythene sheet in shade for a few hours.

The grains are then mixed with chemicals like 2% calcium sulphate (gypsum) and 0.5% calcium carbonate (chalk) on

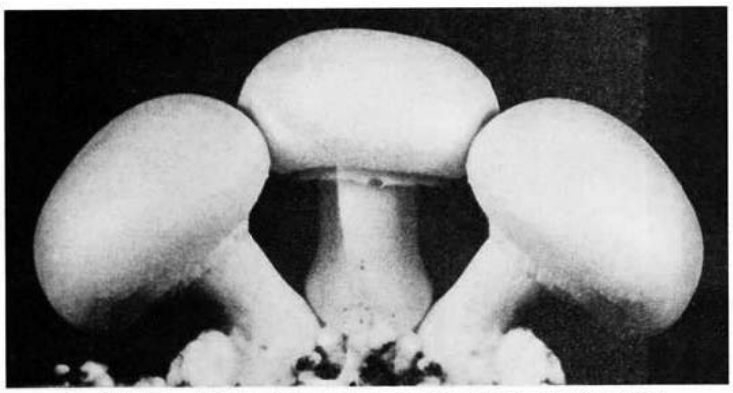


Fig. 4.108 : Mature fruit bodies of *Agaricus brunnescens* (syn. *A. bisporus*) on mushroom bed [Courtesy : Dr Samir Datta]

dry weight basis and adjust the pH of the grain at 7-7.8. About 300-350 gms grains were then filled in milk bottles/ polypropylene bags.

Place a ring of tin (3.5 cm height and 3 cm diametre) towards the inner side of the open-end of polypropylene bag, tighten it with rubber band and then push the margin of the bag towards the inner side and thus a mouth is prepared.

Plug the mouth of the bottle and/or polypropylene bag with non-absorbent cotton. Then cover the mouth with brown paper and tighten it with rubber band. Sterilise the substrate by autoclave at 15lb pressure for 30 minutes for 2 consecutive days. Kept the sterilised substrate in open air to cool down near to room temperature, thus making the substrate ready for inoculation.

Inoculation of Substrate:

The substrate is then inoculated with the mycelial culture (developed earlier, either in Potato Dextrose Agar i.e., PDA or Yeast Potato Dextrose Agar i.e., YPDA or Malt extract Agar and Rice bran decoction medium).

Incubation:

Incubate the inoculated container at 20-25°C in dark for 3 weeks. Shake the contai-ner after a few days, when the mycelial growth becomes visible on the grain.

Storage of Spawn:

Store the spawn at 0-4°C in a refrigerator for a maximum period of 6 months, if it is not nee-ded immediately.

The spawn can be purchased from any spawn-growing centre. (The spawn is also available in "National Centre for Mushroom Research and Training (NCMRT)", Chambaghat, Solan 173 213, Himachal Pradesh, India.

2. Preparation of Compost:

The compost used in the cultivation is of two types:

Natural and Synthetic:

i. Natural Compost:

The natural compost is prepared by mixing barley or wheat straw with fresh and pure horse dung (not with the dung of other animal). Mixed, rain wet or old dung is not sui-table for the preparation of compost. Commonly 100 kg of dung is mixed with 33 kg of straw. The mixture is then stacked a metre high heap.

The heap of mixture should be kept under shade in open air. After 3-4 days, the heap was turned (to release ammonia) and stacked again. The turning process is repeated 4-5 times at an interval of 5-6 days. During this process, gypsum ($CaSO_4.2H_2O$) is added @ 25 kg/tonne (1,000 kg) dung. Finally, 40 ml nemagon is sprayed and added to the mixture. The compost was then filled in the tray of 100 x 50 x 15 cm size.

ii. Synthetic Compost:

The ingredients required for the synthetic compost are:

(a) Chopped wheat straw (3-6 cm size) 300 kg

(b) Wheat bran 30 kg

(c) Calcium ammonium nitrate or Ammonium sulphate 6 kg

(d) Urea 4 kg

(e) Potash 1.5 kg

(f) Calcium sulphate (gypsum) 30 kg

(g) Sawdust 10 kg

Wet the sawdust with water by spraying and mix half of the ingredient, except wheat straw and gypsum. Next day, spread the wheat straw on the cement floor and wet it thoroughly by spraying with water. The sawdust-chemical mix-ture is then mixed thoroughly with wetted wheat straw. This mixture is then stacked under shade into a metre high heap and covered with poly-thene sheet.

After 5 days, the stack is scraped and rest half ingredient is thoroughly mixed with it and the entire mixture is then stacked again. This process is repeated six times. Calcium sulphate is added in the 3rd and 4th turning.

Normally the compost becomes ready to cultivate after 6th turning, but 2 or more turning may be given if the smell of ammonia is yet there in the compost. During last turning, insecticide like malathion (10 ml dissolve in 5 I water) is added to the pre-pared compost. The prepared compost will be brown or dark brown in colour and is sufficient enough to fill 25 trays of 100 x 50 x 15 cm size.

3. Filling of Trays with Compost:

Mix 3 kg of calcium carbonate with the compost prepared earlier. Fill the wooden trays with compost and compress fairly by using a wooden board (1 2 cm x 25 cm), so that a space of about 3 cm deep is left on the top of the tray.

4. Spawning i.e., Inoculation of Compost:

Spread the spawn on the surface of compost and then cover by a thin layer of compost. Little pressure with the fingers is given to make good contact of spawn with compost. Finally the trays are covered with old newspaper. The trays are arranged one after the other in vertical stacks in such a way that sufficient aeration between the trays is maintained.

5. Watering of Inoculated Compost Filled Trays:

Sprinkled water to be given on newspaper to maintain humidity. Water should be applied twice a day or less depending on the availability of moisture. The room temperature should be maintained between 24°C and 25°C for 12-15 days for the good growth of mycelium on the compost. The mycelium appears in the form of white cottony growth on the surface of bed.

6. Casing:

The process of covering the mycelial mat on compost, surface is made with a thin layer of soil mixed with different substances.

The casing can be done with different types of mixture like:

i. Soil : Sand : : 1 : 1;

ii. Well-rotten cow dung: light soil: : 3 : 1 ;

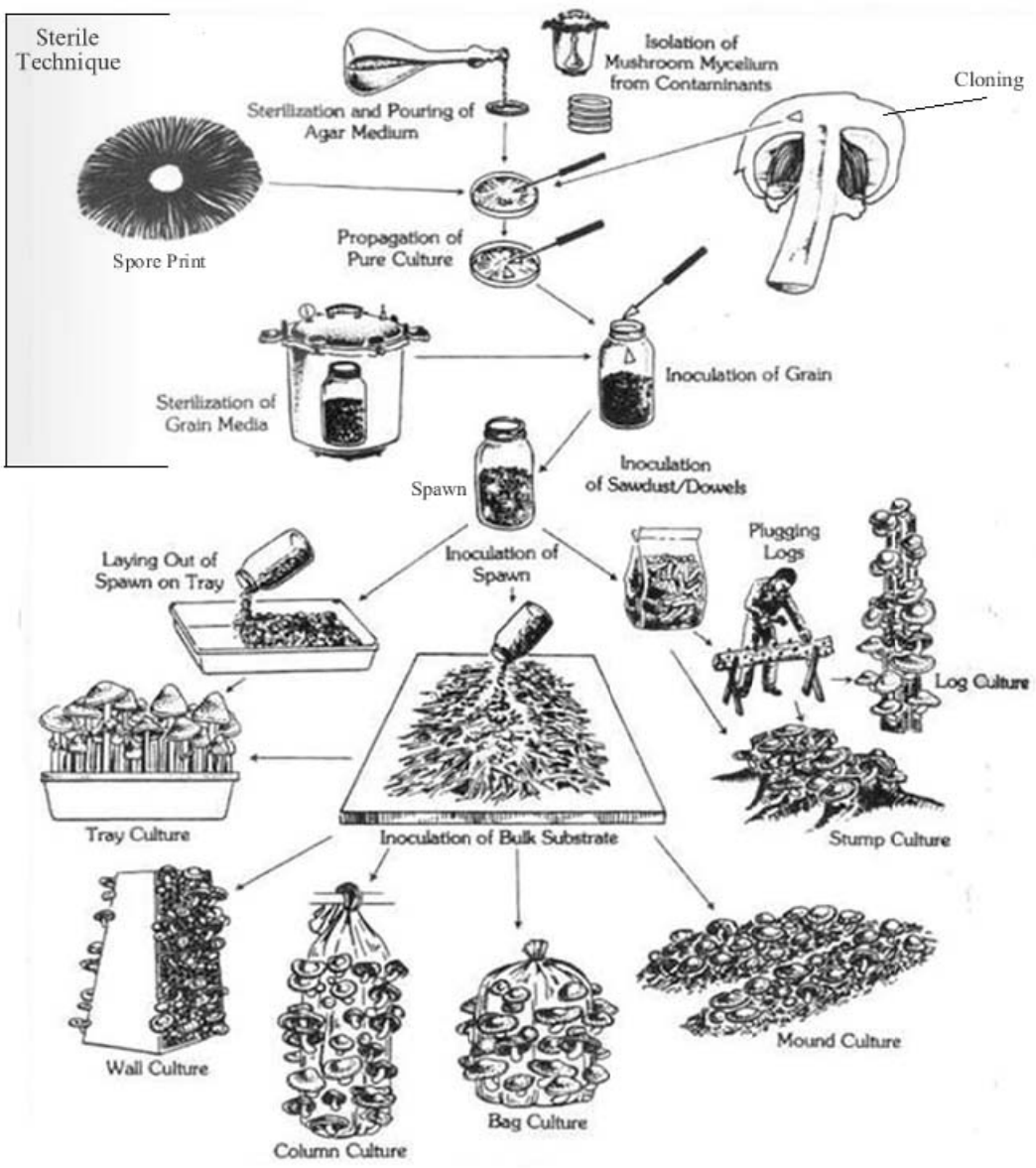
iii. Spent compost: Sand: Slaked lime : : 4:1:1 etc.

Casing soil should be sterilised either by chemicals like methyl bromide, formalin etc. or by heating at 70-75°C temperature for 6 hours to kill the inhabiting fungi, nematodes, insects etc.

The fruit bodies of mushroom are expected to appear after 5-20 days of casing. After casing, the room temperature should be maintained between 14-18°C for the good growth of the fruit body. The fruit bodies attain the size of button stage from pinhead within 7-8 days. Next crop appears at an interval of 8-10 days.

7. Harvesting of Mushrooms i.e., Fruit Bodies:

When the cap of the fruit body is tight with its stalk, the fruit bodies are harvested. The fruit bodies are harvested by twisting and uprooting, after holding the basal region of stalk with fin-gers. The lower part of the stalk is cut out where the compost remains attached.



8. Storage of Mushrooms:

The fruit bodies may be stored at 4°C for a few days, if it is not consumed or marketed immediately.

B. Cultivation of Paddy Straw Mushroom (*Volvariella Volvacea*):

The paddy straw mushroom is also called tropical, straw or Chinese mushroom (Fig. 4.109). In West Bengal, it is called as 'Poal chatu'. The genus Volvariella belongs to the family Pluteaceae under the order Agaricales of Basidiomycotina.

The common edible species under this genus are *V. volvacea, V. diplasia* and *K esculenta*; those are grown commercially in different countries like Burma (Myanmar), China, Philippines, Malaya, India etc.

In addition to paddy straw, other substrates like water hyacinth, cotton waste, banana leaves, sawdust, sugarcane thrash (bagasse) etc., are used as substrate due to the presence cellulose, hemicellulose and lignin.

In India, the cultivation of this mushroom was first initiated in Coimbatore, Tamil Nadu, and now it is popular in different tropical parts due to the requirement of temperature ranges between 30-45°C.

The process of cultivation of straw mush-room is as follows:

1. Requirements,
2. Preparation of spawn,
3. Cultivation procedure,
4. Harvesting of fruit bodies, and
5. Preservation of fruit bodies.

1. Requirements:

i. Spawn of V. volvacea (600-800 gms grain spawn/bed),
ii. Bricks,
iii. Bamboo frame (1 m x 1 m),
iv. Small water tank,
v. Paddy straw (preferably from aman variety), apx. 36 kg,
vi. Loose straw 5-6 kg,
vii. Powder of Gram or Arhar seeds 200-250 gm,
viii. Thermometer (0-100°C scale), and ix. White polythene sheet.

2. Preparation of Spawn:

The spawn can be prepared following the same procedure as adopted in Agaricus brun-nescens (see page 395). But in addition to grains of wheat or sorghum, the rice straw can also be used as substrate.

3. Cultivation Procedure:

Fresh paddy straw, not more than one year old and preferably from the Aman variety, should be collected from farmer or from any store. 24 straw bundles of about 1.5 kg each along with some loose straw are immersed completely in a water-filled tank by putting some weight on the bundles for about 12-15 hours.

Then take out the straw bundles from the tank and keep them in stack on cement floor to drain off excess water.

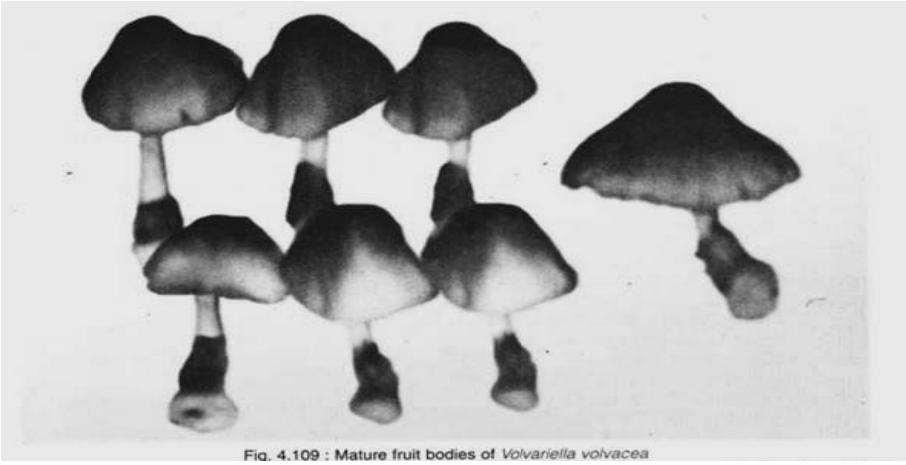


Fig. 4.109 : Mature fruit bodies of *Volvariella volvacea*

4. Preparation of Bed and Spawning:

One square bed of 1 m x 1 m x 1 m or 1 m x 0.75 m x 1 m is prepared with pre-soaked straw, keeping the butt ends (basal region) at one side, placed close to each other and arranged length-wise on a bamboo frame, supported on 4 pillars made of bricks. Same number of soaked straw bundles are placed on the previous one by keeping the butt ends in opposite direction.

Inoculate the bed with spawn. The bids of spawn are placed about 8-10 cm inside the margin, maintaining a space of about 5 cm from each other. About 160-200 grams spawn is required for each layer. Powders of Cram or Arhar seeds of about 50 gms or more are spread along the line of spawning.

Second and third layers are arranged and inoculated in a similar process, but 2nd layer is placed at right angle to the 1st layer and the third layer is like the 1st layer. The spawn and seed powder on the 2nd layer will be given like the 1st layer, but on the 3rd layer those will be dis-tributed uniformly throughout the bed.

Finally, cover the top layer with loose straw. Loosely bind the bed with rope made of wheat straw at the three regions, one in the middle and one on each side. Press the bed with the help of woo-den board to release the internal air and thus the spawn get compressed with the wet straw bun-dles. Cover the bed with polythene sheet.

Watering should be done once or twice with the help of micro-sprayer. The temperature of the bed should remain 30-35°C after spawning and it should not go below 30°C during the growing season. The relative humidity should be between 80-90%.

Polythene sheet should be removed after 7-10 days of spawning for the appearance of button of the mushroom. After that the buttons quickly develop into fruit bodies.

The straw once used in the mushroom culti-vation can be used again. The bed should be pre-pared under shade away from direct sunlight and rain and also in well-aerated condition, but wind should not blow very fast.

5. Harvesting of Mushroom:

The fruit bodies are harvested by gentle twisting when the volva is about to rupture or is just ruptured. The production continues for 25- 30 days, but in two phases. The total production per bed is approximately 3 kg. The production of second phase is comparatively less.

6. Preservation:

The fruit bodies are consumed fresh or can be preserved by drying or in refrigerator for 27- 48 hours. Drying can be done either in the sun or in oven at 50-60°C temperature.

C. Cultivation of Oyster Mushroom (*Pleurotus*):

Species of *Pleurotus* are commonly called Oyster mushroom or Dhingri or Wood fungus (Fig. 4.110). It is the fourth important mushroom in the world ranking with an annual production of about 15,000 tones. It grows commercially in Japan, Taiwan, Italy, France, Thailand, Philippines and India, out of which the first three are the leading countries in its production.

The genus *Pleurotus* contains more than 50 species, of which *P. flabellatus, P. ostreatus, P. sajor-caju, P. sapidus, P. fossulatus, P. cornu- copieae, P. sapathulatus* and *P. florida* have been cultivated in India.

The process of cultivation of Oyster mushroom is as follows:

1. Requirements,
2. Preparation of spawn,
3. Cultivation procedure,
4. Harvesting of fruit bodies,
5. Preservation of fruit bodies.

Requirements:

i. Spawn of *Pleurotus* – 60 gms (30 gms grain spawn/kg of paddy straw),

ii. Chopped and dry paddy straw (1-2 cm) -2 kg,

iii. Gunny or polythene bag (40″ x 24″) – 1 piece.

iv. Horse gram powder – 50 gms (25 gm.s/kg),

v. Thick polythene sheet (5 ft x 5 ft) – 1 piece,

vi. Polythene bags (30″ x 18″) – 2 piece, and

vii. Water sprayer. Preparation of spawn

The spawn can be prepared as per method adopted in Agaricus brunnescens (see page 395). The spawn should not be older than 1 month.

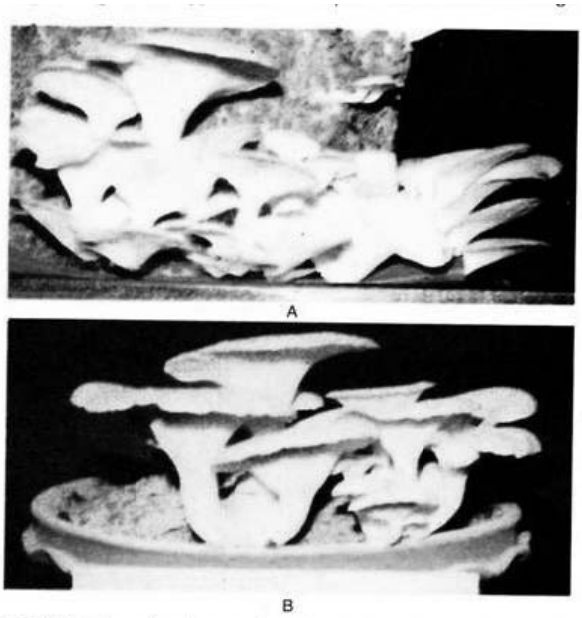


Fig. 4.110 : Fruit bodies of *Pleurotus sajor-caju* on mushroom bed : A. Mushroom bed prepared in polythene bag and B. Mushroom bed prepared on earthern pot

Cultivation Procedure:

Take two kg of chopped straw (preferably of Aman variety) in a gunny or incised polythene bag and tighten the mouth with rope. Immerse the bag completely in water (90 I water contai-ning 7 gm Bavistin, a fungicide along with 125 ml formaldehyde) filled tank by putting some weight on it for approximately 12-15 hours.

Then take out the straw bags from the tank and keep the straw pieces in a wicker basket or a scuttle (Beng. Jhuri). Put more water in the wet straw to remove dirt, rags etc. Wait for one or more hours to drain off excess water.

The wet straw pieces are then kept on polythene sheet and mixed with powder of Horse gram (20-25 gms/kg) and spawn (30 gms/kg) and if possible 10 gms ferti-liser of IFCO or P.P.L (10:26: 26) may be added.

Take 2 polythene bags (appx. 30″ x 18″) and make 6-12 holes at the lower side of each bag. Then the entire mixture is put equally inside the two bags. Keep the filled bags on a bench or table in a room at 21-30°C and 65-80% humidi-ty, with sufficient light and ventilation for 15-16 days, for spawn running.

Spray water on bed twice a day by micro-sprayer. After 15-16 days, the straw pieces are covered with the mycelium and form a solid cylindrical mass. Remove the polythene bag and keep the mass on the same polythene bag in the same place. The compact mass should be watered 4-8 times throughout the day with the micro-sprayer.

The young fruit bodies will be developed after 3-4 days (i.e., 18-20 days of spawning) from all sides of the bed.
Within 2-3 days, the fruit bodies attain the size of harvesting. After harvesting the straw- mycelium mass again put inside the bag and tighten the-mouth with rope. Keep it for 7 days and then again remove the mass from polythene bag and keep the polythene bag as before.
Next crop of mushroom will be available within 7 days i.e., approximately 36 days after starting. Repeat the process again and the third crop will be available in 50 days. During cropping period light should be provided for 15-20 minutes/day for better yield.

Harvesting of Mushroom:
The fruit bodies are harvested by gentle twisting after holding the base of the fruit bodies with fingers. The fruit bodies can be harvested generally 3 times i.e., at 22, 36 and 50 days and the total production will be 2 kg. Afterwards the bed should be destroyed.
Preservation:
After harvesting, the fresh mushrooms can be sold in the market or they can be dried in sun (for three consecutive days) or in oven at 65°C. The cultivation of Pleurotus can also be done on earthen tray or tub.

Some Poisonous Mushrooms:
1. *Amanita phalloides* (death cap): Toxic principles are α and β-amanitin, and phalloidin.
2. *A. virosa* (destroying angles),
3. *A. verna* (fool's cap),
4. *A. muscaria* (fly-agaric) etc.

Diseases of Mushrooms:
Like higher plants, mushrooms also suffer from different diseases caused by fungi, bacteria and viruses.

6. Environmental clean up by plants

Our planet is becoming increasingly polluted with inorganic and organic compounds, primarily as a result of human activities. While inorganic pollutants occur as natural elements in the Earth's crust and atmosphere, human activities such as industry, mining, motorized traffic, agriculture, logging, and military actions promote their release and concentration in the environment, leading to toxicity. Organic pollutants in the environment are mostly man-made and xenobiotic (i.e. not normally produced or expected to be present in organisms).
Many of them are toxic and/or carcinogenic. Sources of organic pollutants in the environment include accidental releases (eg fuels, solvents), industrial activities (eg chemical, petrochemical), agriculture (eg pesticides, herbicides), and military activities (eg explosives, chemical weapons), among others.
Most remediation activity still makes use of conventional methods such as excavation and reburial, capping, and soil washing and burning. However, newly emerging biological cleanup methods, such as phytoremediation, are often simpler in design and cheaper to implement.

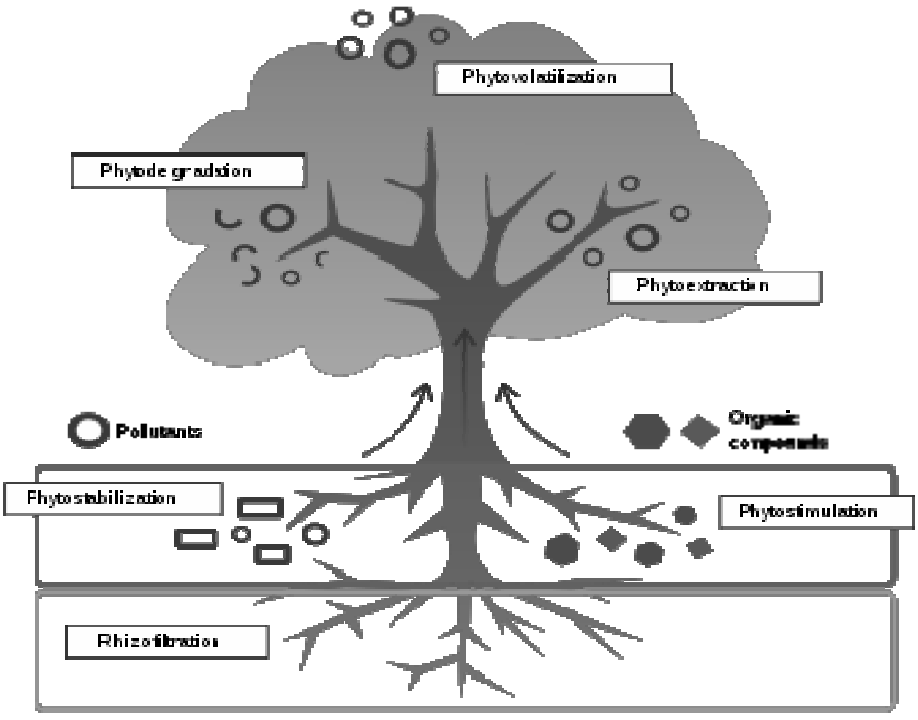
Phytoremediation incorporates a range of technologies that use plants to remove, reduce, degrade, or immobilize environmental pollutants from soil and water, thus restoring contaminated sites to a relatively clean, non-toxic environment. Phytoremediation depends on naturally occurring processes, in which plants detoxify inorganic and organic pollutants, via degradation, sequestration, or transformation. The different uses of plants and their associated microbes for environmental cleanup are summarized below.

Phytoextraction is the removal of pollutants by the roots of plants, followed by translocation to aboveground plant tissues, which are subsequently harvested. Continuous phytoextraction uses plants that accumulate high levels of pollutants over their entire lifetime. Induced phytoextraction enhances pollutant accumulation towards the end of the plant's lifetime, when they attain their maxi-mal biomass, by adding chelators to the soil that reversibly bind the pollutant (usually a metal), releasing it from the soil and making it available for plant uptake. Phytoextraction is especially useful when dealing with toxic pollutants that cannot be biodegraded, such as metals, metalloids, and radionuclides. One category of plants that shows potential for phytoextraction, either as a gene source or for direct use, are the so-called hyperaccumulators, plants that accumulate toxic elements to levels that are at least 100-fold higher than non-accumulator species. Hyperaccumulator plants tend to grow relatively slowly, which limits their usefulness for phytoremediation. Nevertheless, their growth rate may be improved through selective breeding, and the transfer of metal hyperaccumulation genes to high-biomass, fast growing species may also help to circumvent the problem.

Phytodegradation involves the partial or complete degradation of contaminants by internal or secreted plant enzymes. As in phytodegradation, *phytostimulation* also involves enzymatic breakdown, but through microbial activity. Plants can stimulate microbial biodegradation in several ways. Since most organic pollutants can be broken down enzymatically, phytodegradation and phytostimulation are particularly effective for this class of pollutants.

Phytovolatilization depends on the uptake of contaminants by plant roots, followed by their release as volatile chemicals by either the root or the shoot. While some pollutants are volatile to begin with, and are simply dispersed unaffected into the atmosphere, others, such as inorganic selenium, can be transformed enzymatically into volatile forms during the process of plant uptake and release.

Rhizofiltration uses plant roots to filter contaminants directly out of waste streams, in either a hydroponic or a constructed wetland setting. Rhizofiltration is also suitable for inorganics, as the plant material can be replaced periodically. Erosion and leaching often mobilize soil contaminants, resulting in additional aerial or waterborne pollution. *Phytostabilization* through accumulation by plant roots or precipitation in the soil by root exudates immobilizes and reduces the availability of soil pollutants. As an added benefit, plants growing on polluted sites also stabilize the soil and serve as groundcover, thereby reducing both wind and water erosion.



Advantages and limitations of phytoremediation:

A big advantage of phytoremediation over more traditional remediation methods is that in most cases it is less expensive. Depending on the pollutant, substrate, and alternative remediation methods available, phytoremediation is typically 2–10-fold cheaper than conventional remediation methods. It is also less invasive and more aesthetically pleasing compared to excavation and removal, chemical stabilization, or soil washing or incineration. The ability to use plants in various capacities for different synergistic processes, resulting not only in environmental cleanup but also in ecosystem restoration, is the biggest advantage. Tailoring the phytoremediation technique to the specifics of a polluted environment will become more feasible as more information becomes available; for example, in the future it may be possible to select a combination of plant species with different remediation capabilities to clean up sites containing a mix of contaminants. Preferentially, native plant species will be used in order to promote ecosystem restoration during the cleanup process.

A limitation of phytoremediation is that the plant roots have to be able to reach the pollutant and act on it. Thus, the soil characteristics, toxicity level, and climate have to be amenable to plant growth, while the pollutant must not only be within physical reach of the roots, it must be bioavailable for absorption as well. Phytoremediation also usually takes longer than conventional methods. Flowthrough filtration systems and plant degradation of pollutants generally work fairly fast (from days for filtration up to several years for degradation), but soil cleanup via plant accumulation may takes years to decades. Some of these limitations may be circumvented by deep planting and by amending the soil with substances that either make it more amenable to plant growth (eg lime, compost), or that make the pollutant more bioavailable (eg chelators, surfactants).

Mechanical remediation technologies and phytoremediation can be used in conjunction. Pollutant distribution and concentration are heterogeneous at many sites, so the most efficient remediation solution may be a combination of different approaches, eg excavation of the most contaminated spots, followed by phytoremediation.

Developing transgenic plants for phytoremediation:

Transgenic plants are genetically modified organisms. In genetic engineering, plants are induced to take up a piece of DNA containing one or a few genes originating from either the same plant species or from any different species, including bacteria or animals. The foreign piece of DNA is usually integrated into the nuclear genome, but can also be engineered into the genome of the chloroplast.

Foreign DNA may cause an existing enzymatic activity to become up-regulated (over expression) or down-regulated (knockout/knockdown), or may introduce an entirely new enzymatic activity altogether. The expression of the introduced gene can be regulated by using different promoters. The gene product, a protein, may be present at all times, in all tissues (constitutive expression), or only in certain tissues (eg only in roots) or at certain times (eg only in the presence of light or a chemical inducer).

In addition to the gene of interest, a marker gene is usually included in the gene construct so that transgenics can be selected for after the transformation event. Usually these marker genes confer herbicide or antibiotic resistance. The introduced genes integrate into the host DNA and are inherited by the offspring like any other gene.

In the context of phytoremediation, it is desirable to engineer high-biomass producing, fast-growing plants with an enhanced capacity to tolerate pollutants. In addition, if a pollutant is remediated via accumulation, as is often the case for inorganics, transgenics may be engineered to possess improved pollutant uptake and root–shoot translocation abilities. If the pollutant is remediated by degradation, as organics often are, enzymes that facilitate degradation in either the plant tissue or the rhizosphere (the region just outside of the root) may be over expressed. In cases where pollutants are volatilized, enzymes involved in the volatilization process may be over expressed.

If a transgenic approach is to be used to breed plants with superior phytoremediation properties, it is necessary to understand the underlying mechanisms involved. Generally, plant species that are easy to transform and have a short generation time, such as *Arabidopsis*, tobacco (*Nicotiana tabacum*), or Indian mustard (*Brassica juncea*), are used for testing transgenic approaches in initial lab and greenhouse experiments. *B juncea* is also a popular species for phytoremediation, as it is an efficient accumulator of inorganics, grows quickly, and attains a high biomass. Hybrid poplar (*Populus* sp.), a perennial, fast-growing, and environmentally tolerant tree with a high transpiration rate which aids in the translocation of pollutants to the shoot, is another favored species for phytoremediation.

Another tree species that is being tested in remediation studies is yellow poplar (*Liriodendron* sp). Both *Populus* and *Liriodendron* can be genetically engineered. Genetic engineering programs for phytoremediation are also underway for the development of transgenic wetland species, such as *Spartina* spp, reeds, and *Typha* spp.

A variety of genes have been used in genetic engineering for phytoremediation. For instance, bacterial enzymes involved in degradation of the explosive TNT were expressed in plants, resulting in enhanced plant TNT tolerance and degradation (French *et al*. 1999). Furthermore, expression of a mammalian cytochrome P450 in plants resulted in enhanced ability to metabolize the organic solvent trichloroethylene (Doty *et al*. 2000).

Plant volatilization has also been enhanced through genetic engineering. Overexpression of two key enzymes – cystathionine-gamma-synthase and selenocysteine methyltransferase – was shown to promote the con-version of selenocysteine to volatile selenium. Volatilization of mercury by plants was achieved through the introduction of a bacterial mercury reductase (MerA); the resulting plants volatilized elemental mercury and were significantly more mercury-tolerant.

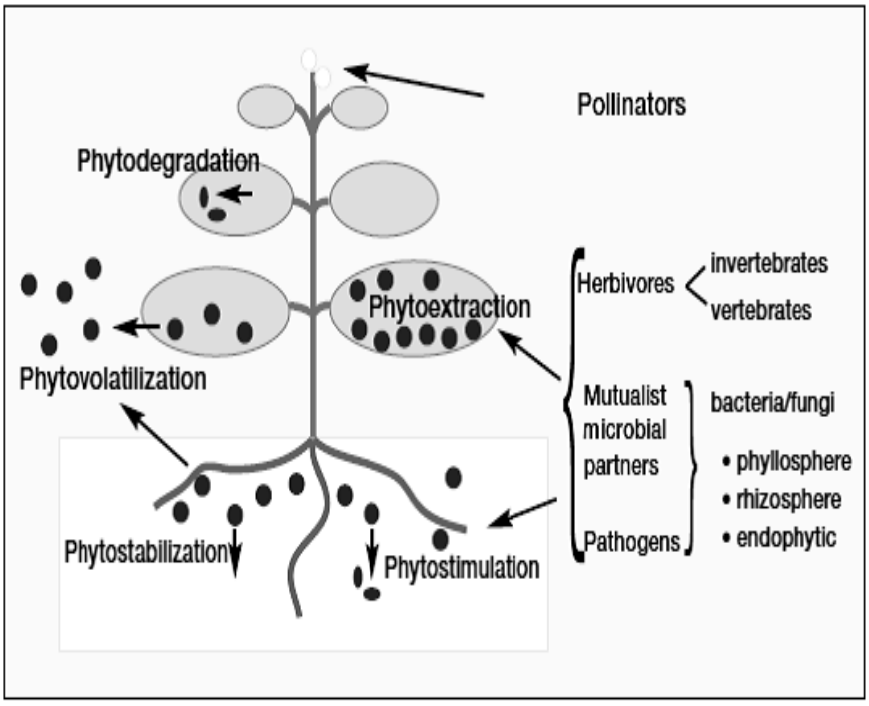


Figure 2. Schematic overview of phytoremediation methods. Shown on the right are some ecological partners of the plants that may influence phytoremediation efficiency and may, in turn, be affected by the phytoremediation process.

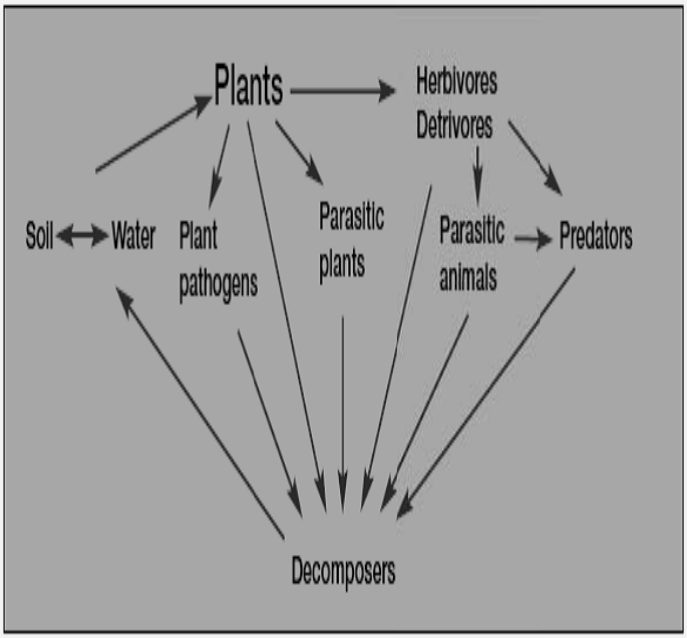


Figure 3. Schematic overview of possible movement of pollutants through the ecosystem. The paths taken and the relative fluxes for each path will depend on the pollutant, the plant species involved, and local conditions.

7. Stress in plants

Stress

The concept of plant stress is often used imprecisely, and stress terminology can be confusing, so it is useful to start our discussion with some definitions. **Stress** is usually defined as an external factor that exerts a disadvantageous influence on the plant. This chapter will concern itself with environmental or abiotic factors that produce stress in plants, although biotic factors such as weeds, pathogens, and insect predation can also produce stress. In most cases, stress is measured in relation to plant survival, crop yield, growth (biomass accumulation), or the primary assimilation processes (CO_2 and mineral uptake), which are related to overall growth. The concept of stress is intimately associated with that of **stress tolerance**, which is the plant's fitness to cope with an unfavorable environment. In the literature the term *stress resistance* is often used interchangeably with *stress tolerance*, although the latter term is preferred. Note that an environment that is stressful for one plant may not be stressful for another. For example, pea (*Pisum sativum*) and soybean (*Glycine max*) grow best at about 20°C and 30°C, respectively. As temperature increases, the pea shows signs of heat stress much sooner than the soybean. Thus the soybean has greater heat stress tolerance.

Water stress

The water-limited productivity of plants depends on the total amount of water available and on the water-use efficiency of the plant. A plant that is capable of acquiring more water or that has higher water-use efficiency will resist drought better. Some plants possess adaptations, such as the C_4 and CAM modes of photosynthesis that allow them to exploit more arid environments. In addition, plants possess acclimation mechanisms that are activated in response to water stress. **Water deficit** can be defined as any water content of a tissue or cell that is below the highest water content exhibited at the

most hydrated state. When water deficit develops slowly enough to allow changes in developmental processes, water stress has several effects on growth, one of which is a limitation in leaf expansion. Leaf area is important because photosynthesis is usually proportional to it. However, rapid leaf expansion can adversely affect water availability.

Response and adaptation to water stress

1. Decreased Leaf Area Is an Early Adaptive Response to Water Deficit

Typically, as the water content of the plant decreases, its cells shrink and the cell walls relax. This decrease in cell volume results in lower turgor pressure and the subsequent concentration of solutes in the cells. The plasma membrane becomes thicker and more compressed because it covers a smaller area than before. Because turgor reduction is the earliest significant biophysical effect of water stress, turgor-dependent activities such as leaf expansion and root elongation are the most sensitive to water deficits.

2. Water Deficit Stimulates Leaf Abscission

The total leaf area of a plant (number of leaves × surface area of each leaf) does not remain constant after all the leaves have matured. If plants become water stressed after a substantial leaf area has developed, leaves will senesce and eventually fall off. Such a leaf area adjustment is an important long-term change that improves the plant's fitness in a water-limited environment. Indeed, many drought-deciduous, desert plants drop all their leaves during a drought and sprout new ones after a rain. This cycle can occur two or more times in a single season. Abscission during water stress results largely from enhanced synthesis of and responsiveness to the endogenous plant hormone ethylene.

3. Water Deficit Enhances Root Extension into Deeper, Moist Soil

Mild water deficits also affect the development of the root system. Root-to-shoot biomass ratio appears to be governed by a functional balance between water uptake by the root and photosynthesis by the shoot. Simply stated, *a shoot will grow until it is so large that water uptake by the roots becomes limiting to further growth*; conversely, *roots will grow until their demand for photosynthate from the shoot equals the supply*. This functional balance is shifted if the water supply decreases. All these factors lead to a preferential root growth into the soil zones that remain moist. As water deficits progress, the upper layers of the soil usually dry first. Thus, plants commonly show a mainly shallow root system when all soil layers are wetted, and a loss of shallow roots and proliferation of deep roots as water in top layers of the soil is depleted. Deeper root growth into wet soil can be considered a second line of defense against drought. Enhanced root growth into moist soil zones during stress requires allocation of assimilates to the growing root tips. During water deficit, assimilates are directed to the fruits and away from the roots. For this reason the enhanced water uptake resulting from root growth is less pronounced in reproductive plants than in vegetative plants. Competition for assimilates between roots and fruits is one explanation for the fact that plants are generally more sensitive to water stress during reproduction.

4. Stomata Close during Water Deficit in Response to Abscisic Acid

The preceding sections focused on changes in plant development during slow, long-term dehydration. When the onset of stress is more rapid or the plant has reached its full leaf area before initiation of stress, other responses protect the plant against immediate desiccation. Under these conditions, stomata closure reduces evaporation from the existing leaf area. Thus, stomatal closure can be considered a third line of defense against drought. Uptake and loss of water in guard cells changes their turgor and modulates stomatal opening and closing. Because guard cells are located in the leaf epidermis, they can lose turgor as a result of a direct loss of water by evaporation to the atmosphere. The decrease in turgor causes stomatal closure by **hydropassive closure**. This closing mechanism is likely to operate in air of low humidity, when direct water loss from the guard cells is too rapid to be

balanced by water movement into the guard cells from adjacent epidermal cells. A second mechanism, called **hydroactive closure**, closes the stomata when the whole leaf or the roots are dehydrated and depends on metabolic processes in the guard cells. A reduction in the solute content of the guard cells results in water loss and decreased turgor, causing the stomata to close; thus the hydraulic mechanism of hydroactive closure is a reversal of the mechanism of stomatal opening. However, the control of hydroactive closure differs in subtle but important ways from stomatal opening. Abscisic acid is synthesized continuously at a low rate in mesophyll cells and tends to accumulate in the chloroplasts. Besides ABA (Sauter et al. 2001), other si gnals, such as pH and inorganic ion redistribution, appear to play a role in long-distance signaling between the roots and the shoots.

5. Water Deficit Limits Photosynthesis within the Chloroplast

The photosynthetic rate of the leaf (expressed per unit leaf area) is seldom as responsive to mild water stress as leaf expansion is because photosynthesis is much less sensitive to turgor than is leaf expansion. However, mild water stress does usually affect both leaf photosynthesis and stomatal conductance. As stomata close during early stages of water stress, water-use efficiency may increase (i.e., more CO_2 may be taken up per unit of water transpired) because stomatal closure inhibits transpiration more than it decreases intercellular CO_2 concentrations. As stress becomes severe, however, the dehydration of mesophyll cells inhibits photosynthesis, mesophyll metabolism is impaired, and water-use efficiency usually decreases. Results from many studies have shown that the relative effect of water stress on stomatal conductance is significantly larger than that on photosynthesis. The response of photosynthesis and stomatal conductance to water stress can be partitioned by exposure of stressed leaves to air containing high concentrations of CO_2. Any effect of the stress on stomatal conductance is eliminated by the high CO_2 supply, and differences between photosynthetic rates of stressed and unstressed plants can be directly attributed to damage from the water stress to photosynthesis.

6. Water Deficit Increases Resistance to Liquid-Phase Water Flow

When a soil dries, its resistance to the flow of water increases very sharply, particularly near the *permanent wilting point*. At the permanent wilting point (usually about –1.5 MPa), plants cannot regain turgor pressure even if all transpiration stops. Because of the very large soil resistance to water flow, water delivery to the roots at the permanent wilting point is too slow to allow the overnight rehydration of plants that have wilted during the day. Rehydration is further hindered by the resistance within the plant, which has been found to be larger than the resistance within the soil over a wide range of water deficits (Blizzard and Boyer 1980). Several factors may contribute to the increased plant resistance to water flow during drying.

As plant cells lose water, they shrink. When roots shrink, the root surface can move away from the soil particles that hold the water, and the delicate root hairs may be damaged. In addition, as root extension slows during soil drying, the outer layer of the root cortex (the hypodermis) often becomes more extensively covered with suberin, a water-impermeable lipid, increasing the resistance to water flow.

Heat stress

Most tissues of higher plants are unable to survive extended exposure to temperatures above 45°C. Non-growing cells or dehydrated tissues (e.g., seeds and pollen) can survive much higher temperatures than hydrated, vegetative, growing cells. Actively growing tissues rarely survive temperatures above 45°C, but dry seeds can endure 120°C, and pollen grains of some species can endure 70°C. In general, only single-celled eukaryotes can complete their life cycle at temperatures above 50°C, and only prokaryotes can divide and grow above 60°C. Periodic brief exposure to sublethal heat stresses often induces tolerance to otherwise lethal temperatures, a phenomenon

referred to as **induced thermo tolerance**. The mechanisms mediating induced thermo tolerance will be discussed later in the chapter. As mentioned earlier, water and temperature stress are interrelated; shoots of most C_3 and C_4 plants with access to abundant water supply are maintained below 45°C by evaporative cooling; if water becomes limiting, evaporative cooling decreases and tissue temperatures increase. Emerging seedlings in moist soil may constitute an exception to this general rule. These seedlings may be exposed to greater heat stress than those in drier soils because wet, bare soil is typically darker and absorbs more solar radiation than drier soil.

Response and adaptation to heat stress

1. High Temperature Reduces Membrane Stability

The stability of various cellular membranes is important during high-temperature stress, just as it is during chilling and freezing. Excessive fluidity of membrane lipids at high temperatures is correlated with loss of physiological function. In oleander (*Nerium oleander*), acclimation to high temperatures is associated with a greater degree of saturation of fatty acids in membrane lipids, which makes the membranes less fluid (Raison et al. 1982). At high temperatures there is a decrease in the strength of hydrogen bonds and electrostatic interactions between polar groups of proteins within the aqueous phase of the membrane. High temperatures thus modify membrane composition and structure and can cause leakage of ions. Membrane disruption also causes the inhibition of processes such as photosynthesis and respiration that depend on the activity of membrane-associated electron carriers and enzymes. Photosynthesis is especially sensitive to high temperature. In their study of *Atriplex* and *Tidestromia*, O. Björkman and colleagues (1980) found that electron transport in photosystem II was more sensitive to high temperature in the cold-adapted *A. sabulosa* than in the heat-adapted *T. oblongifolia*. In these plants the enzymes ribulose-1,5-bisphosphate carboxylase, NADP: glyceraldehyde-3-phosphate dehydrogenase, and phosphoenolpyruvatecarboxylase were less stable at high temperatures in *A. sabulosa* than in *T. oblongifolia*. However, the temperatures at which these enzymes began to denature and lose activity were distinctly higher than the temperatures at which photosynthesis began to decline. These results suggest that early stages of heat injury to photosynthesis are more directly related to changes in membrane properties and to uncoupling of the energy transfer mechanisms in chloroplasts than to a general denaturation of proteins.

2. Several Adaptations Protect Leaves against Excessive Heating

In environments with intense solar radiation and high temperatures, plants avoid excessive heating of their leaves by decreasing their absorption of solar radiation. This adaptation is important in warm, sunny environments in which a transpiring leaf is near its upper limit of temperature tolerance. In these conditions, any further warming arising from decreased evaporation of water or increased energy absorption can damage the leaf. Both drought resistance and heat resistance depend on the same adaptations: reflective leaf hairs and leaf waxes; leaf rolling and vertical leaf orientation; and growth of small, highly dissected leaves to minimize the boundary layer thickness and thus maximize convective and conductive heat loss. Some desert shrubs—for example, white brittlebush (*Encelia farinosa*, family Compositae)—have dimorphic leaves to avoid excessive heating: Green, nearly hairless leaves found in the winter are replaced by white, pubescent leaves in the summer.

3. At Higher Temperatures, Plants Produce Heat Shock Proteins

In response to sudden, 5 to 10°C rises in temperature, plants produce a unique set of proteins referred to as **heat shock proteins** (**HSPs**). Most HSPs function to help cells withstand heat stress by acting as molecular chaperones. Heat stress causes many cell proteins that function as enzymes or structural components to become unfolded or misfolded, thereby leading to loss of proper enzyme structure and activity. Such misfolded proteins often aggregate and precipitate, creating serious problems within the cell. HSPs act as molecular chaperones and serve to attain a proper folding of misfolded, aggregated

proteins and to prevent misfolding of proteins. This facilitates proper cell functioning at elevated, stressful temperatures. Heat shock proteins were discovered in the fruit fly (*Drosophila melanogaster*) and have since been identified in other animals, and in humans, as well as in plants, fungi, and microorganisms. For example, when soybean seedlings are suddenly shifted from 25 to 40°C (just below the lethal temperature), synthesis of the set of mRNAs and proteins commonly found in the cell is suppressed, while transcription and translation of a set of 30 to 50 other proteins (HSPs) is enhanced. New mRNA transcripts for HSPs can be detected 3 to 5 minutes after heat shock (Sachs and Ho 1986). Although plant HSPs were first identified in response to sudden changes in temperature (25 to 40°C) that rarely occur in nature, HSPs are also induced by more gradual rises in temperature that are representative of the natural environment, and they occur in plants under field conditions. Some HSPs are found in normal, unstressed cells, and some essential cellular proteins are homologous to HSPs but do not increase in response to thermal stress (Vierling 1991). Plants and most other organisms make HSPs of different sizes in response to temperature increases. The molecular masses of the HSPs range from 15 to 104 kDa (kilodaltons), and they can be grouped into five classes based on size. Different HSPs are localized to the nucleus, mitochondria, chloroplasts, endoplasmic reticulum, and cytosol. Members of the HSP60, HSP70, HSP90, and HSP100 groups act as molecular chaperones, involving ATP-dependent stabilization and folding of proteins, and the assembly of oligomeric proteins. Some HSPs assist in polypeptide transport across membranes into cellular compartments. HSP90s are associated with hormone receptors in animal cells and may be required for their activation, but there is no comparable information for plants.

Low-molecular-weight (15–30 kDa) HSPs are more abundant in higher plants than in other organisms. Whereas plants contain five to six classes of low-molecular- weight HSPs, other eukaryotes show only one class (Buchanan et al. 2000). The different classes of 15–30 kDa molecular-weight HSPs (smHSPs) in plants are distributed in the cytosol, chloroplasts, ER and mitochondria. The function of these small HSPs is not understood. Cells that have been induced to synthesize HSPs show improved thermal tolerance and can tolerate exposure to

temperatures that are otherwise lethal. Some of the HSPs are not unique to high-temperature stress. They are also induced by widely different environmental stresses or conditions, including water deficit, ABA treatment, wounding, low temperature, and salinity. Thus, cells previously exposed to one stress may gain cross-protection against another stress. Such is the case with tomato fruits, in which heat shock (48 hours at 38°C) has been observed to promote HSP accumulation and to protect cells for 21 days from chilling at 2°C.

4. Adaptation to Heat Stress Is Mediated by Cytosolic Calcium

Heat stress causes a reduction in cytosolic pH from the normal slightly alkaline value, probably by inhibiting proton-pumping ATPases and pyrophosphatases that pump protons across the plasma membrane or into the vacuole. Additionally, heat stress effects a change in calcium homeostasis inside the cell by affecting the influx of calcium into the cytosol through either plasma membrane or vacuolar calcium channels, or by action on efflux ATPases or proton cotransporters. This increase in cytosolic calcium leads to the activation of calmodulin (CaM), which binds to glutamate decarboxylase (GAD) converting it from the inactive to the active form. Glutamate conversion to γ− aminobutyric acid (GABA) is then accomplished consuming protons in the process and mediating an increase in cytosolic pH. CAX1 and CAX2 are transport proteins, ACA: Ca_2^+ ATPase.

Cold stress Chilling temperatures are too low for normal growth but not low enough for ice to form. Typically, tropical and subtropical species are susceptible to chilling injury. Among crops, maize, *Phaseolus* bean, rice, tomato, cucumber, sweet potato, and cotton are chilling sensitive. *Passiflora*, *Coleus*, and *Gloxinia* are examples of susceptible ornamentals. When plants growing at relatively

warm temperatures (25 to 35°C) are cooled to 10 to 15°C, **chilling injury** occurs: Growth is slowed, discoloration or lesions appear on leaves, and the foliage looks soggy, as if soaked in water for a long time. If roots are chilled, the plants may wilt. Species that are generally sensitive to chilling can show appreciable variation in their response to chilling temperatures. Genetic adaptation to the colder temperatures associated with high altitude improves chilling resistance. In addition, resistance often increases if plants are first hardened (acclimated) by exposure to cool, but noninjurious, temperatures. Chilling damage thus can be minimized if exposure is slow and gradual. Sudden exposure to temperatures near 0°C, called *cold shock*, greatly increases the chances of injury.

Response and adaptation to cold stress

1. Membrane Properties Change in Response to Chilling Injury

Leaves from plants injured by chilling show inhibition of photosynthesis, slower carbohydrate translocation, lower respiration rates, inhibition of protein synthesis, and increased degradation of existing proteins. All of these responses appear to depend on a common primary mechanism involving loss of membrane function during chilling. For instance, solutes leak from the leaves of chillig sensitive *Passiflora maliformis* (conch apple) floated on water at 0°C, but not from those of chilling-resistant *Passiflora caerulea* (passionflower). Loss of solutes to the water reflects damage to the plasma membrane and possibly also to the tonoplast. In turn, inhibition of photosynthesis and of respiration reflects injury to chloroplast and mitochondrial membranes. Why are membranes affected by chilling? Plant membranes consist of a lipid bilayer interspersed with proteins and sterols. The physical properties of the lipids greatly influence the activities of the integral membrane proteins, including H+-ATPases, carriers, and channel-forming proteins that regulate the transport of ions and other solutes, as well as the transport of enzymes on which metabolism depends. As the membranes become less fluid, their protein components can no longer function normally. The result is inhibition of H+-ATPase activity, of solute transport into and out of cells, of energy transduction, and of enzyme-dependent metabolism. In addition, chilling- sensitive leaves exposed to high photon fluxes and chilling temperatures are photo-inhibited, causing acute damage to the photosynthetic machinery.

2. Ice Crystal Formation and Protoplast Dehydration Kill Cells

The ability to tolerate freezing temperatures under natural conditions varies greatly among tissues. Seeds, other partly dehydrated tissues, and fungal spores can be kept indefinitely at temperatures near absolute zero (0 K, or –273°C), indicating that these very low temperatures are not intrinsically harmful. Fully hydrated, vegetative cells can also retain viability if they are cooled very quickly to avoid the formation of large, slow-growing ice crystals that would puncture and destroy subcellular structures. Ice crystals that form during very rapid freezing are too small to cause mechanical damage. Conversely, rapid warming of frozen tissue is required to prevent the growth of small ice crystals into crystals of a damaging size, or to prevent loss of water vapor by sublimation, both of which take place at intermediate temperatures (–100 to –10°C).

3. ABA and Protein Synthesis Are Involved in Acclimation to Freezing

In seedlings of alfalfa (*Medicago sativa* L.), tolerance to freezing at –10°C is greatly improved by previous exposure toncold (4°C) or by treatment with exogenous ABA without exposure to cold. These treatments cause changes in them pattern of newly synthesized proteins that can be resolved on two-dimensional gels. Some of the changes are unique to the particular treatment (cold or ABA), but some of the newly synthesized proteins induced by cold appear to be the same as those induced by ABA or by mild water deficit. Protein synthesis is necessary for the development of freezing tolerance, and several distinct proteins accumulateduring acclimation to cold, as a result of changes in gene expression (Guy 1999). Isolation of the genes for these proteins reveals that several of the proteins that are induced by low temperature share homology with the RAB/LEA/DHN (responsive to ABA, late embryo abundant, and dehydrin, respectively) protein family. As described earlier in the

section on gene regulation by osmotic stress, these proteins accumulate in tissues exposed to different stresses, such as osmotic stress. Their functions are under investigation.

4. Numerous Genes Are Induced during Cold Acclimation

Expression of certain genes and synthesis of specific proteins are common to both heat and cold stress, but some aspects of cold-inducible gene expression differ from that produced by heat stress (Thomashow 2001). Whereas during cold episodes the synthesis of "housekeeping" proteins (proteins made in the absence of stress) is not substantially down-regulated, during heat stress housekeeping-protein synthesis is essentially shut down. On the other hand, the synthesis of several heat shock proteins that can act as molecular chaperones is up-regulated under cold stress in the same way that it is during heat stress. This suggests that protein destabilization accompanies both heat and cold stress and that mechanisms for stabilizing protein structure during both heat and cold episodes are important for survival.

Stress induced gene expression

Heat stress related gene expression

The five classes of heat shock proteins found in plants

HSP class	Size (kDa)	Examples (Arabidopsis / prokaryotic)	Cellular location
HSP100	100–114	AtHSP101 / ClpB, ClpA/C	Cytosol, mitochondria, chloroplasts
HSP90	80–94	AtHSP90 / HtpG	Cytosol, endoplasmic reticulum
HSP70	69–71	AtHSP70 / DnaK	Cytosol/nucleus, mitochondria, chloroplasts
HSP60	57–60	AtTCP-1 / GroEL, GroES	Mitochondria, chloroplasts
smHSP	15–30	Various AtHSP22, AtHSP20, AtHSP18.2, AtHSP17.6 / IBPA/B	Cytosol, mitochondria, chloroplasts, endoplasmic reticulum

Source: After Boston et al. 1996.

Cold stress related gene expression

More than 100 genes are up-regulated by cold stress. Because cold stress is clearly related to ABA responses and to osmotic stress, not all the genes up-regulated by cold stress necessarily need to be associated with cold tolerance, but most likely many of them are. Many cold stress–induced genes are activated by transcriptional activators called **C-repeat binding factors** (**CBF1**, **CBF2**, **CBF3**; also called DREB1b, DREB1c, and DREB1a, respectively) (Shinozaki and Yamaguchi-Shinozaki 2000). CBF/DREB1-type transcription factors bind to **CRT/DRE elements** (C-repeat/dehydration-responsive, ABA-independent sequence elements) in gene promoter sequences, which were discussed earlier in the chapter. CBF/DREB1 is involved in the coordinate transcriptional response of numerous cold and osmotic stress–regulated genes, all of which contain the CRT/DRE elements in their promoters. CBF1/DREB1b is unique in that it is specifically induced by cold stress and not by osmotic or salinity stress, whereas the DRE-binding elements of the DREB2 type (discussed earlier in the section on osmotic stresses) are induced only by osmotic and salinity stresses and not by cold. The expression of CBF1/DREB1b is controlled by a separate transcription factor, called ICE (*i*nducer of *C*BF *e*xpression). ICE transcription factors do not appear to be induced by cold, and it is presumed that ICE or an associated protein is post transcriptionally activated, permitting activation of CBF1/DRE1b, but the precise signaling pathway(s) of cold perception, calcium signaling, and the activation of ICE are presently under investigation. Transgenic plants constitutively expressing CBF1 have more cold–up-regulated gene transcripts than wild-type plants have, suggesting that numerous cold–up-regulated proteins that may be involved in cold acclimation are being produced in the absence of cold in these CBF1 transgenic plants. In addition, CBF1 tansgenic plants are more coldtolerant than control plants.

8. Genetically modified crops

Genetically modified crops (GMCs, GM crops, or biotech crops) are defined as crops whose genomes have been altered in ways that do not occur naturally. Although the definition of GMCs includes organisms that have been genetically modified by selective breeding, the most commonly used definition refers to organisms modified through genetic engineering or recombinant DNA technologies. Genetic engineering allows one or more genes to be cloned and transferred from one organism to another—either between individuals of the same species or between those of unrelated species. It also allows an organism's endogenous genes to be altered in ways that lead to enhanced or reduced expression levels. When genes are transferred between unrelated species, the resulting organism is called **transgenic**. The term **cisgenic** is sometimes used to describe gene transfers within a species. In contrast, the term **biotechnology** is a more general one, encompassing a wide range of methods that manipulate organisms or their components—such as isolating enzymes or producing wine, cheese, or yogurt. Genetic modification of plants or animals is one aspect of biotechnology.

Examples in food crops include resistance to certain pests, diseases, or environmental conditions, reduction of spoilage, or resistance to chemical treatments (e.g. resistance to an herbicide), or improving the nutrient profile of the crop. Examples in non-food crops include production of pharmaceutical agents, biofuels, and other industrially useful goods, as well as for bioremediation.

Farmers have widely adopted GM technology. It is the fastest adopted crop technology in the world. Acreage increased from 1.7 million hectares in 1996 to 185.1 million hectares in 2016, some 12% of global cropland. As of 2016, major crop (soybean, maize, canola and cotton) traits consist of herbicide tolerance (95.9 million hectares) insect resistance (25.2 million hectares), or both (58.5 million hectares). In 2015, 53.6 million ha of GM maize were under cultivation (almost 1/3 of the maize crop). GM maize outperformed its predecessors: yield was 5.6 to 24.5% higher with less mycotoxins (−28.8%), fumonisin (−30.6%) and thricotecens (−36.5%). Non-target organisms were unaffected, except for Braconidae, represented by a parasitoid of European corn borer, the target of Lepidoptera active Bt maize. Biogeochemical parameters such as lignin content did not vary, while biomass decomposition was higher.

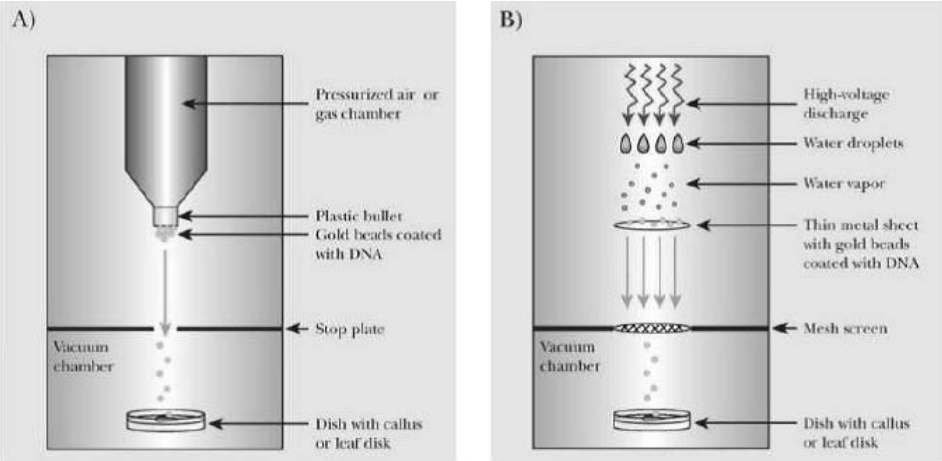
There is a scientific consensus that currently available food derived from GM crops poses no greater risk to human health than conventional food, but that each GM food needs to be tested on a case-by-case basis before introduction. Nonetheless, members of the public are much less likely than scientists to perceive GM foods as safe. The legal and regulatory status of GM foods varies by country, with some nations banning or restricting them, and others permitting them with widely differing degrees of regulation.

However, opponents have objected to GM crops on grounds including environmental impacts, food safety, whether GM crops are needed to address food needs, whether they are sufficiently accessible to farmers in developing countries and concerns over subjecting crops to intellectual property law. Safety concerns led 38 countries, including 19 in Europe, to officially prohibit their cultivation.

Methods

Genetically engineered crops have genes added or removed using genetic engineering techniques, originally including **gene guns**, **electroporation**, **microinjection** and ***Agrobacterium***. More recently, **CRISPR** and **TALEN** offered much more precise and convenient editing techniques.

Gene guns (also known as biolistics) target genes into plant cells. It is the most common method. DNA is bound to tiny particles of gold or tungsten which is subsequently shot into plant tissue or single plant cells under high pressure. The accelerated particles penetrate both the cell wall and membranes. The DNA separates from the metal and is integrated into plant DNA inside the nucleus. This method has been applied successfully for many cultivated crops, especially monocots like wheat or maize, for which transformation using *Agrobacterium tumefaciens* has been less successful. The major disadvantage of this procedure is that serious damage can be done to the cellular tissue.



***Agrobacterium tumefaciens*-mediated transformation** is another common technique. *Agrobacteria* are natural plant parasites. Their natural ability to transfer genes provides another engineering method. To create a suitable environment for themselves, these *Agrobacteria* insert their genes into plant hosts, resulting in a proliferation of modified plant cells near the soil level (crown gall). The genetic information for tumor growth is encoded on a mobile, circular DNA fragment (plasmid). When *Agrobacterium* infects a plant, it transfers this T-DNA to a random site in the plant genome. When used in genetic engineering the bacterial T-DNA is removed from the bacterial plasmid and replaced with the desired foreign gene. The bacterium is a vector, enabling transportation of foreign genes into plants. This method works especially well for dicotyledonous plants like potatoes, tomatoes, and tobacco. *Agrobacteria* infection is less successful in crops like wheat and maize.

Ti plamid:

The size of Ti plasmid is approx. 200 kb.

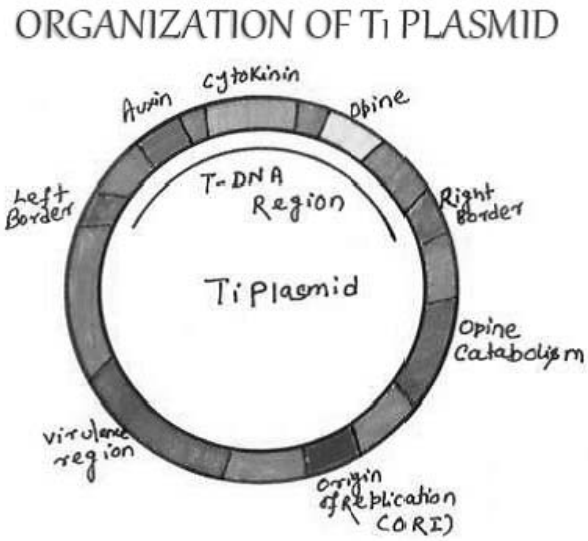
The Ti plasmid has three important regions:

(i) T-DNA region

(ii) Virulence region

(iii) Opine catabolism region

There is ori region that is responsible for the origin of DNA replication.



T-DNA transfer and integration

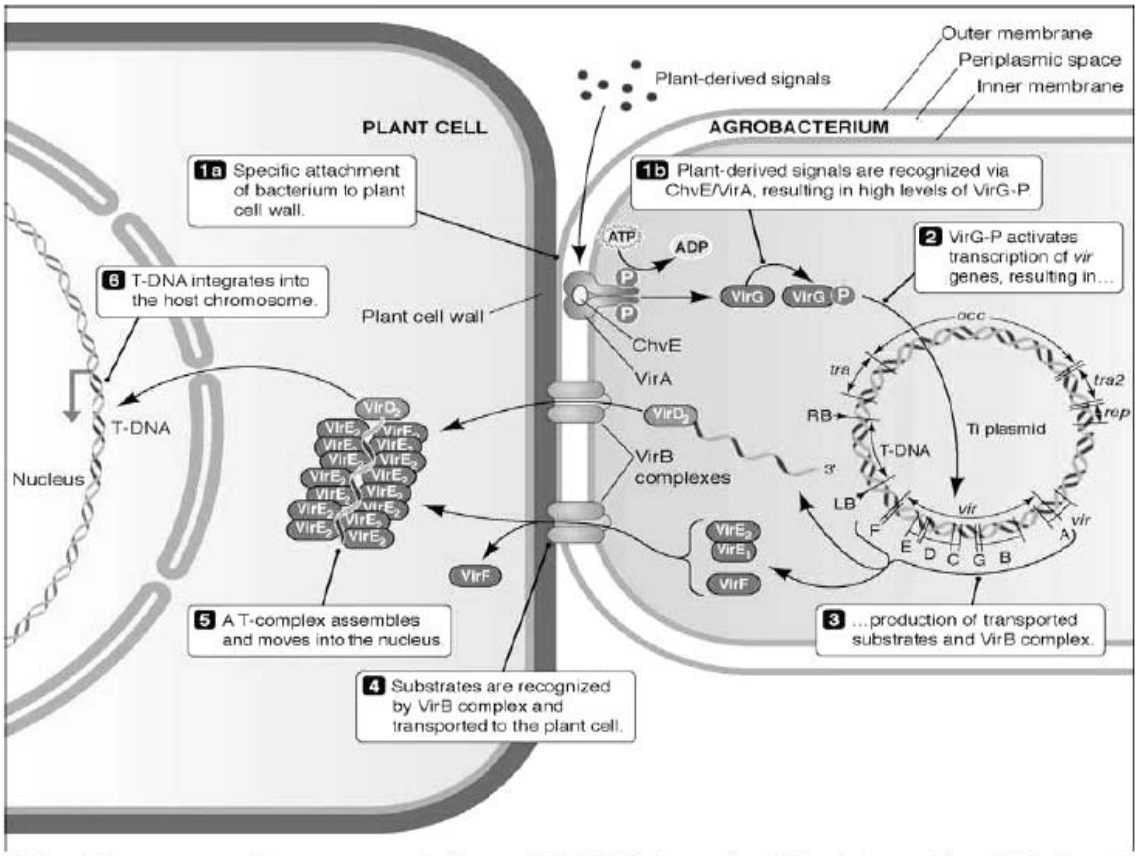


Fig: Diagrammatic representation of T-DNA transfer &its integration into host plant cell genome

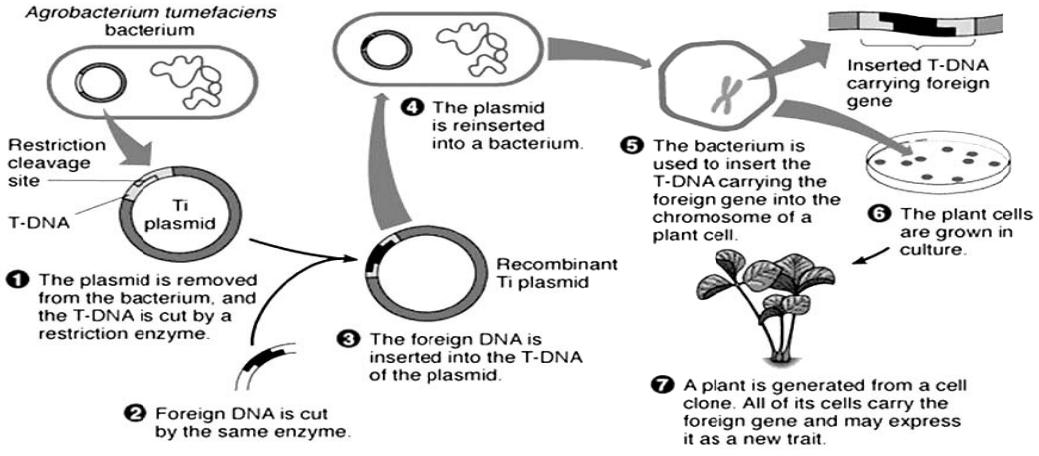
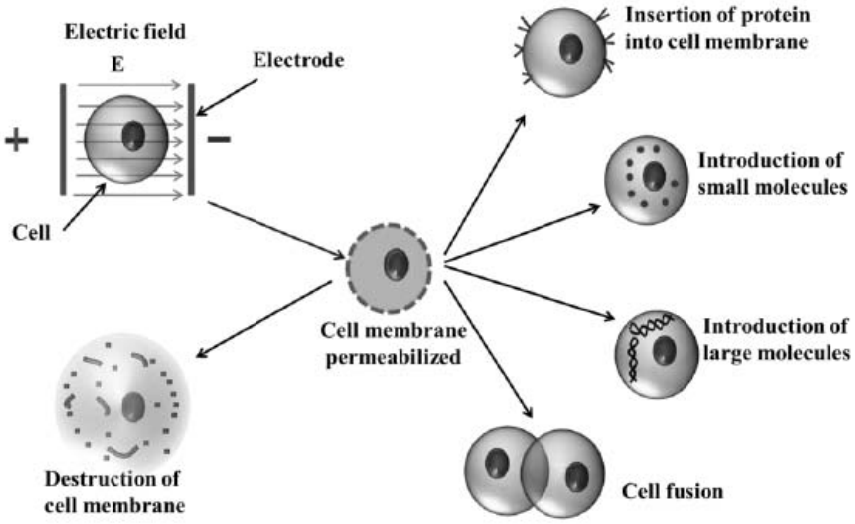


Fig.1: *Agrobacterium*-mediated genetic transformation.

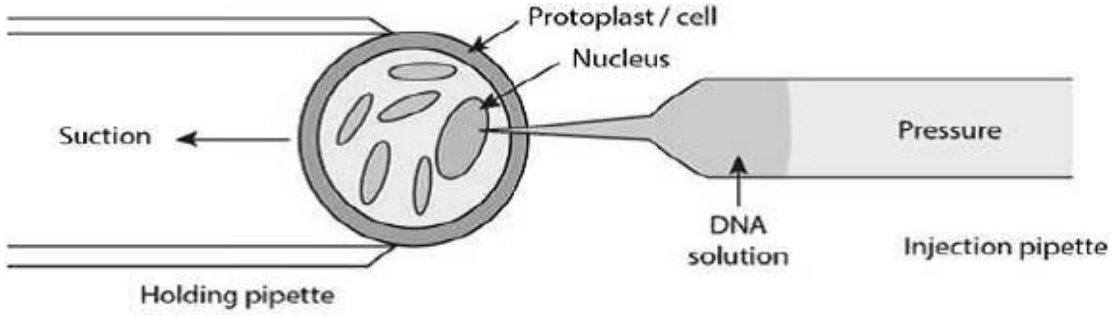
Electroporation is used when the plant tissue does not contain cell walls. Electroporation involves the creation of pores in the cell membrane using electric pulse of high field strength. If DNA is present in the buffer solution at sufficient concentration, it will be taken up through these pores.



Microinjection is a direct physical method involving the mechanical insertion of the desirable DNA into a target cell.

- The technique of microinjection involves the transfer of the gene through a micropipette into the cytoplasm or nucleus of a plant cell or protoplast.
- The most significant use of this is the introduction of DNA into the oocyte and the eggs of animals, either the transient expression analysis or to generate transgenic animals.
- The major limitations of microinjection are that it is slow, expensive, and has to be performed by trained and skilled personnel.

Types of modifications



Transgenic

Transgenic plants have genes inserted into them that are derived from another species. The inserted genes can come from species within the same kingdom (plant to plant), or between kingdoms (for example, bacteria to plant). In many cases the inserted DNA has to be modified slightly in order to be correctly and efficiently expressed in the host organism. Transgenic plants are used to express proteins, like the cry toxins from *B. thuringiensis*, herbicide-resistant genes, antibodies etc.

Cisgenic

Cisgenic plants are made using genes found within the same species or a closely related one, where conventional plant breeding can occur. Some breeders and scientists argue that cisgenic modification is useful for plants that are difficult to crossbreed by conventional means (such as potatoes), and that plants in the cisgenic category should not require the same regulatory scrutiny as transgenics.

Subgenic

Genetically modified plants can also be developed using **gene knockdown** or **gene knockout** to alter the genetic makeup of a plant without incorporating genes from other plants. In 2014, Chinese researcher Gao Caixia filed patents on the creation of a strain of wheat that is resistant to powdery mildew. The strain lacks genes that encode proteins that repress defenses against the mildew. The researchers deleted all three copies of the genes from wheat's hexaploid genome. Gao used the TALENs and CRISPR gene editing tools without adding or changing any other genes.

Multiple trait integration

With multiple trait integration, several new traits may be integrated into a new crop.

Herbicide Resistant GM crops:

Weed infestations destroy about 10 percent of crops worldwide. To combat weeds, farmers often apply herbicides before seeding a crop and between rows after the crops are growing. As the most efficient broad-spectrum herbicides also kill crop plants, herbicide use may be difficult and limited. Farmers also use tillage to control weeds; however, tillage damages soil structure and increases erosion.

A good or an ideal herbicide is expected to possess the following characteristics:

i. Capable of killing weeds without affecting crop plants.
ii. Not toxic to animals and microorganisms.
iii. Rapidly translocated within the target plant.
iv. Rapidly degraded in the soil.

Glyphosate Resistance:

Glyphosate, is a glycine derivative. It acts as a broad-spectrum herbicide and is effective against 76 of the world's worst 78 weeds. Glyphosate is less toxic to animals and is rapidly degraded by microorganisms. In addition, it has a short half-life. The American chemical company Monsanto markets glyphosate as **Round up**.

Mechanism of action of glyphosate:

Glyphosate is rapidly transported to the growing points of plants. It is capable of killing the plants even at a low concentration. Glyphosate acts as a competitive inhibitor of the enzyme 5-enoylpyruvylshikimate 3-phosphate synthase (EPSPS). This is a key enzyme in shikimic acid pathway that results in the formation of aromatic amino acids (tryptophan, phenylalanine and tyrosine), phenols and certain secondary metabolites.

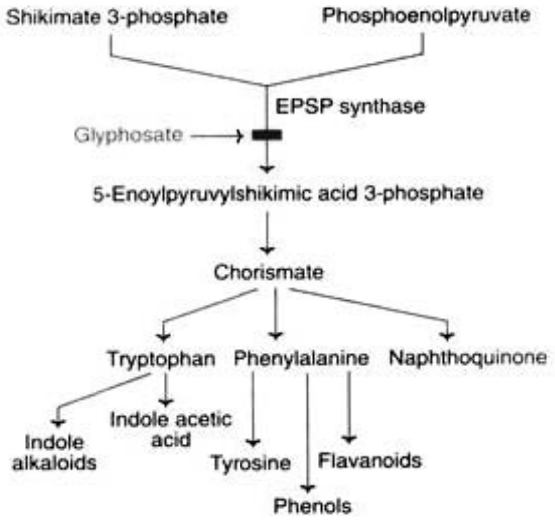


Fig. 50.5 : Shikimate pathway indicating the action of the herbicide glyphosate (EPSP synthase-5-Enolylpyruvylshikimate 3-phosphate synthase)

The enzyme EPSPS catalyses the synthesis of 5-enoylpyruvylshikimate 3-phosphate from shikimate 3-phosphate and phosphoenoylpyruvate. Glyphosate has some structural similarly with the substrate phosphoenol pyruvate. Consequently, glyphosate binds more tightly with EPSPS and blocks the normal shikimic acid pathway. Thus, the herbicide glyphosate inhibits the biosynthesis of aromatic amino acids and other important products.

This results in inhibition of protein biosynthesis (due to lack of aromatic amino acids). As a consequence, cell division and plant growth are blocked. Further, the plant growth regulator indole acetic acid (an auxin) is also produced from tryptophan. The net result of glyphosate is the death of the plants. Glyphosate is toxic to microorganisms as they also possess shikimate pathway.

Glyphosate is non-toxic to animals (including humans), since they do not possess shikimate pathway.

Strategies for glyphosate resistance:

There are three distinct strategies to provide glyphosphate resistance to plants:

1. Overexpression of crop plant EPSPS gene:

An overexpressing gene of EPSPS was detected in *Petunia*. This expression was found to be due to gene amplification rather than an increased expression of the gene. EPSPS gene from *Petunia* was isolated and introduced into other plants. The increased synthesis of EPSPS (by about 40 fold) in transgenic plants provides resistance to glyphosate. These plants can tolerate glyphosate at a dose of 2-4 times higher than that required to kill wild-type plants.

2. Use of mutant EPSPS genes:

An EPSPS mutant gene that conferred resistance to glyphosate was first detected in the bacterium Salmonella typhimurium. It was found that a single base substitution (C to 7) resulted in the change of an amino acid from proline to serine in EPSPS. This modified enzyme cannot bind to glyphosate, and thus provides resistance.

The mutant EPSPS gene was introduced into tobacco plants using *Agrobacterium* Ti plasmid vectors. The transgene produced high quantities of the enzyme EPSPS. However, the transformed tobacco plants provided only marginal resistance to glyphosate. The reason for this was not immediately identified.

3. Detoxification of glyphosate:

The soil microorganisms possess the enzyme glyphosate oxidase that converts glyphosate to glyoxylate and aminomethylphosponic acid. The gene encoding glyphosate oxidase has been isolated from a soil organism Ochrobactrum anthropi. With suitable modifications, this gene was introduced into crop plants e.g. oilseed rape. The transgenic plants were found to exhibit very good glyphosate resistance in the field.

Use of a combined strategy:

More efficient resistance of plants against glyphosate can be provided by employing a combined strategy. Thus, resistant (i.e. mutant) EPSPS gene in combination with glyphosate oxidase gene are used. By this approach, there occurs glyphosate resistance (due to mutant EPSPS gene) as well as its detoxification (due to glyphosate oxidase gene).

Phosphinothricin Resistance:

Phosphinothricin (or glufosinate) is also a broad spectrum herbicide like glyphosate. Phosphinothricin is more effective against broad-leafed weeds but least effective against perennials.

Phosphinothricin-a natural herbicide:

Phosphinothricin is an unusual herbicide, being a derivative of a natural product namely bialaphos.

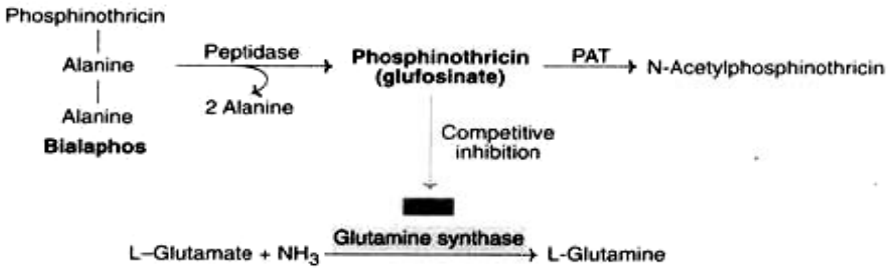


Fig. 50.7 : The formation, mode of action and detoxification of phosphinothricin (PAT-Phosphinothricin acetyl transferase).

Certain species of Streptomyces produce bialaphos which is a combination of phosphinothricin bound to two alanine residues, forming a tripeptide. By the action of a peptidase, bialaphos is converted to active phosphinothricin.

Mechanism of action of phosphinothricin:

Phosphinothricin acts as a competitive inhibitor of the enzyme glutamine synthase. This is possible since phosphinothricin has some structural similarity with the substrate glutamate. As a consequence of the inhibition of glutamine synthase, ammonia accumulates and kills the plant cells. Further, disturbance in glutamine synthesis also inhibits photosynthesis. Thus, the herbicidal activity of phosphinothricin is due to the combined effects of ammonia toxicity and inhibition of photosynthesis.

Strategy for phosphinothricin resistance:

The natural detoxifying mechanism of phosphinothricin observed in Streptomyces sp has prompted scientists to develop resistant plants against this herbicide. The enzyme phosphinothricin acetyl transferase (of *Streptomyces* sp) acetylates phosphinothricin, and thus inactivates the herbicide.

The gene responsible for coding phosphinothricin acetyl transferase (bar gene) has been identified in *Streptomyces hygroscopicus*. Some success has been reported in developing transgenic maize and oilseed rape by introducing bar gene. These plants were found to provide resistance to phosphinothricin.

Bromoxynil

Tobacco plants have been engineered to be resistant to the herbicide bromoxynil.

Glufosinate

Crops have been commercialized that are resistant to the herbicide glufosinate, as well. Crops engineered for resistance to multiple herbicides to allow farmers to use a mixed group of two, three, or four different chemicals are under development to combat growing herbicide resistance.

2,4-D

In October 2014 the US EPA registered Dow's Enlist Duo maize, which is genetically modified to be resistant to both glyphosate and 2,4-D, in six states. Inserting a bacterial aryloxyalkanoate dioxygenase gene, aad1 makes the corn resistant to 2,4-D. The USDA had approved maize and soybeans with the mutation in September 2014.

Insect (Pest) Resistance GM crops:

It is estimated that about 15% of the world's crop yield is lost to insects or pests. The damage to crops is mainly caused by insect larvae and to some extent adult insects.

The majority of the insects that damage crops belong to the following orders (with examples):

i. Lepidoptera (bollworms).

ii. Coleoptera (beetles).

iii. Orthoptera (grasshoppers).

iv. Homoptera (aphids).

Till some time ago, chemical pesticides are the only means of pest control. Transgenic plants with insect resistance transgenes have been developed. About 40 genes obtained from microorganisms of higher plants and animals have been used to provide insect resistance in crop plants.

Resistance Genes from Microorganisms:

***Bacillus thuringiensis* (Bt) toxin:**

Bacillus thuringiensis was first discovered by Ishiwaki in 1901, although its commercial importance was ignored until 1951. *B. thuringiensis* is a Gram negative, soil bacterium. This bacterium produces a parasporal crystalline proteinous toxin with insecticidal activity. The protein produced by B .thuringiensis is referred to as insecticidal crystalline protein (ICP). ICPs are among the endotoxins

produced by sporulating bacteria, and were originally classified as δ-endotoxins (to distinguish them from other classes of α-, β- and y-endotoxins).

Bt toxin genes:
Several strains of B. thuringiensis producing a wide range of crystal (cry) proteins have been identified. Further, the structure of cry genes and their corresponding toxin (δ-endotoxin) products have been characterized. The cry genes are classified into a large number of distinct families (about 40) designated as cry 1…… cry 40, based on their size and sequence similarities. And within each family, there may be sub-families. Thus, the total number of genes producing Bt toxins (Cry proteins) is more than 100.

There are differences in the structure of different Cry proteins, besides certain sequence similarities. The molecular weights of Cry proteins may be either large (~130 KDa) or small (~70KDa). Despite the differences in the Cry proteins, they share a common active core of three domains.

Mode of action of Cry proteins:
Most of the Bt toxins (Cry proteins) are active against Lepidopteran larvae, while some of them are specific against Dipteran and Coleopteran insects. The pro-toxin of Cry I toxin group has a molecular mass of 130 kilo Daltons (130 KDa).

When this parasporal crystal is ingested by the target insect, the pro-toxin gets activated within its gut by a combination of alkaline pH (7.5 to 8.5) and proteolytic enzymes. This results in the conversion of pro-toxin into an active toxin with a molecular weight of 68 KDa. The active form of toxin protein gets itself inserted into the membrane of the gut epithelial cells of the insect. This result in the formation of ion channels through which there occurs an excessive loss of cellular ATP. As a consequence, cellular metabolism ceases, insect stops feeding, and becomes dehydrated and finally dies.

Some workers in the recent years suggest that the Bt toxin opens cation-selective pores in the membranes, leading to the inflow of cations into the cells that causes osmotic lysis and destruction of epithelial cells (and finally the death of insect larvae). The Bt toxin is not toxic to humans and animals since the conversion of pro-toxin to toxin requires alkaline pH and specific proteases.

The usage Bt is commonly used for a transgenic crop with a cry gene e.g. Bt cotton. In the same way, Cry proteins are also referred to as Bt proteins. It may also be stated here that the authors use four different names for the same group of proteins-δ-endotoxin, insecticidal crystal protein (ICP), Cry and now Bt.

Bt-based genetic transformation of plants:

It has been possible to genetically modify (GM) plants by inserting Bt genes and provide pest resistance to these transformed plants. For an effective pest resistance, the bacterial gene in transgenic plants must possess high level expression. This obviously means that the transgene transcription should be under the effective control of promoter and terminator sequences. The early attempts to express cry 1A and cry 3A proteins under the control of CaMV 35S or Agrobacterium T-DNA promoters resulted in a very low expression in tobacco, tomato and potato plants.

In March 1995, the first Bt crop deregulated in the U.S. were seven lines of Colorado Potato Beetle Resistant Bt Potato by Monsanto. Since then, many more Bt crops have been deregulated, engineered to produce a variety of different Bt proteins from various subspecies of Bt. Bt crops include:

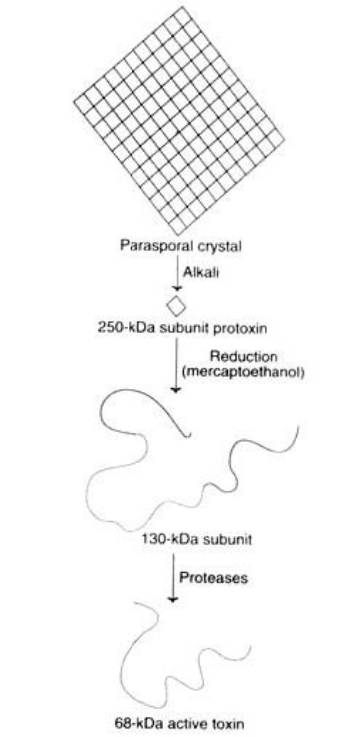
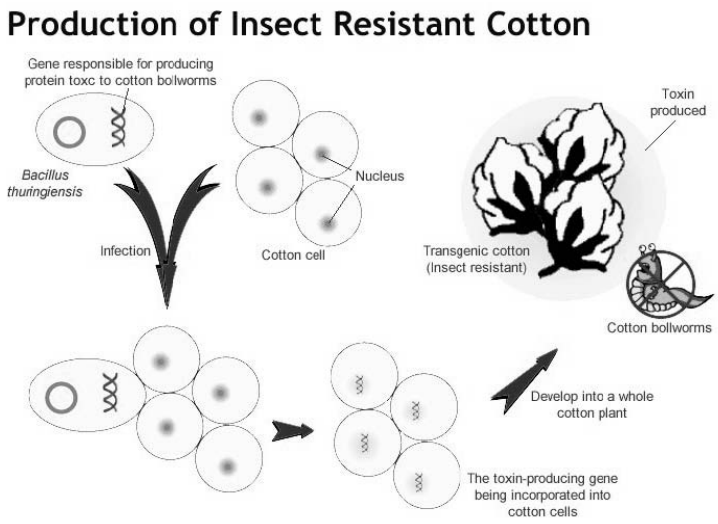


Fig. 50.2 : A diagrammatic representation of the formation of active toxin from the parasporal crystal of **Bacillus thuringiensis.**



Corn:
European Corn Borer Resistant Corn (first deregulated in the U.S. in May 1995)
Corn Rootworm Resistant Corn (first deregulated in the U.S. in October 2002)
Cotton:
Lepidopteran Resistant Cotton (first deregulated in the U.S. in June 1995)
Potato:
Colorado Potato Beetle Resistant Bt Potato (first deregulated in the U.S. in March 1995)
Potato Tuber Moth Resistant Bt Potato (being developed in South Africa)
Soybean:
Bt Soybean (first deregulated in the U.S. in October 2011, not yet sold commercially)
Tomato:
Lepidopteran Resistant Tomato (first deregulated in the U.S. in March 1998, not yet sold commercially)

Golden Rice —The Provitamin A Enriched Rice:

About one-third of the world's population is dependent on rice as staple food. The milled rice that is usually consumed is almost deficit in P-carotene, the pro-vitamin A. As such, vitamin A deficiency (causing night blindness) is major nutritional disorder world over, particularly in people subsisting on rice.

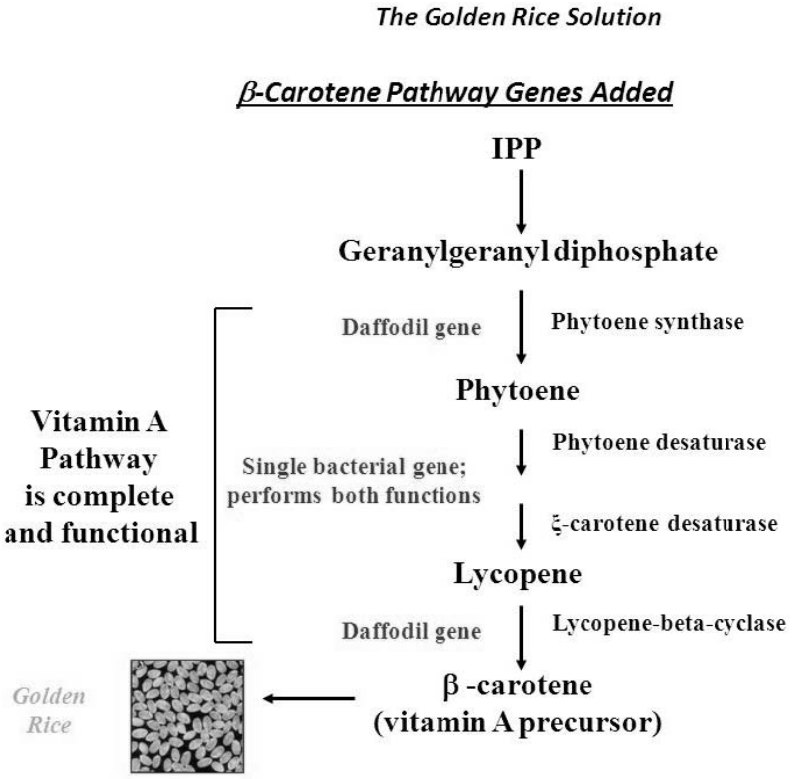
To overcome vitamin A deficiency, it was proposed to genetically manipulate rice to produce β-carotene, in the rice endosperm. The presence of β-carotene in the rice gives a characteristic yellow/orange colour, hence the pro-vitamin A-enriched rice are appropriately considered as Golden Rice.

The genetic manipulation to produce Golden Rice required the introduction of three genes encoding the enzymes phytoene synthase, carotene desaturase and lycopene β-cyclase. It took about 7 years to insert three genes for developing Golden Rice.

Golden Rice has met almost all the objections raised by the opponents of GM foods. However, many people are still against the large scale production of Golden Rice, as this will open door to the entry of many other GM foods.

Another argument put forth against the consumption of Golden Rice is that it can supply only about 20% of daily requirement of vitamin A. But the proponents justify that since rice is a part of a mixed diet consumed (along with many other foods), the contribution of pro-vitamin A through Golden Rice is quite substantial.

Recently (in 2004), a group of British scientists have developed an improved version of Golden Rice. The new strain, Golden Rice 2 contains more than 20 times the amount of pro-vitamin A than its predecessor. It is claimed that a daily consumption of 70 g rice can meet the recommended dietary allowance for vitamin A.



Genetically modified tomato:

Flavr Savr is a genetically modified tomato, was the first commercially grown genetically engineered food to be granted a license for human consumption. It was produced by Californian company Calgene 1992. Calgene introduced a gene in plant which synthesize a complementary mRNA to PG gene and inhibiting the synthesis of PG enzyme. On May 21, 1994, the genetically engineered Flavr Savr tomato was introduced.

Fruit ripening is an active process characterized by increased respiration accompanied by a rapid increase in ethylene synthesis. As the chlorophyll gets degraded, the green color of fruit disappears and a red pigment, lycopene is synthesized. The fruit gets softened as a result of the activity of cell wall degrading enzymes namely polygalacturonase (PG) and methyl

esterase. The phyto hormone ethylene production is linked to fruit ripening as the same is known to trigger the ripening effect. The breakdown of starch to sugars and accumulation of large number of secondary products improves the flavor, taste and smell of the fruits.

Genes involved in tomatoes ripening:

i) **pTOM5** encodes for phytoene synthase which promote lycopene synthesis that gives red coloration

ii) **pTOM6** gene encodes for polygalacturonase. This enzyme degrades the cell wall, resulting in fruit softening.

iii) **pTOM** gene encodes for ACC oxidase. This enzyme catalyzes the ethylene formation that triggers the fruit ripening.

Development of flavr savr tomato

Softening of fruits is largely due to degradation of cell wall (pectin) by enzyme polygalacturonase (PG). The gene encoding PG has been isolated and cloned (pTOM6).

Procedure involves: -

1) Isolation of DNA from tomato plant that encodes the enzyme polygalacturonase (PG).

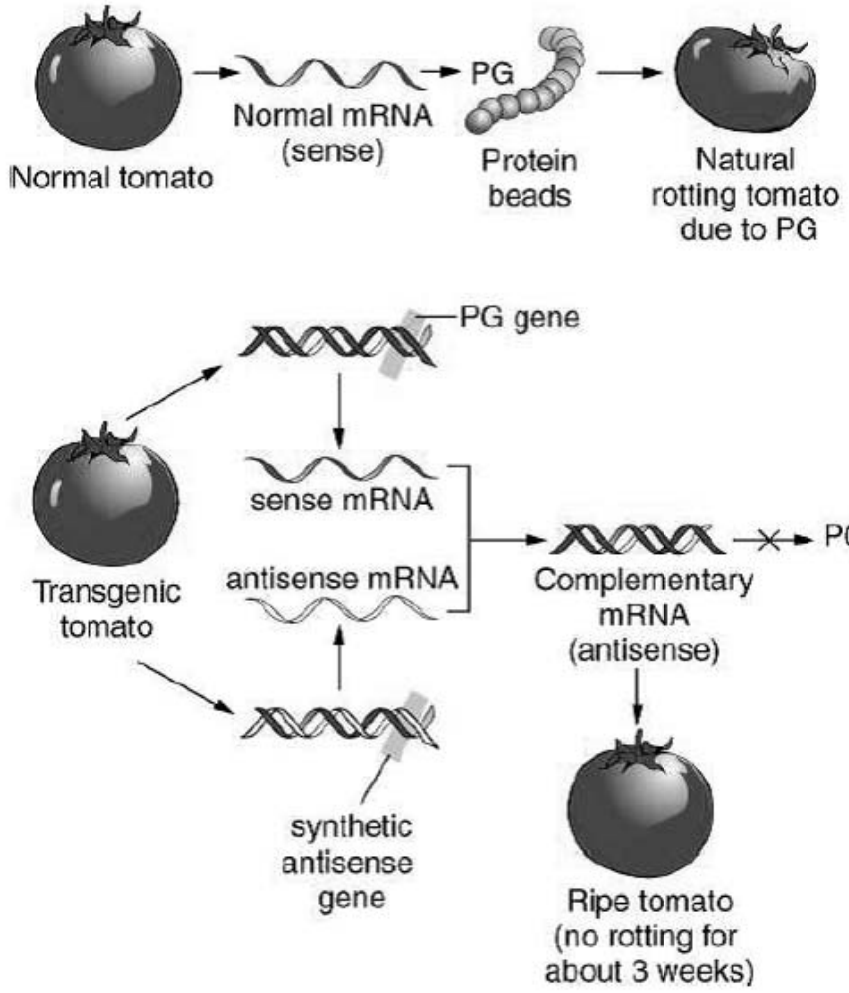
2) Transfer of PG gene to a vector bacteria and production of complementary DNA (cDNA) molecules.

3) Introduction of cDNA into a fresh tomato plant to produce transgenic plant.

Mechanism of pg antisense RNA approach

In normal plants, PG gene encodes a normal or sense mRNA that produce the enzyme PG and it is actively involved in fruit ripening.

- The cDNA of PG encodes for antisense mRNA, which is complementary to sense mRNA.
- The hybridization between sense and antisense mRNA render the sense mRNA ineffective.
- Consequently, no polygalacturonase is produced hence fruit ripening is delayed.



Advantages:

1. Slower ripen rate
2. Ripen longer on vine
3. Fully developed flavors
4. Increase the shelf life

The rise and fall of Flavr Savr Tomato:

The genetically engineered tomato, known as Flavr Savr (pronounced flavour saver) by employing PC antisense RNA was approved by U.S. Food and Drug Administration on 18th May 1994. The FDA ruled that Flavr Savr tomatoes are as safe as tomatoes that are bred by conventional means, and therefore no special labeling is required. The new tomato could be shipped without refrigeration too far off places, as it was capable of resisting rot for more than three weeks (double the time of a conventional tomato).

Although Flavr Savr was launched with a great fanfare in 1995, it did not fulfill the expectation for the following reasons:

i. Transgenic tomatoes could not be grown properly in different parts of U.S.A.

ii. The yield of tomatoes was low.

iii. The cost of Flavr Savr was high.

It is argued that the company that developed Flavr Savr, in its overenthusiasm to become the first Biotech Company to market a bioengineered food had not taken adequate care in developing the transgenic plant. And unfortunately, within a year after its entry, Flavr Savr was withdrawn, and it is now almost forgotten!

Virus resistance

Papaya, potatoes, and squash have been engineered to resist viral pathogens such as **cucumber mosaic virus** which, despite its name, infects a wide variety of plants. Virus resistant papaya were developed in response to a papaya ringspot virus (PRV) outbreak in Hawaii in the late 1990s. They incorporate PRV DNA. By 2010, 80% of Hawaiian papaya plants were genetically modified.

Potatoes were engineered for resistance to **potato leaf roll virus** and **Potato virus Y** in 1998. Poor sales led to their market withdrawal after three years.

Yellow squash that were resistant to at first two, then three viruses were developed, beginning in the 1990s. The viruses are watermelon, cucumber and zucchini/courgette yellow mosaic. Squash was the second GM crop to be approved by US regulators.

Edible oils

Some **GM soybeans** offer improved oil profiles for processing. *Camelina sativa* has been modified to produce plants that accumulate high levels of oils similar to fish oils.

Toxin reduction

A genetically modified *Cassava* under development offers lower **cyanogen glucosides** and enhanced protein and other nutrients (called BioCassava).

In November 2014, the USDA approved a potato that prevents bruising and produces less acrylamide when fried. They do not employ genes from non-potato species. The trait was added to the Russet Burbank, Ranger Russet and Atlantic varieties.

Stress resistance

Plants have been engineered to tolerate non-biological stressors, such as drought, frost, and high soil salinity. In 2011, Monsanto's DroughtGard maize became the first drought-resistant GM crop to receive US marketing approval.

Drought resistance occurs by modifying the plant's genes responsible for the mechanism known as the crassulacean acid metabolism (CAM), which allows the plants to survive despite low water levels.

This holds promise for water-heavy crops such as rice, wheat, soybeans and poplar to accelerate their adaptation to water-limited environments. Several salinity tolerance mechanisms have been identified in salt-tolerant crops. For example, rice, canola and tomato crops have been genetically modified to increase their tolerance to salt stress.

Drugs

In 2012, the FDA approved the first plant-produced pharmaceutical, a treatment for **Gaucher's Disease**. Tobacco plants have been modified to produce therapeutic antibodies.

Biofuel

Algae is under development for use in **biofuels**. Researchers in Singapore were working on **GM *Jatropha*** for biofuel production. Syngenta has USDA approval to market a maize trademarked Enogen that has been genetically modified to convert its starch to sugar for ethanol. Some trees have been genetically modified to either have less lignin, or to express lignin with chemically labile bonds. Lignin is the critical limiting factor when using wood to make bio-ethanol because lignin limits the accessibility of cellulose microfibrils to depolymerization by enzymes. Besides with trees, the chemically labile lignin bonds are also very useful for cereal crops such as maize, barley, and oats.

Materials

Companies and labs are working on plants that can be used to make bioplastics. Potatoes that produce industrially useful starches have been developed as well. Oilseed can be modified to produce fatty acids for detergents, substitute fuels and petrochemicals.

Bioremediation

Scientists at the University of York developed a weed (*Arabidopsis thaliana*) that contains genes from bacteria that could clean TNT and RDX-explosive soil contaminants in 2011. 16 million hectares in the US (1.5% of the total surface) are estimated to be contaminated with TNT and RDX. However *A. thaliana* was not tough enough for use on military test grounds. Modifications in 2016 included switchgrass and bentgrass.

Genetically modified plants have been used for bioremediation of contaminated soils. Mercury, selenium and organic pollutants such as polychlorinated biphenyls (PCBs).

Marine environments are especially vulnerable since pollution such as oil spills are not containable. In addition to anthropogenic pollution, millions of tons of petroleum annually enter the marine environment from natural seepages. Despite its toxicity, a considerable fraction of petroleum oil entering marine systems is eliminated by the hydrocarbon-degrading activities of microbial communities. Particularly successful is a recently discovered group of specialists, the so-called hydrocarbonoclastic bacteria (HCCB) that may offer useful genes.

Advantages of GM crops:

There is a need to produce inexpensive, safe and nutritious foods to help feed the world’s growing population.

Genetic modification may provide:

1. Better quality food.
2. Higher nutritional yields.
3. Inexpensive and nutritious food, like carrots with more antioxidants.
4. Foods with a greater shelf life, like tomatoes that taste better and last longer.
5. Food with medicinal benefits, such as edible vaccines - for example, bananas with bacterial or rotavirus antigens.
6. Crops and produce that require less chemical application, such as herbicide resistant canola.

Disadvantages of GM crops:

Food regulatory authorities require that GM foods receive individual pre-market safety assessments. Also, the principle of ‘substantial equivalence’ is used. This means that an existing food is compared

with its genetically modified counterpart to find any differences between the existing food and the new product. The assessment investigates:

1. Toxicity (using similar methods to those used for conventional foods).
2. Tendency to provoke any allergic reaction.
3. Stability of the inserted gene.
4. Whether there is any nutritional deficit or change in the GM food.
5. Any other unintended effects of the gene insertion.

Economic concerns:

Bringing a GM food to market is a lengthy and costly process, and of course agri-biotech companies wish to ensure a profitable return on their investment. Many new plant genetic engineering technologies and GM plants have been patented, and patent infringement is a big concern of agribusiness. Yet consumer advocates are worried that patenting these new plant varieties will raise the price of seeds so high that small farmers and third world countries will

not be able to afford seeds for GM crops, thus widening the gap between the wealthy and the poor. One way to combat possible patent infringement is to introduce a "suicide gene" into GM plants. These plants would be viable for only one growing season and would produce sterile seeds that do not germinate. Farmers would need to buy a fresh supply of seeds each year. However, this would be financially disastrous for farmers in third world countries who cannot afford to buy seed each year and traditionally set aside a portion of their harvest to plant in the next growing season.

---

9. Exploitation of plant resources

Introduction:

Plants are a vital component of biodiversity and healthy ecosystems. They provide a range of ecosystem services, from production of oxygen and removal of atmospheric carbon dioxide emissions, creation and stabilization of soil, protection of watersheds and provision of natural resources including food, fibre, fuel, shelter and medicine.

Food yielding Crops:

Wheat

Botanical name: *Triticum aestivum*

Family: Gramineae

Origin and Evolution

The origin of wheat is believed at Hindukush mountainous regions adjoining to India and Afganistan. According to De Candolle Valley of Euphrates and Tigris was the origin of wheat. But Vavilov stated origin of Durum wheat is probably Abyssinia and soft wheat groups are in the region of Western Pakistan, South West Afghanistan and Southern parts of mountainous Babshara. Wheat has undergone domestication under selection pressures which changed it from a wild grass to a cultivated species with the present level of production.

Botanical characters

Wheat is an annual plant. Roots fibrous; stem erect (divided in long internodes and conspicuous nodes); leaves are alternate and with sheathing base, it can be divided into 2 parts, the sheath and the blade; inflorescence spike of spikelet; flowers axillary, zygomorphic, hypogynous, sessile and bisexual; tepals 2; stamens 3 and versatile; gynoecium tricarpellary showing pseudomonomery, unilocular, ovary superior, parietal placentation with one ovule; fruit caryopsis and seeds endospermic.

Cultivation

Wheat is one of the oldest and most important of the cereal crops. Though grown under a wide range of climates and soils, wheat is best adapted to temperate regions with rainfall between 30 and 90 cm. Winter and spring wheats are the two major types of the crop, with the severity of the winter determining whether a winter or spring type is cultivated. Winter wheat is always sown in the fall; spring wheat is generally sown in the spring but can be sown in the fall where winters are mild. Ideal sowing time is mid October to starting of November.

Uses

1. Wheat is eaten in various forms by more than 1000 million people in the world.
2. In India, it is second important staple food crop next to rice.
3. Soft wheat is used for making chapatee, bread, cake, biscuits, pastry and other bakery products.
4. In areas where rice is the staple cereal food, wheat is eaten in the form of 'puris' or in the form of 'upma'.
5. In addition to this, wheat is also consumed in various other preparations such as 'dalia', 'halwa', 'sweet meals', etc.
6. In most of the urban areas of the country, the use of backed leavened bread, flakes, cakes, biscuits etc. is increasing at a fast rate.
7. Wheat straw is used as fooder, padding material and mulching material.
8. Wheat grain is used for preparing starch.

Rice

Botanical Name: *Oryza sativa*

Family: Poaceae (Gramineae)

Origin and Evolution

Rice is known to have been grown in China nearly 5000 years ago. Archeological studies also indicate that the remains of rice were found in yung shao excavations from China, of 2600 BC. According to de Candolle various wild relatives of rice such as *Oryza rufipogan* and *O. nivara* are available in India abundantly. Archaeological remains of rice available from Indus valley excavations also support the fact that rice was grown here before 2300 BC.

Nowadays there are two cultivated species of rice viz - *O. sativa* and *O. glaberrima* are widely grown in most of the regions. Their close resemblance and availability of their intermediate varieties is an indication of the fact that both the above mentioned species would have originated from a common ancestor *Oryza perennis*. It spread east ward from India, China and then gradually to Japan, and west ward to Iran, Iraq, turkey and Egypt.

Botanical Characters

Rice is a semi-aquatic annual grass. Plant body is having the height of 50-150 cm. Stem is cylindrical and differentiated into nodes and internodes. In the plant body tuft formation takes place due to the process of tillering. The inter nodes are hollow and of smaller size in the basal region of the plant body but gradually become elongated in the upper regions of the plant. Intercalary meristem is present above the each node. The basal leaf of each tiller and main stem is rudimentary and known as protophyll. Leaves are alternately arranged. Each leaf is differentiated into leaf sheath, lamina, ligule, and auricle. Node is partially or completely enclosed by leaf sheath. Lamina is 30-50 cm in length, and 1-2 cms broad. The margins of lamina are hairy.

Inflorescence of rice plant is panicle of spikelets. The spikelets are solitary and single flowered. Flowers are usually self-pollinated. The flowers are enclosed in lemma and palea and on maturity of plant, they form hull which is attached with grain. The grains of rice enclosed by hull are known as paddy or Dhann Lemma and palea are either owned or awnless. Each flower consists of six stamens (arranged in two whorls and a carpel) which are enclosed by lemma and palea. Grain or fruit is one seeded caryopsis. The grains of rice show variability in size and shape. These are either white or

yellow coloured. In the cereals maximum amount of starch is found in rice grains as reserve food after barley. Each rice grain contains 78% starch, 7.5% protein, along with 1.8% fat and vitamins.

Cultivation

About 90% of total rice production of the world comes from China, India, Bangladesh, Japan, Pakistan, Myanmar, Thailand, Vietnam, Korea, Philippines, Indonesia and Srilanka. However, the cultivation of rice is also common in Italy, Spain, Egypt and America. China is the country which provides maximum production of the world, India is next in number. India has the largest area under rice cultivation about 29% of the total world. More or less it is practically grown in all states of India. However the production of rice in our country is mainly concentrated in the river valleys, deltas and in low-lying coastal areas. The leading

rice producing states of India are West Bengal, Uttar Pradesh, Andhra Pradesh, Punjab, Tamil Nadu, Orissa, Bihar, Madhya Pradesh and Assam. Rice is an essentially the crop of tropical climate. However it is grown over extremely wide range of climatic conditions spread over throughout the world, but it is best suited to the regions which have high temperature, high humidity, prolonged sunshine and assured with continuous supply of water. An annual rainfall of 60-120 cm is favourable for upland varieties, and 180-240 cm for low land varieties. Before the cultivation, field is thoroughly ploughed. Usually the field is having the soil of high water holding capacity and good water drainage. After the proper ploughing the field is flooded with water. However in the non irrigated areas the paddy cultivation process is started when these fields are filled by rain water. The sowing process of rice is undertaken at varied times in different parts of India depending on the climatic conditions. On the basis of harvesting period rice is placed under three categories. These are:

(1) Winter rice: This variety is sowed in the months of June-July and its harvesting is undertaken in November-December. This is mainly a crop of low lying areas and known as Aman.

(2) Autumn rice: This variety is sown in May-June and harvested by September- October. It is mostly cultivated in highlands.

(3) Spring rice: It is sown in December-January and harvested by March- April.

Two main systems for cultivation of rice are followed in India:

(a) Dry Cultivation: Similarly to other cereals in this system, the crop is raised on dry soil. The field is repeatedly ploughed and harrowed in the summer season to obtain the sufficient tilth. The field is ploughed five to seven times at an interval of seven days. Then the seeds are sown by broadcasting, dibbling or drilling in lines. The method of sowing in lines is mostly preferred as it ensures a uniform stand, requires a lower seed rate and facilitates inter culture operations. In this method line to line distance is kept 20-25 cm.

(b) Wet Cultivation: In this system of cultivation the crop is grown under an assured, sufficient and adequately regular supply of water and the plants remain flooded by water from the time of transplanting under harvesting. Wet cultivation system covers about 90% of rice cultivation in India. The land is thoroughly ploughed and puddled with 5-6 cm of standing water, under this system. The major aim of puddling is to get a soft soil so that transplanted seedlings can establish themselves adequately and quickly. Now this wet field is repeatedly ploughed 4-6 times with an interval of four to five days between two ploughing and then levelled up by planking. Here in this system, either the sprouted seeds are directly sown in a puddle or leveled field or alternatively seedlings are grown in a nursery and then

transplanted. In the traditional rice growing areas where there is properly favourable rainfall, temperature and humidity are observed, the seedlings are grown on raised seed beds.

Harvesting and Threshing

When culms of the crop plants turn brown or yellow, the crop is considered to be ready for harvesting. The premature harvesting decreases the crop yield and also affects the milling quality. On the other hand in the delayed harvesting, there are chances of loss of grains by shattering.

The task of harvesting is carried out by the help of hand sickles. After it, the harvested crop is dried immediately and threshed. Normally threshing is done by beating the sheaves or by trempling under the feet of bullocks. In some regions, the threshing is done by machines also, pedal and power threshers are used for this purpose. After the threshing grains are winnowed and properly dried before

bagging. The rice grain covered by husk, as we know is known as paddy. This husk is separated from the grain by beating. After the separation from grain it is
known as bran.

Uses

1. In various countries such as India, China, Japan, and Korea, rice is used as major or staple food. Since its bread or chapatti cannot be prepared because of
glutinine deficiency in the grains hence it is taken directly as food after cooking or boiling.
2. Various food delicacies such as Idli, Dosa and Upma are prepared after fermentation generally in most of the parts of our country, but particularly in south India.
3. Rice is also used in the preparation of biscuits, ice-cream and pastry.
4. It is also used for making alcoholic beverages and as a source of starch.
5. Rice starch is widely used in cosmetic industry, as thickener in calico printing, in the finish of textiles.
6. Rice starch is also used for making dextrins, glucose and adhesives.
7. Paddy husk is used as a fuel and for making hard boards and as a raw material for alcohol and furfural.
8. Rice bran is used as cattle feed.
9. Edible fatty oil is also obtained from rice, bran, which is known as "bran oil".
10. It is used in soap industry and for making cosmetics.
11. Paddy straw is used as a fodder, in the manufacture of straw boards, and as soil mulch. Not only is this but also used for thatching, making hats, sacks, mats ropes and baskets.

3 Barley

Botanical Name: *Hordium vulgare*

Family: Poaceae (Gramineae)

Uses

1. Barley is important next to rice, wheat, maize in area and production.
2. It is more suitable than wheat in India. Due to hardy nature, it can withstand adverse agro-environments like, drought, salinity, alkalinity, varied topography like plain, hill, under rainfed and irrigated conditions etc.
3. It is a Rabi cereal crop and food for people of cooler and semi-arid part of the world.
4. In India, 90% of barley is used as human food.
5. It is also used for preparation of malt, beer, whisky, industrial alcohol and vinegar.
6. Energy rich drinks like bournvita, boost, horlicks and biscuit are form by barley malt.
7. Medicinal value of barley reduces cholesterol level in liver and also stimulates
fatty acid synthesis in liver.
8. In USA as cattle feed and horse feed.
9. Barley has great demand to malting industry.

4 Maize

Botanical Name: *Zea mays*

Family: Poaceae

Uses

1. Maize is extensively used for food, forage and cattle feed.
2. The grain of maize is quite nutritious with high percentage of easily digestable carbohydrates, fats and proteins. The grains of maize are ground into flour and baked into chapattis.

3. The grains of maize are roasted and eaten.

4. Pop corn and corn flakes are prepared from the grains of maize.

5. The oil obtained from maize is known as corn oil. It is used in making food and corn syrup also. The corn sugar is used in making jams and jellies and other confectionary sweets.

6. The grains are directly fed to milk producing and meat providing live stock.

7. The whole plant is used as cattle feed.

8. The maize starch is widely used as a sizing material in the textile and paper industry.

9. Corn oil, which is obtained from the embryo of maize, is used as lubricant and in soap industry.

10. The culm of maize plant is used for paper making raw material.

11. A specific protein zein remains present in maize which is used for the formation of artificial fibre.

12. By the fermentation of maize grains an alcoholic liquor "chichi" is prepared in Bolivia.

Millets:

Millets are the food crops of small grains which are basically used by the population of rural and tribal areas as staple feed, cattle feed and fodder. The production of millets is highest in India. Millets are generally different genera and species of family Poaceae. On the basis of their edible value millets are divided into two categories- major and minor millets.

Major millets are used for food crops as well as fodder. It includes Sorghum, Pearl millet, Finger millet etc. Minor millets are mainly used for fodder purposes. It includes Kodo millet, Little millet, Fox-tail millet and Barnyard millet etc.

The common names and botanical names of prominent millet crops are given in the table 12.1.

Table 12.1: Common and Botanical names of prominent Millet crops

S. No.	Common name	Botanical name
1.	Pearl millet (Bajra)	*Pennisetum typhoides*
2.	Sorghum (Jowar)	*Sorghum vulgare*
3.	Common millet	*Panicum miliaceum*
4.	Little millet	*Panicum miliare*
5.	Australian millet	*Echinochloa decompositum*
6.	Hungary rice	*Digitaria exilis*
7.	Finger millet (Ragi)	*Eleusine coracana*

Forage Crops

Livestock feed includes all the food requirements of not only domesticated livestock, poultry and fishes, but also wild life. Three major food classes for livestock are recognized: forage, fodder and concentrates. Forage refers to all browse (the tender shoots and fruits of shrubs and trees) and herbaceous animal feed, including silage and green feed.

Berseem
Botanical Name: *Trifolium alexandrinum*
Family: Fabaceae
Lucerne (Alfalfa)
Botanical Name: *Medicago sativa*
Family: Fabaceae

Timber yeiding plant:

1. Sissoo:
Botanical Name:
Dalbergia sissoo Roxb.
Family:
Papilionaceae
Characteristics and uses:
1. A large-sized tree planted along roadsides for shade and for its precious wood.
2. It ranks amongst the finest Indian woods used for cabinet and furniture works.
3. It is used for bent-wood articles, calico-printing blocks, picture frames, musical instruments, boat building, tool handles, hookah, etc.
4. Wood it also used for charcoal making.
5. Its wood pulp is used for making good quality of writing and printing paper.

2. Sal:
Botanical Name:
Shorea robusta Gaertn.
Family:
Dipterocarpaceae
Characteristics and uses:
1. Sal wood ranks second to teak in importance and is widely used for construction work and for railway sleepers.
2. It is also used for ship building, furniture, cabinet works, musical instruments, etc.
3. Saldammar, an oleoresin obtained on tapping its trunk, is used in making paints and varnishes
4. Saldammar is also used for preparing shoe polishes, carbon papers, typewriter ribbons, and as an ingredient of samagri which is burnt in religious ceremonies.
5. Saldammar is used as a cementing material for plywood's, asbestos sheets and also for similar purposes in building boats and shops.
6. Sal trees serve as good host for tasar silkworms.
7. Leaves of sal trees are also sometimes used for making Bidies.

3. Teak:
Botanical Name:
Tectona grandis L.
Family:
Verbenaceae
Characteristics and uses:
1. This large tree is distributed widely in central India, Bihar and Western Peninsula.
2. Wood is used on a very large scale in building ships, bridges, railway sleepers, furniture and cabinet work and for making high class models.
3. It is also used for musical instruments including body of Harmonium, Violin keys and long neck of Sitars.
4. Several types of plywood and blackboards are made from teak wood.
5. Its wood-waste is used for making particle board, fibre board, chip board and plastic board.

Sins: *Albizzia lebbeck.*
Arjun: *Terminalia arjuna.*
Indian Almond: *Terminalia catappa*.
Harir: *Terminalia chebula*.
Hollock: *Terminalia myriocarpa*.
Deodar: *Cedrus deodara*.

Fiber yielding plant:

1. Cotton:

Botanical Names:

Gossypium sp. (*G. arboreum, G. barbadense, G. berbaceum* and *G. hirsutum*).

Family:

Malvaceae.

Characteristics and uses:

1. It is the chief fibre plant which supplies more than 70% of the world consumption of fibres.
2. Fibres occur on the seeds in the form of flattened, twisted and tubular hairs (Fig. 77).
3. Raw cotton consists of cellulose (94%), protein (1.3%), pectic substances (0.9% to 1.2%), water (0.6%), sugar (0.3%) and some pigment traces.
4. Chief use of the cotton is in the textile industry. Other uses include stuffing of pillows, cushions, and also in rubber tyre fabrics.
5. Short fibres, called linters or fuzz, are used for superior acetates and viscose rayons, cellulose ethers and esters, and nitrocellulose lacquers.
6. Hull, the outer covering of cotton seeds, is used for cattle feeding, fertilizers, fillers in plastics, and manufacture of insulating materials.
7. A most important semidrying oil (cotton seed oil) is obtained from the seeds of cotton. It is used as a cooking oil. Other uses of this oil include in the preparation of soaps, paints and varnishes, washing powders, artificial leather, glycerine, nitroglycerin, etc.
8. Seed cake is used as fertilizer and as cattle feed.
9. Gossypium roots are used in some fevers.
10. Its seeds are used in diseases like gonorrhoea, catarrh and gleet.
11. Leaf juice of *G. berbaceum* is used in scorpion and snake bites.
12. Cotton fibre is also used in paper industry, specially in preparing fine quality paper.

2. Jute:

Botanical Name:

Corcborus capsularis, C. olitorius.

Family:

Tiliaceae.

Characteristics and uses:

1. It is a chief fibre-yielding annual shrub.
2. Fibres are obtained from the stem of two species, i.e., *C. capsularis* and *C. olitorius*.
3. It is a bast fibre, obtained from the secondary phloem by the process of retting.
4. Jute is cultivated in West Bengal, Assam, Orissa, Bihar and U.P.
5. Jute fibre is used in making carpets, coarse material, twine, gunny bags, paper and many similar articles.
6. Jute forms the base of linoleum and wool carpet industry.

7. High quality grease-proof paper is prepared from the fibres of jute sticks. It is used commonly is confectionery industry for wrapping greasy materials.

8. Dried leaves of *C. capsularis* are also used as a remedy for dysentry.

9. Besides India, Bangladesh is also a chief jute- producing country of the world.

3. Sunnhemp:

Botanical Name:

Crotalaria juncea L.

Family:

Papilionaceae.

Characteristics and uses:

1. This tall shrub is cultivated throughout India, particularly in Maharashtra, Tamil Nadu, West Bengal and U.P.

2. The valuable fibre is obtained from its long stem.

3- The fibre is light coloured, strong, thick and very durable.

4. The fibre is used for twine, fishing nets, mattings, cot stringing, bags, ropes and coarse canvas.

5. Fibre is particularly suitable for cigarette paper and high quality tissue paper, mainly because of its high cellulose and low ash content.

4. Hemp:

Botanical Name:

Cannabis sativa L.

Family:

Cannabinaceae

Characteristics and uses:

1. It is an annual plant with grooved stem and palmately divided leaves.

2. Plants are dioecious, and yield three products namely bast fibre from its stem, oil from its seeds, and narcotics (Bhaang, Charas and Ganja) from its leaves and flowers.

3. The fibre is long, strong and durable but lacks flexibility and elasticity, and is used for the manufacture of ropes, twine, carpets, bags, nets, etc. Finer fibres are produced from male plants, while its female plants produce coarse fibres.

4. Oil of hemp is obtained from its seeds. It is used in making paints, varnishes and soft soap.

5. Narcotic substances such as Bhaang, Ganja and Charas are obtained from various parts of this plant:

5. Flax:

Botanical Name:

Linum usitatissimum L.

Family:

Linaceae

Characteristics and uses:

1. This valuable fiber plant is much superior to cotton in quality, and provides a finer fabric.

2. In India, it is grown in U.P., M.P., Bihar, Rajasthan and Maharashtra as an oilseed crop.

3. Fibers of flax are obtained from its stem.

4. Fibers are remarkable for their great tensile strength, length of staple, fineness and durability. They are used in the manufacture of linen cloth, canvas, carpets, threads, strong twine, cigarette paper, finest writing paper, etc.

5. Oil obtained from its seeds is used for preparing varnish, paints, printers ink, soft soap, etc.

6. Oil is used for edible purposes. It is also used in lubricants, greases, polishes, etc.

7. Oilcake is a valuable cattle feed and also used as manure.

8. Oil is a good laxative for cattle and horses.

9. A poultice of crushed seeds is also useful on ulcers, inflammations, etc.

Coir

Botanical name: *Cocos nucifera* L.

Family: Palmaceae

Uses

1. Besides its main use as floor covering and in rope making, coir fibre extends extensive use as packaging material to protect goods against shock in transport.

2. Coir fibre finds its use in the production of activated carbon, artificial horse hair, paper pulp, roofing tiles, writing boards, thermal insulation, high stretch paper, manufacture of olive oil filters etc.

3. The coir is rubberised for making cushion seating for automobiles and railways.

4. Coir yarn has been found to be idea1 for hop wines, used in breweries in the U.S.A.

5. Coir bags are used in tea estates for collecting tea leaves and for transportation.

6. Coir yarn in used for making fenders which are attached to ships and boats for absorbing the shock of collision.

7. Coir mats are used for commercial packing purposes and circular mats are used for packing.

8. Coir matting, after bituminisation, offer possibilities of being used as floor covering in godowns to withstand moisture absorption by stored goods.

9. Rubber-backed coir mats are soil-proof, sound absorbent and do not scratch polished floors.

10. Heavy matting made out of thick coir rope is being used for transporting gas cylinders. It is used as a strainer in tube wells in place of wire mesh.

11. Hardboard made of coconut husk shorts and coir dust is durable, smooth, insect proof, fire retarding and water-repellent. It can be sawed, nailed, glued and finished for specific requirements.

12. Coir waste has been used in the manufacture of coirolite by incorporating it with resins and other ingredients by the usual techniques of plastic manufacture. The powder obtained is hot-pressed to obtain articles of any shape using appropriate moulds. It is a tough and hard material and possesses good strength and electrical resistance.

Silk Cotton

Botanical Name: *Bombax cieba*

Family: Malvaceae

Uses

1. The fibre of Bombax cieba is light, very buoyant, resilient, resistant to water, but it is very flammable.

2. It is used as an alternative to down as filling in mattresses, pillows, upholstery and stuffed toys such as teddy bears and for insulation.

3. Previously, it was much used in life jackets and similar devices until synthetic materials largely replaced the fibre.

4. The seeds produce oil that is used in soap industries.

5. The fibres create a seal that allows the pressure to force the dart through the tube.

6. The flowers are an important source of nectar and pollen for honey bees.

Oil yielding plant

Castor Seed or Castor Bean:

Botanical Name:

Ricinus communis L.

Family:

Eupborbiaceae

Characteristics and uses:

1. These are the seeds of *Ricinus communis*, a member of family Euphcrbiaceae.
2. Plant is a tall shrub with large rounded, partly lobed leaves and big terminal branches of flowers.
3. Seeds are oblong with crustaceous seed coat.
4. The leaves are applied on head to relieve headache.
5. Oil obtained from seeds, called castor oil, is used as a purgative.
6. Castor oil is also used in making contraceptive jellies, washing powders and creams.
7. Because of a very low freezing point, castor oil ideally suits for the lubrication of aeroplane engines.
8. The oil is also used for transparent soaps, typewriter-inks, paints and varnishes.
9. It is also used for preparation of hair oils, hair fixers, hair lotions, lipsticks and aromatic perfumes.
10. Seed cake is used as fertilizer.
11. Writing- and printing papers are made from its wood pulp.
12. The alkaloids ricinine and toxalhuminricin are obtained from the plant.

2. Mustard:

Botanical Name:

Brassica campestris var. sarson (Now *Brassica napus* L. var. glauca (Roxb.) Schulz.

Family:

Cruciferae or Brassicaceae.

Characteristics and uses:

1. It is the most common edible, oil-yielding plant.
2. Oil is obtained from the seeds.
3. Oil content varies from 30 to 45%.
4. Oil is widely used for cooking.
5. Oil is also used in lamps, in the manufacture of soap and rubber substitutes.
6. Oil cake is used as a cattle feed.
7. Seeds are used as condiment in preparation of pickles and for flavouring curries.
8. The leaves and tender twigs are cooked as a vegetable named “Sarson-Ka-Saag”.

Groundnut

Botanical Name: *Arachis hypogaea*

Family: Fabaceae

Uses

1. The oil of ground nut is predominantly used for culinary purposes.
2. Vegetable ghee is prepared from the hydrogenation of the oil which mainly used for cooking and other food purposes.
3. The lower grade oil is used in soap industries and as lubricant.
4. The oil is also used as emollinent and laxative.

5. The residue left after the oil extraction is known as oil cake or khali and used as cattle feed.

6. The superior quality oil cake is often mixed in wheat flour after milling and used for making chapattis, which are rich in proteins.

7. Seeds are commonly eaten raw or fried before use, these serve the purpose of food supplement because of richness in proteins and vitamins.

8. The crop plants left after the harvesting of crop are used as fodder.

9. The pericarp of pod is used as manure.

10. The peeled of kernels are used for making peanut butter.

11. The groundnut oil emulsion is used for the control of many insect pests of the plants. This emulsion further increases the toxicity of some common insecticides like rotenine, nicotine and nicotine sulphate.

Soyabean

Botanical Name: *Glycine max*

Family: Fabaceae

Uses

1. Approximately 85% of the world's Soyabean crop is processed into Soyabean meal and vegetable oil.

2. Soyabeans can be broadly classified as "vegetable" (garden) or field (oil) types.

3. Soyabean is most excellent for its high (38–45%) protein content as well as its high (approximately 20%) oil content.

4. The bulk of the Soyabean crop is grown for oil production, with the highprotein defatted and "toasted" soy meal used as livestock feed.

5. A smaller percentage of Soyabeans are used directly for human consumption.

6. Immature Soyabeans may be boiled whole in their green pods and served with salt, under the Japanese name ‘edamame’.

Coconut

Botanical Name: *Cocos nucifera*

Family: Arecaceae (Palmae)

Coconut is an important plant of which every plant part is useful. It is also said to be one of the greatest gift provided by nature to mankind. It is the chief source of coir fibres, timber wood and vegetable oil.

Uses

1. Coconut oil is used for culinary purposes.

2. It is also used as hair oil.

3. Coconut oil is popularly used for the manufacture of superior quality soaps, shampoo, shaving cream and other cosmetics.

4. Coconut oil is commonly used for candle sticks and making lubricants.

5. Endosperm is eaten raw and its powder is also used in the preparation of sweets, confectionary and other delicacies.

6. Growth hormones are present in liquid endosperm; therefore it is used as a component for making culture media, in plant tissue culture experiments. Besides this coconut milk is also taken as favourite drink.

7. Special type of sap or juice is obtained by making incision in closed spadix of coconut. It is boiled for making "Jaggery". Alocoholic liquor and vinegar is also prepared by its fermentation.

8. Fibrous mesocarp is an exclusive source of coir, which is used in the manufacture of various products.

9. Tree trunks of coconut are used as girdles for making huts and leaves are used for thatching, or for making mats, baskets and brooms.

10. The dried coconut fruits are used in various religious purposes and offerings in temples, exclusively in Hindus.

Sunflower

Botanical Name: *Helianthus annuus*

Family: Asteraceae

Uses

1. The seeds of sunflower are edible. They can be eaten raw, cooked, roasted or dried. They are a popular, nutritious snack containing a good source of protein, vitamins A, B and E, Ca, N and Fe.
2. The petals are also edible; they can be cooked and eaten like artichokes.
3. The seed heads are also a source of food for birds and animals.
4. Sunflower seeds are a major ingredient in commercial birdseed.
5. Sunflower leaves are used as feed for livestock.
6. Sunflower oil is a popular vegetable oil known for its light colour, mild flavour, low levels of saturated fats and ability to withstand high cooking temperatures.
7. The oil can also be added to soap, lubricants and candles.
8. Sunflower oil can help relieve skin conditions, haemorrhoids and ulcers.
9. Sunflower roots can remove radiation from soils and water. They were used to clean up the Chernobyl disaster.
10. The flowers can be used to make an all natural dye.
11. The stalks are used to make paper and clothes.

3. Sesame or Gingelly Oil:

Botanical Name:

Sesamum indicum L.

Family:

Pedaliaceae

Characteristics and uses:

1. The seeds of this herb yield an edible oil used in confectionery and for making margarine, soaps, cosmetics, insecticides, perfumes and several medicines.
2. Seeds are used as topping the bread and rolls and in religious ceremonies by Hindus.
3. Oil cake is a good cattle feed.
4. Sesame flour is also used as protein source in nitrogen balance among children.
5. Hair lices are killed by the mucilaginous juice of the plant.
6. India, China, Sudan, Myanmar, Pakistan and Mexico are the major sesame oil-producing countries. In India, it is grown In U.P., M.P., Rajasthan, Gujarat, Orissa, Maharashtra, Andhra Pradesh and Tamil Nadu.

4. Eucalyptus Oil:

Botanical Names:

Eucalyptus citridora Hook., *E. globulus* Labill, *E. rostrata* Schlecht**.**

Family:

Myrtaceae

Characteristics and uses:

1. Essential oil obtained from the leaves of these and several other species of Eucalyptus is used in perfumery.

2. The oil is also a source of citronellal used for manufacture of citronellol and menthol.
3. The eucalyptus oil is also used in several germicides and disinfectants.
4. The oil from the leaves and terminal branchlets of *E. globulus* is used as mosquito repellent.
5. In cinema halls, eucalyptus oil is used as an ingredient of deodorizing and asepticizing compositions.
6. Oil is also used in curing bronchitis and asthma.
7. Wood pulp of these species is used in manufacture of good quality writing- and printing paper.
8. These trees are also valuable as shade and shelter trees.
9. An excellent charcoal is prepared from *E. rostrata*.
10. Extensive plantations of *E. citridora* has been undertaken during last few decades in U.P., Haryana, Punjab and Andhra Pradesh. E.globulus is cultivated mainly at hill stations of India. Large scale plantations of *E. rostrata* have recently been made in U.P., M.P. and Karnataka.

5. Peppermint:
Botanical Name:
Mentha piperita
Family:
Labiatae or Lamiaceae
Characteristics and uses:
1. The oil obtained from the leaves is widely used in perfumes and various types of soaps.
2. Medicinally the oil is used as carminative, stimulant and for treating nausea and vomiting.
3. Oil is also used widely in rheumatic pains, cough syrups, mouth washes and inhalations.
4. Several headache ointments are also prepared from peppermint.
5. Its leaves are also used for flavouring purposes.
6. It is widely cultivated in Punjab, Maharashtra and Kashmir.

Beverage yielding plant:

1. Tea:
Botanical Name:
Camellia sinensis (L). O. Kuntze (Syn. *Thea sinensis* L.)
Family:
Theaceae
Characteristics and uses:
1. It is the most common non-alcoholic beverage, used by more than one-half of the world's population.
2. It is obtained from the dried leaves of Camellia sinensis, a native of Assam or China.
3. Tea plant is a small shrub with leathery, lanceolate leaves having serrate margin and many oil glands.
4. A volatile oil, tannin (13 to 18%), and an alkaloid theine (2-5%), are present in tea.
5. Tea leaves, when infused in hot water, dissolve their alkaloid and oil, and the beverage thus resulted has a stimulating effect. If boiled for a longer period, tannin dissolves making the beverage less beneficial.
6. India is a leading tea-exporting country.
7. For preparing green tea, the leaves are steamed and dried without fermenting, while for black tea the leaves are withered, rolled, fermented and dried.
8. Tannins of tea waste are used in plywood industry.
9. Assam, Darjeeling, Nilgiris, Dehradun and Kumaon are the main tea-producing regions in India.

2. Coffee:
Botanical Names:
Coffea arabica (Arabian Coffee); *C. canepbora* syn. *C. robusta* (Congo Coffee); *C. liberica* (Liberian Coffee).

Family:

Rubiaceae

Characteristics and uses:

1. It is an important non-alcoholic beverage, like tea.

2. More than 90% coffee is obtained from the berries of *Coffea arabica*.

3. Coffee beans are roasted for developing the aroma, flavour and colour and finally ground before they reach to the consumer.

4. "Beans" contain caffeine (0.75 to 1.5%), a volatile oil, glucose, dextrins, proteins and a fatty oil.

5. Caffeine provide stimulating effect while the volatile oil (caffeole) is responsible for aroma and flavour.

6. Leaves and fruits of Coffee also contain some alkaloids.

7. In India, *C. arabica* is grown in Nilgiris and Kamataka.

8. Seeds of *C. canepbora* (syn. *C. robusta*), a robust evergreen shrub, are used in making "instant coffee".

Alcoholic Beverages:

These are obtained from anaerobic fermentation of sugars by yeasts. The three important sugar containing crops are grapes (*Vitis vinefera*) used in making wines and brandy; barley (*Hordeum vulgare*) whose germinating seeds used for making beer and whiskies and molasses of sugarcane (*Saccharum officinarum*) used in making rum.

Spices yielding plant:

These are pungent or aromatic plant products which are used for flavoring food and making it more palatable and tasty. Species are derived from plant parts other than leaves.

Some of them are:

i. Chillies – fruits of *Capsicum frutescens*.

ii. Coriander (Dhania) – fruits of *Coriandrum sativum*.

iii. Black pepper (Kali mirach) – dried fruits of *Piper nigrum*.

iv. Cinnamon (Dal-chini) – dried inner bark of *Cinnamomum zeylanicum*.

v. Cardamons (Elaichi) – dried seeds *Elettaria cardenomum*.

vi. Cloves (Laung) – dried flower buds of *Syzygium aromaticum*.

vii. Cumin (Zeera) – fruits of *Cuminum cyminum*.

viii. Turmeric (Haldi) – rhizome of *Curcuma domestica*.

ix. Saffron (Kesar) – dried stigmas and style-tops of the flower of *Crocus sativus*.

x. Thyme (Ajwain) – dried fruits of *Thymus vulgaris*.

xi. Nutmeg (Jaiphal) – kernel of dried fruit of *Myristica fragrans*.

xii. Vanilla – dried unripe-fruit of *Vanilla plantifolia*.

Medicinal plant:

1. Sarpagandha or Serpentine:

Botanical Name:

Rauvolfia serpentina (L.) Benth.ex Kurz.

Family:

Apocynaceae

Characteristics and uses:

1. It is a small shrub with long leaves, white or pinkish flowers, and small dark purple to black fruits.

2. It is an age-old well-known medicinal plant in the Indian medicine, mentioned even in Charak Samhita.

3. The drug is obtained from air died roots.

4. It is well known for reducing high blood pressure and also as a sedative and hypnotic. The alkaloids found in its roots have tranquillizing effects.

5. Due to the tranquillizing effects of the drug it is also used for chronic mental illness.

6. Extracts of roots are useful for treatment of intestinal disorders.

7. Roots mixed with some other plant extracts, are used in the treatment of cholera and fever.

8. Its roots stimulate uterine contraction and are thus used during child birth.

9. Plants are widely distributed in Sub-Himalayan tracts from Punjab to Nepal, Sikkim, Bhutan, Assam, Western Ghats and Andamans.

2. Opium Poppy:

Botanical Name:

Papaver somniferum L.

Family:

Papaveraceae

Characteristics and uses:

1. It is a herb, now grown on a large scale in UP., Jullundur and Hoshiarpur districts of Punjab, M.P. and Rajasthan.

2. Opium is the dried juice which exudes from the injured immature capsules (Fig. 88) of this plant.

3. It is highly useful for mankind in relieving the pain, if used in limited doses, mainly due to the presence of some alkaloids. But its heavy doses and to take it as a habit can be so fatal that no other drug has caused so much corruption and tragedy as opium.

4. Opium is used to induce sleep, relieve pain and relax spasm.

5. Besides other resins and oils, the important alkaloids obtained from the dried latex of the opium plant are morphine, codeine, papaverine and narcotine.

6. Morphine is a powerful analgesic, narcotic and stimulant.

7. Besides many European countries, India and China are two great sufferors of opium.

3. Belladona:

Botanical Name:

Atropa belladona L.

Family:

Solanaceae

Characteristics and uses:

1. This plant, the roots of which are used as sedative, stimulant and antispasmodic, is cultivated in Kashmir and some other Indian states.

2. Roots and leaves are also used as tonic.

3. The drug atropine, obtained from this plant, is used to dilate pupils of eye at the time of eye testing.

4. In certain poisoning cases of snakebites and scorpionbites, it is used as an antidote.

5. Two famous alkaloids found in this drug are atropine and hyoscyamine.

4. Datura or Stramonium:

Botanical Name:

Datura stramonium L.

Family:

Solanaceae

Characteristics and uses:

1. It is an annual plant, the leaves and seeds of which are narcotic.
2. The drug, obtained from its dried leaves, flowering tops and seeds, is used in treatment of asthma.
3. Atropine, an alkaloid obtained from Stramonium, is used to stimulate central nervous system and also for dilating the pupils of the eye in eye testing.
4. Leaves and seeds are used sometimes for criminal poisoning.
5. Kanaka Asava, an Ayurvedic medicine prepared from Stramonium, is used as an antispasmodic, expectorant and demulcent.
6. Its seeds are smoked by the patients suffering from asthma.

5. Quinine:

Botanical Name:

Cinchona sp.

Family:

Rubiaceae

Characteristics and uses:

1. Quinine, a well-known medicine for malarial fever, is obtained from the dried bark of *Cinchona calisaya, C. officinalis, C. ledgeriana and C. succirubra*.
2. In India, *C. calisaya* is found in Nilgiris and Sikkim, *C. ledgeriana* in West Bengal, Khasi Hills and South India, and *C. succirubra* in hilly regions of Sikkim and South India.
3. Quinine also checks certain bacterial infections, and some of its preparations are useful in amoebic dysentry.
4. Quinine, if used in high quantity, may cause deafness, blindness and nausea.
5. Its use should be avoided by heart patients and pregnant women.

6. Ashvagandha:

Botanical Name:

Withania somnifera L. Dunal.

Family:

Solanaceae

Characteristics and uses:

1. It is a small under-shrub with ovate leaves and pale green small flowers arranged in axillary clusters.
2. From the roots of the plant, the drug Ashvagandha is obtained.
3. It is useful for sexual weakness.
4. It is also used in rheumatism and general weakness, and functions as a narcotic and diuretic.
5. Roots and leaves are antibacterial and antibiotic in activity.

7. Ocimum or Holy Basil:

Botanical Name:

Ocimum sanctum L.

Family:

Labiatae or Lamiaceae

Characteristics and uses:

1. The Sacred basil or Holy basil is a well-known sacred plant of Indian houses, gardens and temples all over the country.
2. An infusion of its leaves in hot water, and specially along with tea, is useful in cold, bronchitis and digestive disorders.

3. The oil present in its leaves has the ability to destroy bacteria and some insects.
4. A poultice of its fresh leaves in applied on ringworm and other diseases of skin.
5. Its seeds are used in disorders of the urinary system.
6. Seeds are also useful in dysentery and chronic diarrhoea.

8. Ephedra:
Botanical Names:
Ephedra gerardiana Wall., *E. major* Host.
Family:
Ephedraceae (Gnetales, Gymnosperms)
Characteristics and uses:
1. Ephedra is a small xerophytic shrub of Gymnosperms found in the drier regions of Himalayas from Kashmir to Sikkim.
2. The plants resemble *Equisetum* in their external morphology and bear profusely branched jointed stem which bears nodes and internodes. Foliage leaves are absent. Scaly leaves are present only on the nodes.
3. The famous antibiotic drug ephedrine, is obtained from its dried stems.
4. Ephedrine is used to treat asthma, cold and hay fever.
5. It is also effective as a good cardiac stimulant.
6. Nasal spray, prepared from ephedrine, is effective in inflammation of mucous membrane and also in asthmatic attacks.
7. Stems of *E. major* are the richest source of ephedrine.

Vasaka:
Adhatoda vasica Nees., of family Acanthaceae**.**
This is a perennial shrub. An evergreen shrub about 4-8 feet in height. Leaves elliptic, lanceolate, acuminate, dark green above, pale beneath. Flowers in short dense-axillary peduncled spikes, white with pink or purple stripes. Flowers after rainy season.
Uses:
The leaves contain the alkaloid vascine; they are a powerful expectorant and antispasmodic; they are commonly used in chest diseases, especially phthisis. A decoction of the leaves or their powder is also given, especially for chronic bronchitis and asthma. The leaf juice is given in diarrhoea and dysentery. The leaves are given as a febrifuge in malarial fevers. For relief of asthma the dried leaves are smoked. A poultice of the leaves is applied over fresh wounds, rheumatic joints and inflammatory swellings. A warm decoction of the leaves is used for scabies and other skin diseases.
The flowers and fruits are bitter, aromatic and antispasmodic.
The root is expectorant, antispasmodic, antiseptic, antiperiodic and anthelmintic. It is given in malarial fevers, diseases of the respiratory system, diphtheria and gonorrhoea. The root and the bark have the same medicinal uses as the leaves.
The preparations are—Vansa Khand and Chyavanprash.

Indian Aloe:
Aloe barbedensis Mill., of family Liliaceae.
The plant is about 2-3 feet high. The leaves are fleshly tapering to a blunt point, smooth, pale green, having horny prickles on their margins. The plant is found throughout our country.
Uses:
After removing the skin of leaves they are given in fevers, enlargement of the liver, spleen and other glands, skin diseases, gonorrhoea, constipation, menstrual suppression, piles, jaundice and rheumatic affections. In the diseases of liver and spleen the pulp of one leaf is administered with black salt and ginger. The juice of roasted leaf is being given with honey for cough and cold.
The pulp of one leaf is being given daily in abdominal tumors, dropsy, carbuncles, piles, sciatica, rheumatism and retention of urine in fevers. A salad of leaves is eaten in indigestion, constipation and

flatulence. The leaf juice is given as a remedy for intestinal worms in children. A poultice of the leaves is applied to tumours, cysts, inflamed parts and scalds.

Indian Pennywort:
Centella asiatica (L.) Urban., of family Umbelliferae (Apiaceae).
This is a common trailing herb, rooting at the nodes. Commonly found throughout India.
Uses:
The weed is alterative, tonic, diuretic, antiphlogistic, blood purifier and local stimulant. It is a remedy for skin diseases, like chronic eczema, chronic ulcers etc., enlargement of glands, chronic rheumatism, chronic nervous diseases, madness, cholera, amenorrhoea and piles. The leaves are a household remedy in early stages of dysentery of children.
The powder of leaves is given with milk in small doses in mental weakness and to improve memory. The fresh juice of leaves mixed with milk is given as an alternative in gonorrhoea, jaundice and fevers; this is also useful for children in skin diseases and for improving the blood and nervous system. An ointment made of leaf juice and lanoline is of great value in elephantiasis.

Betel Pepper:
Piper betel Linn., of family Piperaceae;
This is a climbing shrub cultivated for its leaves.
Uses:
The leaves are astringent, aromatic, antiseptic, carminative, aphrodisiac, stimulant, expectorant and sialogogue. The leaf juice with honey is given to children in colic, indigestion, diarrhoea and laryngitis. The leaves are chewed to remove foul odour from the mouth.
The leaf juice is used as eye drops in ophthalmia, and other painful eye diseases and night blindness. Oil coated leaves are a useful dressing for blistered surfaces and ulcers. The essential oil extracted from the leaves, is given in catarrhal and pulmonary diseases.

Foxglove (*Digitalis purpurea*) – contain glycosides (digitonin and digoxin) which are effective in treatment of heart failure.

Dyes:

i. Amla:
Botanical name: *Emblica officinalis* Gaertn.
Family: Euphorbiaceae
Plant part used: Fruit
Uses:
Dried fruit is used as dye after boiling is water and yields bluish black colour called abnusi and is used to dye hair.

ii. Arusa:
Botanical name: *Justicia adhatoda* L.
Family: Acanthaceae
Plant part used: Leaves
Uses:
Yellow dye is obtained from leaves by boiling in water. Where combined with indigo the dye becomes dark-blue green.

iii. Curcuma:
Botanical name: *Curcuma longa* L.
Family: Zingiberaceae
Plant part used: Rhizome

Use:
1. Yields yellow dye
2. When added to Alkali turns red and is known as Kumkum.

iv. Dhak:
Botanical name: *Butea monosperma* (Lam.) Kuntze.
Family: Fabaceae
Plant part used: Flower
Uses:
Flowers yield a yellow dye called tesu.

v. Dhai:
Botanical name: *Woodfordia floribunda* Salisb.
Family: Lythraceae
Plant Part used: Flower
Use:
Red flowers and leaves yield yellow dye used is dyeing silk.

vi. Genda:
Botanical name: *Tagetes erecta* Linn.
Family: Asteraceae
Plant part used: Flower
Uses: Flower yields yellow dye.

vii. Harr:
Botanical name: *Terminalia chebula* Retz.
Family: Combretaceae
Plant part used: Rind of dried fruit.
Uses:
1. Fruit of chebulic myrobalans is black and used to dye and tan.
2. Galls of leaves with alum yield permanent yellow dye.

viii. Kamel:
Botanical name: *Mallotus phillipinensis* Muell.
Family: Euphorbiaceae
Plant part used: Ripe fruit
Uses:
Ripe fruit covered with a powder yielding yellow dye or orange dye used in dyeing silk and wool.

ix. Khair:
Botanical name: *Acacia catechu* wild
Family: Mimosaceae
Plant part used: Red heart wood.
Uses: Kattha is obtained (Red or Brown colour) used in paan and medicine.

x. Nagarmotha:
Botanical name: *Mariscus cyperinus*
Family: Cyperaceae
Plant part used: Root.
Uses: Roots yield dye used to dye cloths.

xi. Rose Lichen:

Botanical name: *Parmelia kamschadalis* Ach.

Family: Parmeliaceae

Plant part used: Thallus.

Uses: Lichen is used in dyes and calico printing of cloth in rose colour.

Rubber:

i. Para Rubber:

Botanical Name: *Hevea brasiliensis* (H Bqk) Muell-Arg.

Family: Euphoribiaceae

Plant part used: Latex

Uses:

1. Rubber is used to make structural materials, soft comfortable. Materials, elastic, conductors, shock absorbers etc.
2. It is used in making mountings for motors and other machinery, gasket, transport materials, translucent materials, sports goods, paints, tyres etc.
3. It is also used in pharmaceutical industries.

ii. Guayule:

Botanical name: *Parthenium argentatum* Gray

Family: Asteraceae

Plant part used: Rubber is accumulated in both tops and roots of the plant. Plants are harvested to get it.

Uses:

1. It is low quality rubber in comparison to Para rubber.
2. It is used for making floor covering, rugs, rubber backs, gloves, hot water bottles, ice caps etc.

Major Rubber producing plants

1.	**Para Rubber**	*Hevea brasiliensis*	Euphorbiaceae	Latex
2.	**Panama Rubber**	*Castilla elastica*	Moraceae	Latex
3.	**Assam Rubber**	*Ficus elastica*	Moraceae	Latex from roots and stems.
4.	**Ceara Rubber**	*Manihot glaziovii*	Euphorbiacae	Latex
	Substitutes of Rubber			
1.	**Guayule**	*Parthenium argentatum*	Asteraceae	Latex from roots
2.	**Russian dandalion**	*Taraxacum koksaghyz*	Asteraceae	Latex from roots
3.	**Balatas Gutta parcha**	*Palaquium gutta*		Coagulated latex for chewing gum
4.	**Sapodilla Chick**	*Achras zapota*	Sapotaceae	Chewing gum

Characteristics of Rubber:

1. It is tough despite of its softness.

2. It is non-conductor of electricity.

3. It is versatile material.

4. Natural rubber is polymer of Isoprene.

5. It is shock absorbent and moisture proof or water proof.

Uses of Rubber:

1. It is used to make many products like washers, gaskets, eraser, sole, heel, balls, bands, roller, elastic, stopper, gloves etc.

2. It is used to make tyres, tubes of cycle, car two wheelers, jeep, truck, three wheelers etc.

3. It is used in making surgical instruments.

4. It is used to make water proof substances, wire insulation and in cushioning.

5. Neoprene is a synthetic product resembling natural rubber in its tensile strength and widely used in electric industry in making sheathing jackets of wires, cables etc.

6. It is used in making conveyor belts, hosepipes, fabrics, coats etc.

7. Silicone rubber is used in stationary seals for high temperature oil lines, gaskets, cable insulation etc.

Gum:

Gums are found naturally as cementing substances between cells i.e., middle lamella or decomposition products of cellulose. The process of disintegrating internal tissue is called gummosis. Gums are colloidal in nature.

They are water soluble but insoluble in alcohol or oils. Gums exude naturally from stem, root or leaves due to wounds or burns. Commercial gums are in the form of dried exudation.

Uses:

Gums are used as adhesives in printing and finishing textiles, candy industry, in medicine, as sizing agent for paper and in food industry. Most common and useful gums are Gum arabic, gum tragacanth and Karayan gum.

i. Gum Arabic:

Botanical name: *Acacia Senegal* (L.) Willd.

Family: Mimosaceae

Plant part used: Gum from bark of trees.

Table. Gums

Some important gum producing plants

S. No.	Name	Botanical name	Family	Use
1.	**Gum Arabic**	*Acacia senagal*	Mimosaceae	It is used as adhesive in textile, mucilage part, polish, confectionary, deserts, painting, emulsifier and medicine.
2.	**Gum. Traga-canth**	*Astragalus gummifer*	Fabacace	Used in calico printing, medicines, adhesive for pills and torches.
3.	**Babool gum**	*Acacia arabica*	Mimosaceae	Used in confectionary
4.	**Guar gum**	*Cyamopsis tetragonoloba*	Fabaceae	In food, paper, textile industry
5.	**Kinogum**	*Pterocarpus marsupium*	Fabaceae	In toothache and diarrhoea
6.	**Bengal kino gum**	*Butea frondosa*	Fabaceae	Adultarant of kino gum

Uses:

1. Used as office glue.
2. In food industry.
3. As thickener of sweets, jellies and chewing gum.

ii. Guar Gum:

Botanical name: *Cyamopsis tetragonolobus* Taub.

Family: Fabaceae

Plant part used: Gum from seeds of cluster bean.

Uses:

1. Emulsifier in salad.
2. As stabilizer in ice cream.

Some Important Gum Producing Plants

Some Important Gum Producing Plants

Resin:

These are oxidative products of essential oils, secreted in definite cavities or passages, typically derived from stems or roots as exudates due to incision, bruising, chipping etc.

Characters:

1. Resins are brittle, amorphous and transparent substances.
2. Insoluble in water but soluble in alcohol, either and turpentine.
3. They are made up of resin, acid, anhydride and ester mixtures.
4. They are antiseptic so can prevent decaying.
5. They can lessen the amount of water loss.

Uses:

1. Useful in making soaps, oil cloth, linoleum, medicine, plastic, varnish, firework, floor coating etc.

Resins may be of three types:

A. Hard resin:

Little amount of essential oil, solid, transparent brittle no odour or taste, non-volatile, poor conductor of electricity, burn in air with smoky flame.

Hard resins are of following type:

1. Copals:

No oil, yield hard elastic varnish used in out door works.

a. East African Copal:

Zangibar copal is obtained from *Trachylobium verrucosum* and Inharubane copal is obtained from *Capaifera conjugata* tree found in south east African coast. The resin is natural exudates from tree trunk.

b. West African Copal:

Congo copal is obtained from *Copaifera demeusii* and *Copaifera mopane*. The trees are common in Congo basin. Accra and Benin Copals are obtained from *Daniella ogea*.

c. Kauri Copal:

It is obtained from the kauri bine i.e., *Agathis australis*. The plant is found in New Zealand. It is yellow, transparent and very hard, used in varnish and in making linoleum.

d. South American Copal:

It is obtained from South American locust (*Hymenaea courbaril*) which is a tall tree of Brazil. It is obtained from the stem twings and fruits exudes which drop on the ground.

e. Manila Copal:

It is obtained from Agathis alba which is a tall conifer. Resin is used in enamels and interior work.

2. Amber:

It is fossil resin found along the shores of Baltic Sea. It was obtained from *Pinus succinifera* (extinct sp.) It is used to make mouth piece of pipes, Holder of cigar and to increase elasticity of rayon fibres.

3. Damar:

Damar is obtained from the members of Dipterocarpaceae. It is abundantly found in Malaya in Sumatra. The chief Genera producing damar are *Shorea robusta* (Saal damar), *Vateria indica* (white damar), *Canaricum strictum* (black damar) and *Balanocarpus* etc. Damar is used in spirit, varnishes because of their lustere and light colour.

4. Lacquer:

It is the natural varnish obtained from *Rhus vemiciflua* (Lacquer tree). The tree is native of China. It is cultivated in Japan.

B. Oleoresin:

The oil content is more in addition to resin.

More or less liquid in nature with distinct aroma and flavour such as:

1. Canada Balsam:

It is obtained from *Abies balsamea* (Balsam fir) of Canada and North United states. The resin is collected on elongated blisters on the bark and only small amounts are obtained. It is yellowish or greenish substances usually used as a mounting medium while making permanent slides. It acts as a cement for optical lenses. It is stimulant, antiseptic, irritant and component of collodion. It is fixative of soaps and perfumes.

2. Terpentine:

It is obtained from coniferous trees. It is viscous honey like soft and brittle solid. It is secreted and accumulated in ducts near cambium and exudes naturally as soft, sticky substance. It is used in manufacture of linoleum, oilcloth, printers ink, floor covering, sealing wax, paints, varnishes, solvent of rubber and printing of cloth.

C. Gum Resin:

It is a mixture of true gum and resin combining the characters of both. They contain small quantity of essential oil and large of colouring substance.

Generally obtained from members of Apiaceae and Burseraceae e.g.:

1. Asafoetide (*Ferula asafoetidia*):

The cortex of thick fleshy roots exudes milky juice during rainy season. The root is cut and dried in the shade. The gum resin is collected on the surface in the form of tears. It has powerful odour and bitter arid taste due to sulphur compounds of essential oil. Asafoetida is a flavourant of curries and other food products and a medicine for cough, asthama, digestion etc.

2. Galbanum (*Ferula galbuniflua*):

The gumresin is extracted from lower part of stem. It is used as medicine. It has tenacious aromatic odour.

3. Opopanax (*Commiphora kataf*):

A member of family Burseraceae used in perfumery. It is also used as medicine. Opopanax is also obtained from *Opopanax chironium* of family Apiaceae.

4. Bdellium (*Commiphora mukul*):

Also known as guggulu; used in perfumery, Hawan samagri and as medicine also.

5. Bisabal myrrh (*Commiphora erythoracea*):

Also known as Sweet myrrh. A member of family Burseraceae, this is a myrrh of antiquity and is used in incense, perfumes and embalming. Also used in perfumes and religious ceremonies.

6. Ammaniaceum (*Dorema ammoniaceum*):

It exudes a milk latex from the stem and flowering branches as tears, which are collected and used as medicine in circulatory stimulant. It is also used in perfume industry.

Plants Used as a source for Insecticides

Insecticide kill or repel insects. Natural insecticides include

(1) *Azadirachta indica* (Magosa/Neem)
(2) *Boenighauscniaalhiflora*.
(3) *Peganum harmala*.
(4) *Derris* (rotenone)
(5) *Chrysanthemum* (Pyrethrum).

10. Conservation of plants

Threats to Biodiversity:

Biodiversity is considered as a reservoir of resources to be used for the manufacture of food, medicine, industrial products, etc. But with an increased demand of rapid population growth,

biodiversity is gradually depleting. A number of plants" and ani-mal species have already become extinct and many are endangered.

The different factors responsible for causing threat to biodiversity are as follows:

1. Habitat destruction:

The primary cause of loss of biodiversity is habitat loss or destruction which is resulted due to the large industrial and commercial activities associated with agriculture, irrigation, construction of dams, mining, fishing etc.

2. Habitat fragmentation:

With increased population, the habitats are fragmented into pieces by roads, fields, canals, power lines, towns etc. The isolated fragment of habitats restricts the potential of species for dispersal and colonization. In addition, the habitat fragmentation also brings about microclimatic changes in light, temperature, wind etc.

3. Pollution:

The most dreaded factor inducing loss of biodiversity is environmental pollution which include air pollution, Water pollution, industrial pollution, pollution due to chemical Pastes, pesticides radioactive materials etc.

4. Overexploitation:

The natural resources are over exploited to meet growing rural poverty, intensive technological growth and globalization of economy. All these factors together may be responsible for the extinction of a number of species.

5**. Introduction of exotic species:**

The introduction of exotic species are due to:

(i) horticulture

(ii) agriculture;

(iii) European colonisation and

(iv) accidental transport.

It is seen that some exotic species may kill or eat the native species thereby causing its extinction.

6. Diseases:

Since the animals are more vulnerable to infection, the anthropological activities may increase the incidence of diseases in wild species, leading to their extinction.

7. Shifting or Jhum cultivation:

The shifting or Jhum cultivation by poor tribal people greatly affects the forest structure which is a store house of biodiversity.

8. Poaching of wild life:

A number of wildlife species are becoming extinct due to poaching and hunting.

6. Conservation of Biodiversity:

Biodiversity is being depleted by the loss of habitat, fragmentation of habitat, over exploitation of resources, human sponsored ecosystems, climatic changes, pollution invasive exotic spices, diseases, shifting cultivation, poaching of wild life etc.

Since the human beings are enjoying all the benefits from biodiversity, they should take proper care for the preservation of biodiversity in all its form and good health for the future generation i.e., the human being should prevent the degradation and destruction of the habitats thereby maintaining the biodiversity at its optimum level.

Conservation of biodiversity is protection, upliftment and scientific management of biodiversity so as to maintain it at its threshold level and derive sustainable benefits for the present and future generation. In other words, conservation of bio-diversity is the proper management of the biosphere by human beings in such a way that it gives maximum benefits for the present generation and also develops its potential so as to meet the needs of the future generations.

Mainly the conservation of biodiversity has three basic objectives:

(a) To maintain essential ecological processes and life supporting systems.

(b) To preserve the diversity of species.

(c) To make sustainable utilisation of species and ecosystems.

Strategies for Conservation of Biodiversity:

The following strategies should be undertaken in order to con-serve biodiversity:

(1) All the possible varieties (old or new) of food, forage and timber plants, live stock, agriculture animals and microbes should be conserved.

(2) All the economically important organisms in protected areas should be identified and conserved.

(3) Critical habitats for each species should be identified and safeguarded.

(4) Priority should be given to preserve unique ecosystems.

(5) There should be sustainable utilisation of resources.

(6) International trade in wild life should be highly regulated.

(7) The poaching and hunting of wildlife should be prevented as far as practicable.

(8) Care should be taken for the development of reserves and protected areas.

(9) Efforts should be made to reduce the level of pollutants in the environment.

(10) Public awareness should be created regarding biodiversity and its importance for the living organisms.

(11) Priority should be given in wildlife conservation programme to endangered species over vulnerable species and to vulnerable species over rare species.

(12) The habitats of migratory birds should be protected by bilateral and multilateral agreement.

(13) The over exploitation of useful products of wild life should be prevented.

(14) The useful animals, plants and their wild relatives should be protected both in their natural habitat (in-situ) and in zoological botanical gardens (ex-situ)

(15) Efforts should be made for setting up of National parks and wild life sanctuaries to safeguard the genetic diversity and their continuing evolution.

(16) Environmental laws should be strictly followed.

Conservation Methods:

There are two types of conservation methods namely in-situ and ex-situ conservations. Let us discuss the different conservation methods along with their importance.

(a) *In situ* conservation:

The conservation of species in their natural habitat or natural ecosystem is known as *in situ* conservation. In the process, the natural surrounding or ecosystem is protected and maintained so that all the constituent species (known or unknown) are conserved and benefited. The factors which are detrimental to the existence of species concerned are eliminated by suitable mechanism.

The different **advantages** of *in situ* conservation are as follows:

(a) If is a cheap and convenient way of conserving biological diversity.

(b) It offers a way to preserve a large number of organisms simultaneously, known or unknown to us.

(c) The existence in natural ecosystem provides opportunity to the living organisms to adjust to differed' environmental conditions and to evolve in to a better life form.

The only **disadvantage** of in situ conservation is that it requires large space of earth which is often difficult because of growing demand for space. The protection and management of biodiversity through in situ conservation involve certain specific areas known as protected areas which include national parks, Sanctuaries and Biosphere reserves.

1. Protected areas:

The protected areas are biogeographical areas where biological diversity along with natural and cultural resources are protected, maintained and managed through legal and administrative measures. The demarcation of biodiversity in each area is determined on the basis of climatic and physiological conditions.

In these areas, hunting, firewood collection, timber harvesting etc. are prohibited so that the wild plants and animals can grow and multiply freely without any hindrance. Some protected areas are: Cold desert (Ladakh and Spiti), Hot desert (Thar), Saline Swampy area (Sunderban and Rann of Kutch), Tropical moist deciduous forest (Western Ghats and north East) etc. Protected areas include national parks, sanctuaries and biosphere reserves. There are 37,000 protected areas throughout the world. As per World Conservation Monitoring Centre, India has 581 protected areas, national parks and sanctuaries.

2. National parks:

These are the small reserves meant for the protection of wild life and their natural habitats. These are maintained by government. The area of national parks ranges between 0.04 to 3162 km. The boundaries are well demarcated and circumscribed. The activities like grazing forestry, cultivation and habitat manipulation are not permitted in these areas. There are about 89 national parks in India.

Some important national Parks of India are:

(i) Biological Park, Nandankanan, Orissa,

(ii) Corbett national Park Nainital, U.P. (First national Park)

(iii) Koziranga national Park, Jorhat, Assam

(iv) Tudula national Park, Maharashtra

(v) Hazaribagh national Park, Hazaribagh, Bihar

(vi) Band havgarh national park, M.P.

(vii) Bandipur national park, Karnataka.

(viii) Kanha National Park, M.P.

(ix) Reibul Lamjao National Park, Manipur

(x) Nawgaon National Park, Maharashtra

3. Sanctuaries:

These are the areas where only wild animals (fauna) are present. The activities like harvesting of timbers, collection of forest products, cultivation of lands etc. are permitted as long as these do not interfere with the project. That is, controlled biotic interference is permitted in sanctuaries, which allows visiting of tourists for recreation. The area under a sanctuary remains in between 0.61 to 7818 km.

Some important sanctuaries of India are as follows:

(i) Nandankanan Zoological Park

(ii) Chandaka Elephant reserve

(iii) Simlipal Tiger Reserve

(iv) Bhitarkanika Wild life Sanctuary

(v) Gharial project at Tikarpada

(vi) Chilika (Nalaban) Sanctuary

4. Biosphere reserves:

Biosphere reserves or natural reserves are multipurpose protected areas with boundaries circumscribed by legislation. The main aim of biosphere reserve is to preserve genetic diversity in representative ecosystems by protecting wild animals, traditional life style of inhabitant and domesticated plant/ animal genetic resources. These are scientifically managed allowing only the tourists to visit.

Some importance of biosphere reserves are as follows:

(a) These help in the restoration of degraded ecosystem.

(b) The main role of these reserves is to preserve genetic resources, species, ecosystems, and habitats without disturbing the habitants.

(c) These maintain cultural, social and ecologically sustainable economic developments.
(d) These support education and research in various ecological aspects,

Some important biosphere reserves are:

Simlipal, (Orissa), Sunderban (West Bengal), Kanha (M.P Kaziranga (Assam) etc. The biosphere reserve net work was intro-duced by UNESCO 1971.

Biosphere Reserves of India

Name	**States**	**Year**
Nilgiri Biosphere Reserve	Tamil Nadu, Kerala, Karnataka	2000
Gulf of Mannar Biosphere Reserve	Tamil Nadu	2001
Sundarbans Biosphere Reserve	West Bengal	2001
Nanda Devi Biosphere Reserve	Uttarakhand	2004
Nokrek Biosphere Reserve	Meghalaya	2009
Pachmarhi Biosphere Reserve	Madhya Pradesh	2009
Simlipal Biosphere Reserve	Odisha	2009
Great Nicobar Biosphere Reserve	Great Nicobar	2013
Achanakmar-Amarkantak Biosphere Reserve	Chhattisgarh, Madhya Pradesh	2012
Agasthyamalai Biosphere Reserve	Kerala and Tamil Nadu	2016
Khangchendzonga National Park	Sikkim	2018

(b) Ex-situ conservation:

Ex-situ conservation is the preservation of components of biological diversity outside their natural habitats. This involves conservation of genetic resources, as well as wild and cultivated or species, and draws on a diverse body of techniques and facilities. Such strategies include establishment of botanical gardens, zoos, conservation strands and gene, pollen seed, seedling, tissue culture and DNA banks.

Cryopreservation:

This type of in vitro conservation is done in liquid nitrogen at a temperature of -196°C. This is particularly useful for conserving vegetatively propagated crops, e.g., potato.

Cryopreservation is the storage of material at ultra-low temperature (i.e., -196° C) either by very rapid cooling, as used for storing seeds, or by gradual cooling and simultaneous dehydration, as being done in tissue culture.

In cryopreservation, the material can be stored for a considerable long period of time in compact low maintenance refrigeration units.

Botanic gardens:

Botanic gardens are uniquely positioned to help address the issues relevant to restoring ecosystems. They provide knowledge and expertise in plant taxonomy, horticulture, biodiversity inventory, conservation biology, restoration ecology and ethnobotany – all key elements for achieving successful restoration.

Botanic gardens also collectively serve as a global repository for documented plant material, with at least one-third of all flowering plants maintained in living collections or seed banks. Utilizing knowledge gained from these collections, related herbaria and libraries, and combined with landscape knowledge from field surveys and ecological research, botanic gardens bring the understanding necessary to ensure that restoration leads to adequate taxonomic diversity and incorporates appropriate genetic provenance.

Botanic gardens can therefore restore diverse and ecologically resilient places, avoiding the dangers and pitfalls associated with growing inappropriate plants in the wrong environment.

Botanic gardens are also well-placed to raise awareness amongst the general public of the need for, and benefits that can be derived from, successful ecological restoration projects. The new Ecological Restoration Alliance of Botanic Gardens will thus also address the need for greater political commitment, awareness and participation in restoration work.

Gene bank:

Gene banks are a type of biorepository which preserve genetic material. For plants, this could be by in vitro storage, freezing cuttings from the plant, or stocking the seeds (e.g. in a seedbank). For animals, this is the freezing of sperm and eggs in zoological freezers until further need. With corals, fragments are taken which are stored in water tanks under controlled conditions. Plant genetic material in a 'gene bank' is preserved at -196° Celsius in Liquid Nitrogen as mature seed (dry) or tissue (meristems).

Accession is the common term given to an individual sample in a gene bank, such as a distinct species or variety.

In plants, it is possible to unfreeze the material and propagate it, however, in animals, a living female is required for artificial insemination. While it is often difficult to use frozen animal sperm and eggs, there are many examples of it being done successfully.

In an effort to conserve agricultural biodiversity, gene banks are used to store and conserve the plant genetic resources of major crop plants and their crop wild relatives. There are many gene banks all over the world, with the Svalbard Global Seed Vault being the most famous one.

Seed gene bank:

These are cold storages where seeds are kept under controlled temperature and humidity for storage and this is easiest way to store the germ plasma of plants at low temperature. Seeds preserved under controlled conditions (minus temperature) remain viable for long durations of time.

iv. Tissue culture bank:

Cryopreservation of disease free meristems is very helpful. Long term culture of excised roots and shoots are maintained. Meristem culture is very popular in plant propagation as it's a virus and disease free method of multiplication.

Long term captive breeding:

The method involves capture, maintenance and captive breeding on long term basis of individuals of the endangered species which have lost their habitat permanently or certain highly unfavorable conditions are present in their habitat.

Animal Translocation:

Release of animals in a new locality which come from anywhere else.

Translocation is carried in following cases:

1. When a species on which an animal is dependent becomes rare.
2. When a species is endemic or restricted to a particular area.
3. Due to habit destruction and unfavorable environment conditions.
4. Increase in population in an area.

Zoological Gardens:

In zoos wild animals are maintained in captivity and conservation of wild animals (rare, endangered species). The oldest zoo, the Schonbrumm zoo which exists today also, was established in VIENNA in 1759.

In India, the 1st zoo came into existence at BARRACKPORE in 1800. In world there are about 800 zoos. Such zoos have about 3000 species of vertebrates. Some zoos have undertaken captive breeding programmes.

The **strategies** for ex-situ conservations are:

(i) Identification of species to be conserved.

(ii) Adoption of Different ex-situ methods of conservation.

(i) Long-term captive breeding and propagation for the species which have lost their habitats permanently.

(ii) Short-term propagation and release of the animals in their natural habitat

(iii) Animal translocation

(iv) Animal reintroduction

(v) Advanced technology in the service of endangered species.

The different **advantages** of ex-situ conservation are:

(a) It gives longer life time and breeding activity to animals.

(b) Genetic techniques can be utilised in the process.

(c) Captivity breed species can again be reintroduced in the wild.

Some **disadvantages** of this method are:

(a) The favourable conditions may not be maintained always.

(b) Mew life forms cannot evolve.

(c) This technique involves only few species.

Ramsar Sites:

Six internationally significant wetlands of India have been declared as Ramsar Sites under the Ramsar Convention. To focus attention on urban wetlands threatened by pollution and other anthropogenic

activities, state Governments were requested to identify lakes that could be include the National Lake Conservation Plan (NLCP).

World Heritage Sites:

To be included on the World Heritage List, sites must be of outstanding universal value and meet at least one out of ten selection criteria.

These criteria are explained in the Operational Guidelines for the Implementation of the World Heritage Convention which, besides the text of the Convention, is the main working tool on World Heritage. The criteria are regularly revised by the Committee to reflect the evolution of the World Heritage concept itself.

Until the end of 2004, World Heritage sites were selected on the basis of six cultural and four natural criteria. With the adoption of the revised Operational Guidelines for the Implementation of the World Heritage Convention, only one set of ten criteria exists.

World Heritage Sites in India:

According to UNESCO World Heritage Sites located in India are 37, as of June 2018, the sixth most of any country.

Some e.g.: Ajanta cave, Maharastra; Agra Fort, Uttar Pradesh, Taj Mahal, Uttar Pradesh.

Red Data Book:

The red data book is the state document established for documenting rare and endangered species of animals, plant and fungi as well as some local sub-species that exist within the territory of the state or country. This book provides central information for studies and monitoring programmes on rare and endangered species and their habits.

IUCN Red List:

The IUCN Red List of Threatened Species (also known as the IUCN Red List or Red Data List), founded in 1964, has evolved to become the world's most comprehensive inventory of the global conservation status of biological species. It uses a set of criteria to evaluate the extinction risk of thousands of species and subspecies. These criteria are relevant to all species and all regions of the world. With its strong scientific base, the IUCN Red List is recognized as the most authoritative guide to the status of biological diversity. A series of Regional Red Lists are produced by countries or organizations, which assess the risk of extinction to species within a political management unit.

Categories:

Species are classified by the IUCN Red List into nine groups, specified through criteria such as rate of decline, population size, area of geographic distribution, and degree of population and distribution fragmentation. There is an emphasis on the acceptability of applying any criteria in the absence of high quality data including suspicion and potential future threats, so long as these can reasonably be supported.

Extinct (EX) – beyond reasonable doubt that the species is no longer extant.

Extinct in the wild (EW) – survives only in captivity, cultivation and/or outside native range, as presumed after exhaustive surveys.

Critically endangered (CR) – in a particularly and extremely critical state.

Endangered (EN) – very high risk of extinction in the wild, meets any of criteria A to E for Endangered.

Vulnerable (VU) – meets one of the 5 red list criteria and thus considered to be at high risk of unnatural (human-caused) extinction without further human intervention.

Near threatened (NT) – close to being at high risk of extinction in the near future.

Least concern (LC) – unlikely to become extinct in the near future.

Data deficient (DD)

Not evaluated (NE)

In the IUCN Red List, "threatened" embraces the categories of Critically Endangered, Endangered, and Vulnerable.

The major IUCN threatened categories (IUCN Red List Categories, 1995) currently recognised, together with their definitions are:

(i) Extinct (EX):

Species not definitely located in the wild during the past fifty years but which may survive in cultivation (e.g. *Franklinia aloetamha*). Some authors suggest that 'Extinct' should denote those taxa that have been totally lost and that the terminology 'Extinct in the wild' should be used to refer to species lost in the wild, while living under cultivation (dubbed by IUCN as EW).

(ii) Endangered (EN):

Species in danger of extinction (within a few decades) and whose survival is unlikely if the causal factors continue to operate (Areca concinna, Euphorbia obdelkuri). In this category are included those taxa whose numbers have been reduced to a critical level or whose habitats have been so drastically reduced that they are deemed to be in immediate danger of extinction.

Also included are taxa that may now be extinct even though seen in the wild in the past 50 years. The other criteria are 50% decline in the last 10 years; <5,000 km2 area of occupancy or <500 km2 in fragmented areas; 2,500 individuals or subpopulation of 250 mature individuals.

The category Critically Endangered (CR) includes species that face an extremely high risk of extinction in the wild in the immediate future. These are characterized by 80% decline in the last 10 years, 100 km2 occupancy or 10 km2 in fragmented areas.

(iii) Vulnerable (VU):

Taxa likely to move into the endangered category in near future if the causal factors continue to operate (Ranunculus ophioglossifolius). Included in this category are taxa in which most or all populations decrease in size because of overexploitation, extensive destruction of habitat or other environmental disturbances.

Also included are taxa with populations that are still abundant but under threat from severe adverse factors throughout their distribution range. The other criteria include 50% decline in last 20 years; < 20,000 km2 occupancy or < 2,000 km2 in fragmented populations; 10,000 individuals or subpopulation of 1,000 mature individuals.

(iv) Rare (R):

Taxa with small populations that are not endangered or vulnerable at present but are at risk are included under this category (Lactuca saligna, Salvia saxicola). A species may be rare because of restricted geographical range, high habitat specificity and small local population size, or thinly scattered over a more extensive range, or due to a combination of two or more of these characteristics. Rare species have a population of less than 20,000 individuals. Some species are naturally rare and have never occurred in greater numbers, yet they are able to maintain these numbers. Other species become rare through man's action or other natural forces.

Man and Biosphere Programme:

Man and the Biosphere Programme (MAB) is an intergovernmental scientific programme, launched in 1971 by UNESCO, that aims to establish a scientific basis for the improvement of relationships between people and their environments.

MAB's work engages fully with the international development agenda—specially with the Sustainable Development Goals and the Post 2015 Development Agenda—and addresses challenges linked to scientific, environmental, societal and development issues in diverse ecosystems; from mountain regions to marine, coastal and island areas; from tropical forests to dry lands and urban areas. MAB combines the natural and social sciences, economics and education to improve human livelihoods and the equitable sharing of benefits, and to safeguard natural and managed ecosystems, thus promoting innovative approaches to economic development that are socially and culturally appropriate, and environmentally sustainable.

The MAB programme provides a unique platform for cooperation on research and development, capacity-building and networking to share information, knowledge and experience on three interlinked issues: biodiversity loss, climate change and sustainable development. It contributes not only to better understanding of the environment, but also promotes greater involvement of science and scientists in policy development concerning the wise use of biological diversity.
As of March 2016, 669 biosphere reserves in 120 countries, including 20 transboundary sites, have been included in the World Network of Biosphere Reserves.

CITES:
CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) is an international agreement between governments. Its aim is to ensure that international trade in specimens of wild animals and plants does not threaten their survival.
CITES was drafted as a result of a resolution adopted in 1963 at a meeting of members of IUCN (The World Conservation Union). The text of the Convention was finally agreed at a meeting of representatives of 80 countries in Washington, D.C., the United States of America, on 3 March 1973, and on 1 July 1975 CITES entered in force. The original of the Convention was deposited with the Depositary Government in the Chinese, English, French, Russian and Spanish languages, each version being equally authentic.
CITES is an international agreement to which States and regional economic integration organizations adhere voluntarily. States that have agreed to be bound by the Convention ('joined' CITES) are known as Parties. Although CITES is legally binding on the Parties – in other words they have to implement the Convention – it does not take the place of national laws. Rather it provides a framework to be respected by each Party, which has to adopt its own domestic legislation to ensure that CITES is implemented at the national level.

Hot Spots:
Hot spots are the areas with high density of biodiversity or mega diversity which are most threatened at present. There are 16 hot spots in world, out of which two are located in India namely North-East Himalayas and Western Ghats.
Hot spots are generally determined based on four factors:
(i) Number of species/species diversity
(ii) Degree of endemism (species restricted to a particular area or region)
(iii) Degree of threat to the habitat due to its degradation and fragmentation
(iv) Degree of exploitation
The number of hot spots in the world is 34 covering an area less than 2% of land surface with about 20% of human population living there. There are 15 hot spots in tropical forests, 5 in mediterranean type forests and 9 in island. India has 3 hot spots – India-Burma, Himalaya and Western Ghats – Sri Lanka. India is considered as a country of mega-diversity with 2.4% of land area and having 8.1% of global diversity.The global hot spot and endemic species present within them are:
(1) North East Himalayas (3,500);
(ii) Western Ghats (1,600);
(iii) Cape region of South Africa (6,000);
(iv) Upland Western Amazonia (5,000);
(v) Madagascar (4,900);
(vi) Philippines (3,700)
(vii) Boreo (3, 500);
(viii) South West Australia (2,830);
(ix) Western Ecuador (2,500);
(x) Colombian Choco (2,500);
(xi) Peninsular Malaysia) (2, 400);

11. Plants in forensic science

Introduction:

"Forensic" comes from the *Latin* word "*forensis*" meaning forum. During the time of the Romans, a criminal charge meant presenting the case before the public. Both the person accused of the crime & the accuser would give speeches based on their side of the story.

Forensic botany is a marriage of many disciplines and results ultimately in their application to matters of law. The botanical aspects of forensic botany include plant anatomy, plant growth and behavior, plant reproductive cycles and population dynamics, and plant classification schemes (morphological and genetic) for species identification. The forensic aspects require an understanding of what is necessary for botanical evidence to be accepted as evidence in our judicial system. Forensics requires recognition of pertinent evidence at a crime scene, appropriate collection and preservation of evidentiary material, maintenance of a chain of custody, an understanding of scientific testing methods, validation of new forensic techniques, and admissibility criteria for court.

- Application of botany in law enforcement i.e. scientific use of plant materials to solve crimes
- Examples of plant life or plant remains that can be used as evidences are wood, seed, fruit, leaf, twig, trichrome, pollen, spore, algal cell etc.
- Plant allow forensic botanists to identify things such as what season the crime took place or geographical location, whether or not a body has been moved following a murder, and how long a body has been buried if it was buried
- These forms of physical evidences can be sometimes traced to an individual suspect
- The scientific methods utilized in forensic botany, and these methods range from simple techniques (e.g., light microscopy) to more technical molecular biology techniques (e.g., DNA sequencing).
- Plants have been used as evidence in criminal cases for kidnapping, child abuse, hit-and-run motor vehicle accidents, drug enforcement, homicide, sexual and physical assault, the establishment of time of death, and verification of an alibi. In addition, new applications are under development to use plant material in forensics as "tracers" to aid in the identification of missing persons, to track drug distribution patterns, and to link bodies to primary crime scene locations after they have been dumped at secondary sites.

Unique roles of forensic scientists:

The individual with the best argument would determine the outcome of the case.

- Assist in recognition and collection of physical evidence
- Document and maintain chain-of-custody
- Analyze and evaluate the evidence using a variety of scientific approaches
- Interact with the legal system
    - Assist attorneys (and often law enforcement personnel)
    - Testify in Court

What botanical evidences can do?

- Determine the circumstances and cause of death
- Estimate time frames in relations to the death
- Establish where the death could have taken place
- Determine if there were multiple crime scenes

- Prove or disprove an alibi
- Solve crimes by matching crime scene evidence to suspect
- Identify illegal products from endangered species

Advantages of using plant sample:

- Plant evidence is long lasting, which means that plant parts to remain identifiable for a very long periods of time
- Plant cell wall is made of some chemical compounds which are nearly indestructible and do not decay quickly
- Pollen grains and spores also have walls that are made of decay resistant material-sporopollenin
- Ecological and molecular restraints of various plant species allow a forensic botanist to narrow down the possibilities of where a crime was committed, when it was committed and who committed the crime

Plant in our society

To understand the widespread application and potential utility of plants in forensics, we discuss a few brief examples of plant usage in human society. As these examples are presented, consider the number of plant-based items that may be found on your person, among your private possessions, and in your home and workplace — and consider, one day, that they may be useful
as critical trace evidence.

1. **Food**

Apples are generally considered to be a wholesome, healthful addition to the daily diet as a good source of vitamins and fiber. In actuality, this concept was promoted by the apple industry in response to the renouncement of apples by Carry Nation as part of the Prohibition Act. The Women's Christian Temperance Union was opposed to apples because they were, in part, responsible for alcohol use on the frontier. In the early 1900s, the apple industry began promoting the healthful benefits of apples, and today, we have many apple cultivars to choose from at our markets. Interestingly, burglars often sample fruit and other foods in the homes they are invading while pilfering goods.

2. **Fiber**

One prevalent clothing fiber in our society is cotton. Cotton comes from the elongated epidermal hairs on the seeds of the *Gossypium hirsutum* plant. Prior to mechanized harvesting of cotton fibers, flax (*Linum usitatissimum*) was the most common fiber plant for the textile industry. Flax fibers, unlike cotton, are sclerenchyma fibers from the stem of the flax plant. Flax fibers are commonly woven into linen, and certain cultivars are used to produce cigarette papers and linseed oil. As trace evidence, the source of clothing, carpet fibers, rope, twine, and threads can be useful for associating a victim to a suspect or individuals back to a primary crime scene for an investigative lead.

3. **Medicine**

Herbal remedies and folklore investigations to identify active chemical components have long been part of human culture and have played an important role in the discovery of useful medicinal compounds (e.g., aspirin). In addition, well-preserved stomach contents from ancient human remains have yielded insight into rituals involving herbs and food. For example, in 1984, a peat cutter near Manchester, England, discovered a well-preserved human leg and called in the police to investigate. The body of Lindow Man was recovered, and radiocarbon dated to approximately A.D. 50–100, and he was determined to be a member of the Celtic tribes.

4. Beauty

Plants are key components in many herbal shampoos, soaps, cosmetics, and perfumes. Not only are botanicals used in human cosmetics and have appealing scents, but they beautify our environment as well. The *Dahlia* flower, for example, has an unusual history. Originating from Mexico and called *Cocoxochitl* by the Aztec Indians, it was discovered and seeds were sent to a French priest studying botany in Madrid, Spain.

5. Recreation

The American obsession with green lawns can be visualized in the numerous golf courses, city parks, and extensive front and backyards that are ingrained parts of suburban life. Prior to the Civil War, few Americans had lawns. However, in the 1950s, the term "lawn" was used in reference to a portion of land kept closely mown in front or around a house. In the 1950s, turfgrass breeding programs gave rise to several new grass varieties that offered improved heat,

drought, and disease resistance. In the 1970s and '80s, lawn specialists began recommending blends of grasses (e.g., fescue, bluegrass, perennial ryegrass) rather than a monoculture of a single grass species. Today, American homeownersnspend enormous capital and energy on achieving a perfectly groomed, green lawn as a setting for their homes. In fact, an entire lawn-care industry has developed around this particular aspect of suburban homes. Grass samples may be one of the most abundant types of botanical evidence found at crime scenes simply due to the American obsession with the lawn.

6. Law Enforcement

Plants may be present as biological evidence in many ways:

• Seeds caught and carried in a pant cuff

• Grass stains on a dress after a sexual assault

• Plant leaves and stems snagged and carried in a vehicle's undercarriage, grill, wheel wells, hood, or trunk

• Stomach contents with vegetable matter to aid in verification of an alibi

• Use of pollen to date the burial of skeletal remains in a mass grave

All of these examples and more can assist the forensic community in associating a person to an object, a person to a crime scene, or a suspect to a victim. "Every criminal leaves a 'trace' (evidence)" is a phrase with some accuracy. That "trace" may very well be biological plant material.

Analysis of samples

Plant Anatomy

The study of the internal structure of plants is called plant anatomy. When a stem, root, or leaf is dissected, the cells can be arranged in specific patterns that may be useful for classification and identification. For example, the internal arrangement of cells in the root structure of a dicotyledonous (the first true leaves of a seedling occur in pairs) versus a monocotyledous (the first true leaf is a single leaf) plant is characteristic.

Analysis of anatomical samples:

Cell shape and orientation of certain structures within a cell can be helpful in classification of a species. In order to learn about plant anatomy and specific plant structures within the plant body plan, it is important to take a practical approach. In the following sections we describe examples of in expensive laboratory exercises that can be performed as training for forensic botanists.

Plant Anatomical Features used in Forensic Botany

The Vascular Cambium

- Lateral meristematic region
- Division of cells here produces secondary xylem and secondary phloem tissues

- Diameter expansion forces tangential elongation of phloem cells
- Epidermis and primary phloem layers eventually fall off
- Dead phloem cells compose the outer bark

Two Cell Types

Fusiform Initials - divide to produce new xylem or phloem cells that have longitudinally elongated shapes

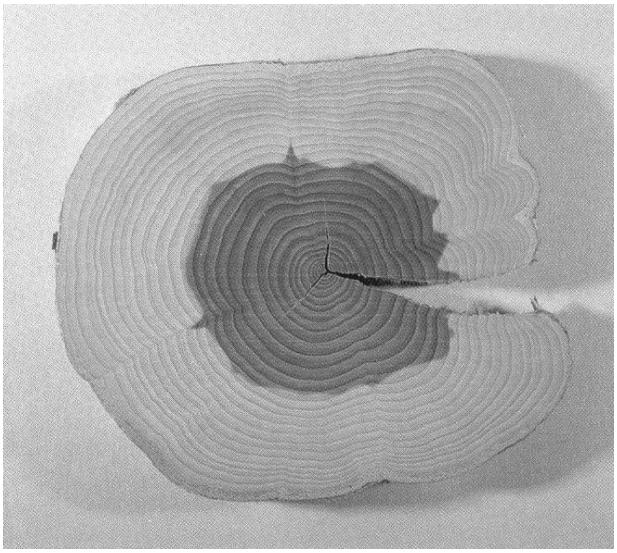
Ray initials - short, rounded cells that divide to produce new xylem or phloem ray cells

Cambium: the growing (generative) layer between the xylem and phloem.

Xylem: principle strengthening and water conducting tissue of the stem, roots, and branches.

Phloem: inner bark, principal function to distribute manufactured foodstuffs.

Bark: dead, outer tissue that protects the cambium from the external environment and exposure to pathogens and physical injury.



Vessel: the composite, tube-like structure found in hardwoods from the fusion of cells in a longitudinal column.

Fibre: an elongated cell with pointed ends and a thick or infrequently thin wall.

Rays: ribbon-shaped tissue extending in a radial direction across the grain of the wood.

Sapwood and Heartwood:

In mature trees, the xylem has both living and dead cells.

Sapwood contains the only living cells in the xylem (not all sapwood cells are alive either) and has a conductive function.

- **Heartwood** is composed of dead cells and lends mechanical support only.

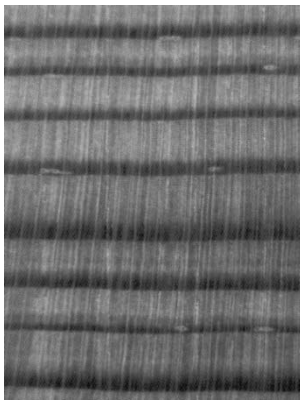
Growth rings:

Mark annual growth boundaries in trees grown in temperate climates
Often composed of 2 distinct segments
Early wood (spring wood)
Late wood (summer wood)
Early wood and latewood cells have different characteristics

Cell Differences Within Growth Rings

Earlywood

- Large Radial diameter cells
- Lower density than latewood



Latewood

- Smaller radial diameter cells
- Thicker cell walls

Irregularities in Annual Ring Formation

False rings

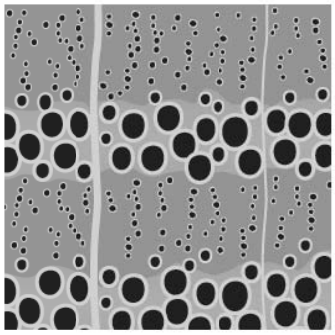
- Growth interrupted by environment (e.g. defoliation)
- Slow growth may cause formation of latewood type cells

Discontinuous rings

- Cambium was dormant in one region
- One-sided crowns, suppressed, or over mature trees

Trees grown in tropical environments

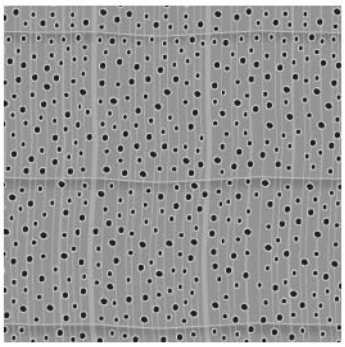
- Almost continual growth can limit occurrence of rings
- In some climates, stopping and restarting of growth can give more than one growth increment in a year



Ring-porous: The largest pores are in the early wood while those in the late wood are more evenly distributed and uniform in size. These woods typically have distinct figures and patterns, and the uneven uptake of stain (the large pores soak up more color).

Semi- ring/diffuse porous- Pores are large in the early wood and smaller toward the latewood, but without the distinct zoning, as seen in ring-porous woods.

Ring porous wood



Diffuse porous- Pores are distributed fairly evenly across the early wood and latewood. Most domestic diffuse-porous woods have relatively small-diameter pores, but some tropical woods of this type (e.g. mahogany) have rather large pores.

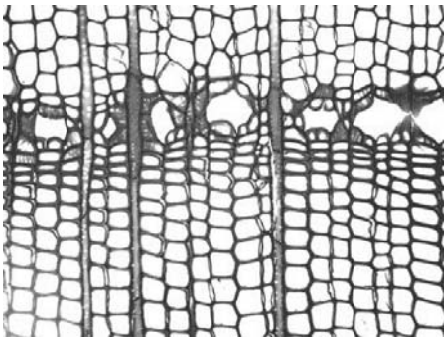
Non porous- Softwoods (gymnospermous wood) don't have vessel cells. However, different softwoods have different growth-ring characteristics. In white pine, the rings are non-distinct, and stain uptake is fairly even, as in diffuse porous woods. In yellow pine, where the rings are clearly visible, stain uptake in early wood is more pronounced than in latewood, as in ring-porous woods**.**

Diffuse porous wood

Criteria for identification…...

- Structure of tracheid walls and xylem rays
- Resin canals
- Pitting in rays
- Crystals

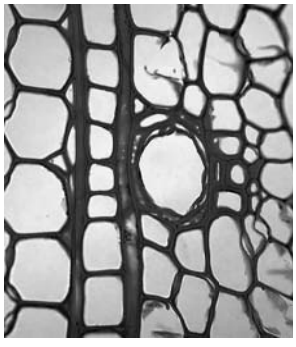
Resin canals:



Traumatic resin canals (produced by injury)



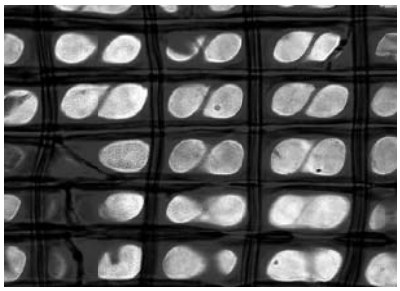
Resin canals with thin-walled epithelial cells



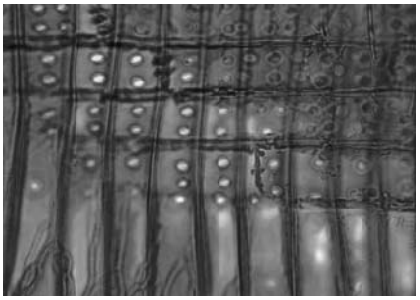
Resin canals with thick-walled epithelial cells

The wood formed immediately after wounding will contain traumatic resin canals. Wounding may be caused by freezing, fires, or mechanical damage. Traumatic resin canals do not have as regular a shape as the normal resin canals, and are formed by some species that normally do not have resin canals.

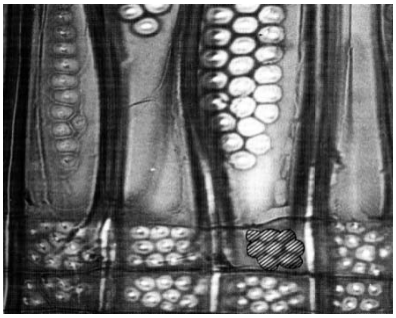
Pits:



Large window-like pits (large pinoid pits-occupying practically the entire cross- field)



Cupressoid pits (The pit aperture is approx. the same size as the pit border)



Araucaroid (pits 2 or more rows alternating pits side by side)

Crystals:

- In ordinary cells
- In chambered cells
- In idioblasts
- Raphides/ druses
- Oil/ mucilage cells

Since 1930s: became increasingly more common in forensic applications (Lane et al., 1990).

Cell wall: particularly important for two reasons:

◊it is not easily digested by most organisms and therefore persists when other plant features are destroyed (Bock and Norris, 1997)

◊ the size, shape, and pattern of cell walls is often taxon-specific (Lane et al., 1990).

Unique cell types: sclereids, trichomes- are useful in identifying botanical material (Lane et al., 1990).

Tissue organization of different plant parts (Willey and Heilman, 1987).

Wood characteristics (secondary tissue organization)

Roots: Frequently found with exposed or shallowly buried bodies

Plant roots, like their above-ground counterparts, exhibit annual growth rings that can be useful in pinning down the post-mortem interval, or at least the time since the body came to be at the location where it was found.

Three ways in which the roots can be used to date the remains or otherwise characterize the burial site:

(1) **Examine root development after it has been damaged.** When a grave is dug or the ground otherwise disturbed, roots can be damaged but still continue growing. If the meristematic zone is damaged, no secondary xylem cells can be produced, leaving a permanent lesion. The number of growth rings laid down after the lesion indicates the number of years since the damage occurred.

(2) **Examine roots in direct contact with the remains.** Roots in contact with the bones or personal effects of the deceased can be cross-sectioned at the point of contact and the annual rings counted, establishing a minimum time frame for time since death. The contact must be penetrative, i.e. the roots must be growing through clothing or bones, in order for the interpretation to be valid.

(3) **Examine branch growth.** Annual longitudinal growth of the roots, in addition to radial growth, can be estimated, and a time frame determined from the length of a root from its point of contact with the remains to its distal end.

Stomach contents:

Characteristic cell types from food plants can be used to identify a victim's last meal (Bock and Norris, 1997). knowledge about which can be useful in determining the victim's whereabouts or actions prior to death

Some of these cell types include (Dickison, 2000):

Sclereids (pears)

Starch grains (potatoes and other tubers)

Raphide crystals (pineapple)

Druse crystals (citrus, beets, spinach)

Silica bodies (cereal grasses and bamboos)

Time since death can be approximated by the state of digestion of the stomach contents

It normally takes at least a couple of hours for food to pass from the stomach to the small intestine

A meal still largely in the stomach implies death shortly after eating, while an empty or nearly-empty stomach suggests a longer time period between eating and death (Batten, 1995).

However, there are numerous mitigating factors to take into account:

- the extent to which the food had been chewed
- the amount of fat and protein present
- physical activity undertaken by the victim prior to death
- mood of the victim
- physiological variation from person to person.

All these factors affect the rate at which food passes through the digestive tract.

A Case Study:

In a case where a young woman had been stabbed to death, witnesses reported that she had eaten her last meal at a particular fast food restaurant.

Provenance

Her stomach contents did not match the limited menu of the restaurant, leading investigators concluded that she had eaten at some point after being seen in the restaurant.

The investigation led to the apprehension of a man whom the victim knew, and with whom she had shared her actual final meal (Dickison, 2000).

Tree ring analysis (Dendrochronology) is a common technique for dating masterworks by European painters, many of which were painted directly on wood.

Given that the samples are in good condition, analysts can pinpoint the exact year when the tree, from which the wood for the painting was taken, was cut down.

Case Study

A Peter Paul Reubens painting originally dated 1616 was shown to be at least 10 years younger, and a painted wall panel recovered from a house in Switzerland in the 1970s was determined to have been painted on spruce harvested in 1497 (Schweingruber, 1988).

Dendrochronology techniques are useful in determining the provenance of wooden art objects and musical instruments

In one case, two violins forming part of an inheritance were claimed to have been made by Antonio Stradivari.

The sounding-boards of the instruments were x-rayed and compared to standard curves for spruce from the Alpine region of northern Italy, where Stradivarius is known to have worked.

The oldest rings from the samples dated to 1902 and 1894 respectively for the two violins.

Furthermore, these oldest rings were not the outermost rings of the wood from which the violins were constructed.

Allowing for a period of seasoning before the wood could be used to make the instruments, analyses showed that the violins could not have been made before 1910.

Given that Stradivari did his best work at the turn of the 17th century, the instruments were deemed to be fakes (Schweingruber, 1988).



The Kidnapping of Charles Lindbergh, Jr. (March 01, 1932)

A critical piece of evidence in the case was a crude homemade wooden ladder left at the scene. Xylotomist Arthur Koehler of the United States Forest Service undertook a meticulous examination of the ladder and when the case finally came to trial four years later, offered the first botanical testimony ever to be heard and accepted in American courts.

The ladder had been constructed in three sections, presumably for ease of transport Koehler identified each side rail and rung with a number and identified each piece to species. Through careful examination of the characteristic milling marks left on each piece and comparisons with local mills, he was able to trace all components of the ladder back to their respective retail sources.



He also noted distinctive marks left on the wood by a dull, nicked hand plane. Of particular interest was rail #16, a piece of low-grade pine which had four distinctive square nail holes. It was also relatively unweathered.

The low grade of the wood, the nail holes, and its unweathered condition suggested that particular piece of wood had been removed from some interior construction.

Without a suspect however, progress on the case was slow. In September of 1934, some of the notes used to pay the ransom were used at a gas station by Bruno Hauptmann, a carpenter who lived in the Bronx, New York City. He was arrested when $14 600 of the ransom money was found in his garage.

Upon searching the attic for more ransom money, police noticed that one of the floorboards was eight feet shorter than the others. The square nail holes in rail 16 lined up exactly with holes in one of the attic floor beams, and the annual ring pattern of rail 16 matched that of the short floorboard. A hand plane recovered from Hauptmann's garage was indeed dull and damaged, and made marks identical to those on the ladder and on a homemade shelf in the Hauptmann garage.

Hauptmann was convicted of kidnapping and murder and was executed on April 3rd, 1936.

Pollen Analysis:

- Pollen and spores are chemically extracted from samples
- To identify pollen and spores, specialists can use a compound light microscope, a scanning electronic microscope, reference collections that may consist of photos and illustrations or perhaps even actual dried specimens arranged systematically (herbariums).
- Pollen and spore evidence that has been collected, analyzed, and interpreted can be presented in court.
- These “fingerprints” can be used to confirm certain aspects of a crime.

How to collect pollen and spore?

- During an investigation, control samples must be collected as well as evidence samples.
- Samples must be collected wearing gloves and with clean tools (such as brushes and cellophane tape) and placed in sterile containers, which then must be sealed and labeled with care.
- Sampling instruments must be cleaned after each use, or new ones must be used.
- Collected evidence must be secured, and the chain of custody must be maintained.

Pollen Fingerprint

Pollen fingerprint is the number and type of pollen grains found in a geographic area at a particular time of year.

Four (4) essential parts-

(1) number of pollen grains
(2) type of pollen grains
(3) found in a certain area
(4) at a particular time

What it does?
Pollen fingerprint can link a piece of evidence to a particular place and time.
Advantages of pollen analysis in forensic botany:
Since both pollen and spores have resistant structures, they at times can help to determine such things as
Whether a victim/suspect was moved or not
Where is the crime's location, whether it occurred in a city or in the village
In which season it may have occurred
How do you think pollen collected here differs from pollen in Darjeeling?
How would pollen collected in the summer differ from pollen collected in the winter?
How should you analyze the pollen?
What instruments or techniques should you use?
Srebrenica massacre or Srebrenica genocide
(part of the Bosnian War)





Some of the more than 6,100 gravestones at the Srebrenica-Potočari Memorial and Cemetery for the Victims of the 1995 massacre	
Location	**Srebrenica, Bosnia and Herzegovina**
Date	**11–22 July 1995**
Target	**Bosniak men and boys**
Attack type	**Military assault, mass murder, ethnic cleansing, genocide**
Deaths	**8,373**
Perpetrators	**Army of Republika Srpska Scorpions paramilitary group**

In July 1995 a massacre of civilians followed the burial in seven mass graves. Three months later the bodies were exhumed and transported to a number of new burial sites in an attempt to conceal evidence of the massacre and to deflect blame.

Could palynology help in relating the secondary burial sites with the original primary burial sites and thereby more closely link the massacre to known or suspected perpetrators

Case study:

"Murder on the Danube" case

The first time police used pollen to solve a crime was in Austria in 1959. A forensic scientist studying the mud on a murder suspect's boot found what turned out to be a 20-million-year-old pollen grain from a hickory tree. That species no longer grew in Austria then. But investigators were able to locate a Miocene sediment outcrop on the Danube River, from which such a pollen grain could have become recycled into the environment.

"We know you killed him," they told the murder suspect, in the best police procedural fashion, "and we know where." Then they took him to the outcrop. The suspect was so unnerved that he led them straight to the victim's grave.

Re-exhumation was commenced by the United Nations International Criminal Tribunal for the former Yugoslavia (ICTY) in 1997.

The objective of the palynological and associated soil analyses was to determine the environmental profile of the original burial sites and to try and find a connection with the secondary sites where different environmental profiles existed. These analyses were done independently of all other forensic investigations being undertaken at the same time to ensure credibility. Five of the original sites and 19 secondary sites were investigated in detail. Analyses indicated that the original mass graves each had a different geological and botanical profile which easily separated each site. Samples from all sites were taken from the fill of the graves close to and from varying distances away from bodies or body parts and from sediment surrounding the mass graves. Over 240 comparator samples were collected from various sources to determine the background pollen profile of each site, the local vegetation was recorded and abundance of major species determined. Results showed that sediments and associated spores and pollen from the original mass graves had indeed been transferred along with the bodies to the numerous secondary burial sites and that even some botanical evidence at the primary burial sites pointed to the original execution site or sites. Pollen found at the original burial sites consisted of cultivated grasses (cereals including wheat and maize), wild grasses (Poaceae), pines (*Pinus*), spruces (*Picea*), sedges (Cyperaceae), beeches (*Fagus*) and walnut (*Juglans*). Various combinations of these pollen types, plus many others, were subsequently found in exotic material sampled from within the graves at the secondary burial sites, proving a link between the original and subsequent burial sites. The accuracy of the evidence provided by the pollen was confirmed by other types of forensic evidence and presented in court. The investigation showed the importance of being able to differentiate between imported and local fill used at grave scenes. This was probably the first time that environmental profiling was used systematically in a war crimes investigation.

An example of the use of forensic palynology in assessing an alibi.

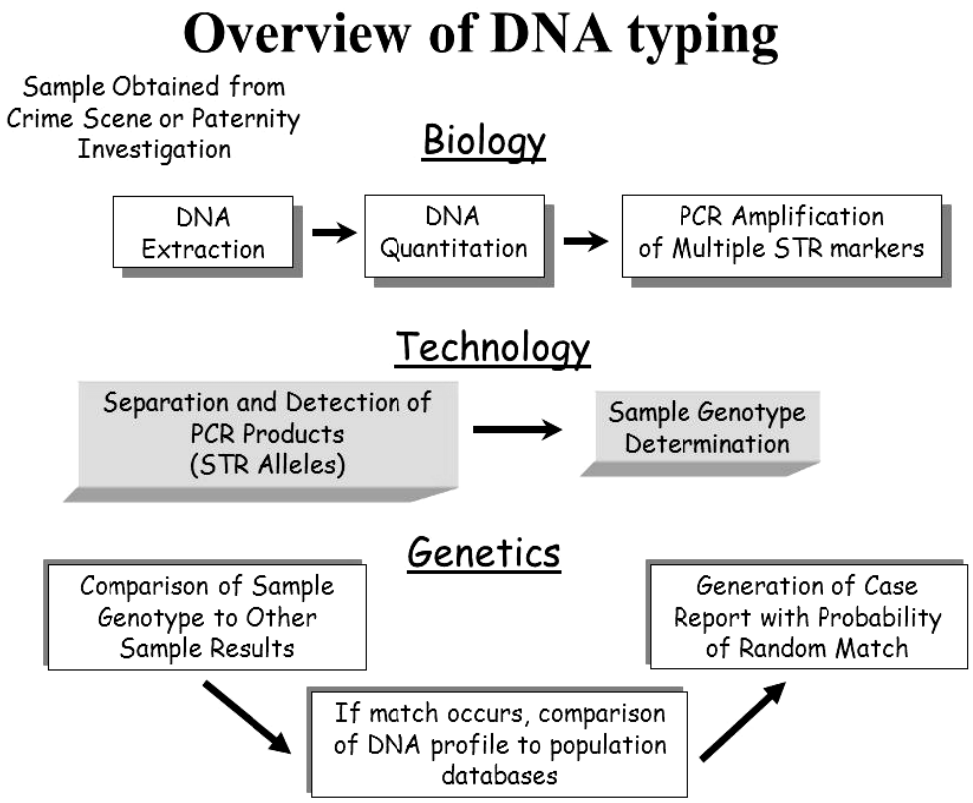
A man was found shot in the back on Mount Holdsworth in the Tararua Ranges north of Wellington, the capital city of New Zealand. Police investigations pinpointed one individual who had been seen in the area, knew, and had the means and motive to kill the victim. His alibi was that an eyewitness was mistaken as he never had been in the area and the jacket he was reported to have been wearing had been purchased in The Netherlands and brought to Wellington, where it never had left the city. Furthermore the distinctive board shorts that he was reported to have been wearing had been purchased in a small coastal New Zealand town after the victim had been murdered. Pollen of *Nothofagus menziesii*, a mountain plant, on the clothing suggested that the alibi was untrue and that the clothing had been in mountains in the vicinity of Mount Holdsworth or a similar mountain scene where *Nothofagus menziesii* was growing.

General DNA Typing Considerations

Many different types of biological evidence are commonly submitted to forensic science laboratories for examination. Initially, evidence that was suitable for DNA analysis was limited to human biological substances that contain nucleated cells. This limitation has been overcome in the last 5 years with the implementation of mitochondrial DNA sequencing, and plant and animal DNA testing in the forensic arena. The quantity of DNA that can be extracted from common biological sources

varies greatly with evidentiary samples. Note that, in practice, crime scenes samples may contain considerably less usable DNA depending on environmental conditions. DNA has been isolated from other sources such asgastric fluids and fecal stains. However, it can be difficult to generate a DNA profile from these sources due to significant degradation.

Four factors affect the ability to obtain a DNA profile. The first issue is sample quantity. The sensitivity of PCR-based DNA typing methods is noteworthy but still limited. The second concern is sample degradation. Prolonged exposure of even a large bloodstain to the environment or to bacterial contamination can degrade the DNA and render it unsuitable for further analysis. The third consideration is sample purity. While most DNA typing methods are robust, dirt, grease, some dyes in fabrics, and so forth can seriously compromise the DNA typing process. Environmental insults will not change DNA type "A" into type "B," but they can adversely affect the ability of the scientist to obtain a complete DNA profile from the sample. The last issue is the ratio of major contributor to the minor contributor(s) from DNA mixtures. Both DNA profiles can readily be detected with, for example, 1:1 and 3:1 DNA mixtures. However, with mixture ratios such as 25:1 or 50:1, the quantity of the major DNA species prevents the detection of the minor source.



Plant DNA Typing Considerations

For DNA analysis of botanical samples, many of the same considerations for human identity testing still apply. The four factors (quantity, quality, purity, and mixture ratios) may all play a role in determining whether further testing should be performed or whether an interpretable DNA profile can be obtained from a plant sample. Two factors need to be considered for obtaining a sufficient quantity of plant DNA for profiling: size of the plant fragment and ability of the analyst to mechanically break the plant cell wall for the sufficient release of nuclear DNA contents. The size of a plant fragment can seldom be enhanced other than to initially collect as much sample as possible from the crime scene. However, new technologies are aiding in the DNA extraction process for improving DNA yield from

plant samples. Traditionally, plant cells have been disrupted by the mechanical pressure of grinding by hand in a mortar and pestle with the addition of liquid nitrogen to increase the fragility of the cell wall. Some useful equipment for breaking plant cell walls may be a mechanical homogenizer (consisting of a rotating blade with a serrated tip) or the addition of metallic beads prior to high-speed oscillation of samples. In addition, several companies manufacture commercial plant DNA extraction kits that minimize the number of centrifugation steps and maximize DNA yields. The use of some of these commercial kits also seems to improve the final purity of the plant DNA so that the PCR amplification process is not inhibited by the presence of secondary plant metabolites (e.g., tannins, resins, phenolic compounds). Finally, care in collection and preservation of botanical evidence as well as the development of plant species-specific probes and PCR primer sets in the future will address mixture interpretation issues.

Preparation of Genomic DNA from Plant Tissue

Although many commercially available plant DNA extraction kits have recently become available, there are some very reliable traditional chemical extractions for plant DNA purification. The following protocol is one example of such a procedure.

Materials:

Cold, sterile water

Liquid nitrogen

Extraction buffer (100 mM Tris-Cl, pH 8; 100 mM EDTA; 250 mM NaCl; 100 µg/mL proteinase K)

10% (wt/vol) N-lauroylsarcosine

Isopropanol

TE buffer (10 mM Tris-Cl, pH 8; 1 mM EDTA)

Cesium chloride (CsCl)

10 mg/mL ethidium bromide

CsCL-saturated isopropanol

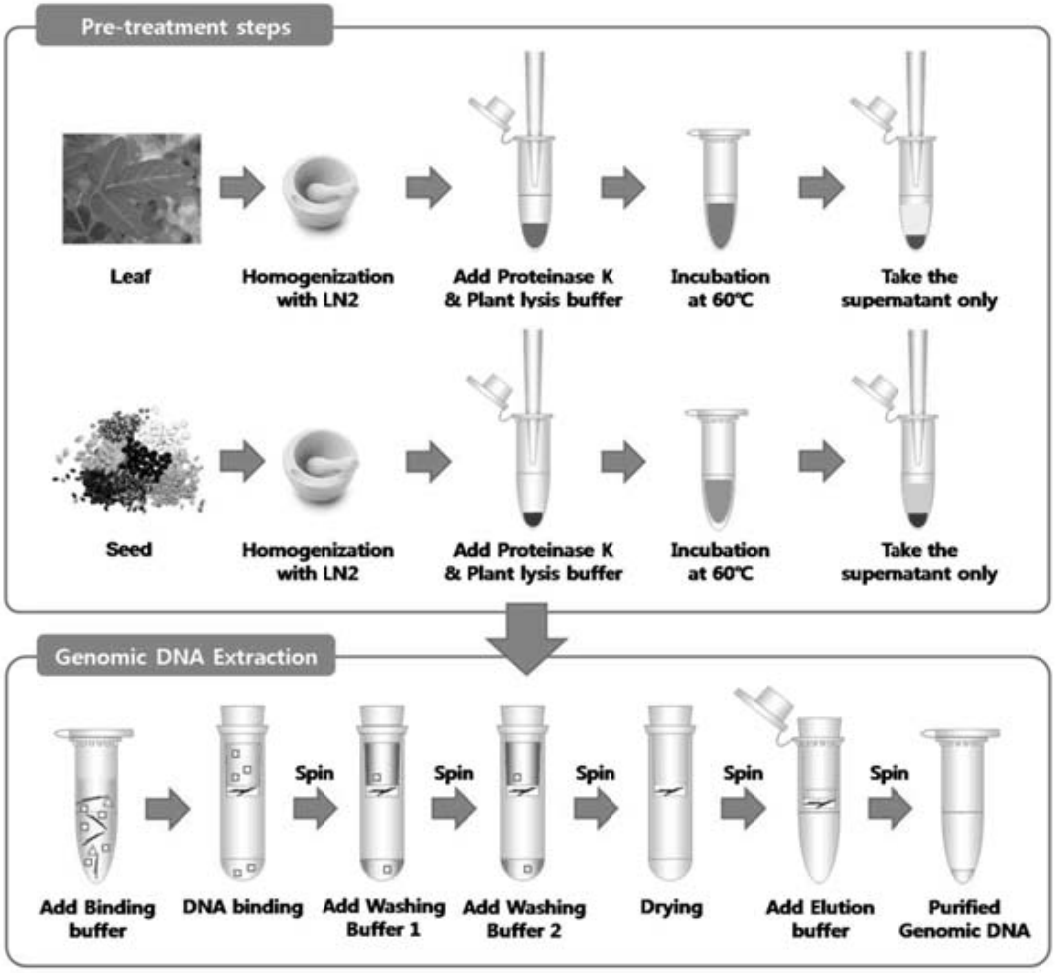
Ethanol

3M sodium acetate, pH 5.2

Beckman JA-14, JA-20, JA-21, and Vti80 rotors

- Harvest 10–50 g of fresh plant tissue.
- Rinse with cold, sterile water; dry with tissues; and freeze with liquid nitrogen. Mechanically grind with a mortar and pestle.
- Transfer frozen powder to a 250-mL centrifuge bottle and immediately add 5 mL of extraction buffer per gram of starting fresh plant tissue; gentle mix. Add 10% N lauroylsarcosine to a final concentration of 1%; incubate 2 h at 55°C.
- Centrifuge for 10 min in a JA-14 rotor at 6000 rpm chilled to 4°C. Save the supernatant and repeat this step if debris is still present.
- Add 0.6 volumes of isopropanol and mix. If no visible precipitate forms, place at –20°C for 30 min. Centrifuge for 15 min in a JA-14 rotor at 8000 rpm at 4°C. Discard the supernatant.
- Resuspend the pellet in 9 mL TE buffer, add 9.7 g of solid CsCl, mix, and incubate 30 min on ice. Centrifuge for 10 min in a JA-20 rotor at 8000 rpm at 4°C and save the supernatant.
- Add 0.5 mL of 10 mg/mL ethidium bromide and incubate 30 min on ice.
- Centrifuge 10 min in a JA-20 rotor at 8000 rpm at 4°C. Transfer supernatant to two 5-mL ultracentrifuge tubes and seal.
- Centrifuge in a Vti80 rotor at 20°C for 4 h at 80,000 rpm. Collect the band of DNA using a 15-G needle and syringe.

- Remove any residual ethidium bromide by repeated extraction of the DNA band with CsCl-saturated isopropanol.
- Add 2 volumes of water and 6 volumes of 100% ethanol, mix, and incubate for 1 h at –20°C. Centrifuge for 10 min in a JA-20 or JA-21 rotor at 8000 rpm at 4°C.
- Resuspend the pellet in TE buffer and precipitate again by adding 1/10 volume of 3M sodium acetate and 2 volumes of 100% ethanol. Repeat the centrifugation step. Air-dry the pellet by inverting the tube over a paper towel for 15 min, then resuspend the pellet in the TE buffer. The expected plant DNA yield should be 104 μg of 50 Kb high molecular- weight DNA per gram of starting plant tissue.



Classic forensic botany cases: Case histories by using Plant anatomy and systematic, Palynology, Plant ecology, Limnology, Plant Molecular Biology and DNA, Drug enforcement and DNA.

Forensic botany is defined as the use of plant evidence in court. It is subdivided into several botanical subspecialties, including plant anatomy (the study of cellular features), plant systematics (taxonomy and species identification), palynology (the study of pollen), plant ecology (plant succession patterns), and limnology (the study of freshwater ecology). In the past decade, molecular biology and the use of DNA methods have been important tools to further the research of these disciplines.

Plant Anatomy and Systematics

Plant systematics is a broad discipline that includes the study of evolutionary relationships between plant species and taxonomy (the identification of plant species). Species identification is a typical first step in analyzing botanical evidence for casework. Plant anatomy uses features such as leaf morphology and tree growth ring patterns to aid in species identification and in performing physical

matches of evidence, respectively. The kidnapping and death of Charles Lindbergh's young son in 1932 was the first modern-era case to use such botanical evidence in court. A wooden ladder was used to gain access to the second-story nursery to kidnap Lindbergh's son.

Arthur Koehler, a wood identification expert for the Forest Products Laboratory of the U.S. Forest Service in Wisconsin, was able to provide critical evidence against Bruno Richard Hauptman, who was later convicted of the crime.

Koehler had an excellent academic record and had provided evidence in several cases prior to the famous Lindbergh trial. His testimony is noteworthy since the use of scientific experts in the mid-1930s was generally limited to fingerprints, handwriting, bullet comparisons, and analyses of stomach contents. Koehler first identified the four tree species used to construct the ladder as yellow pine, ponderosa pine, Douglas fir, and birch, via microscopic analysis of wood-grain patterns. Next, Koehler analyzed the tool marks left on the wood from both the commercial planing mill and the hand plane used by Hauptman during the construction of the ladder. Koehler used oblique light in a darkened room to observe the plane patterns left on the wood. Amazingly, he was able to trace the wood by the mill plane marks to a shipment of yellow pine delivered to the National Lumber and Millwork Company in Bronx, New York. The hand-plane marks on the ladder exactly matched those made by a hand plane found in Hauptman's possession. Finally, Koehler compared the annual growth rings and knot patterns on rail 16 of the ladder to a section of wood in Hauptman's attic. The pattern of knots and growth rings on rail 16 exactly matched the exposed end of wood.

Palynology

Forensic palynology refers to the use of pollen in criminal investigations. The major plant groups identified as pollen sources include flowering plants, conifers, and ferns. Ferns technically produce spores instead of pollen but are included in pollen types. Pollen is microscopic and not visually obvious trace evidence during crime scene collection, but is retained on clothing, embedded in carpets, and pervasive in soil. Pollen grain morphology can be used to identify a plant genus and often the species. Crime scenes that are restricted to a few square meters, such as a rape scene or the entry point of a burglary, are good choices for pollen evidence.

Localized areas have a specific pollen distribution pattern representing the combination of plant species found in the surrounding vegetation. Common pollen types from plants that use wind for distribution (e.g., grass, bracken spores) will be less useful than pollen from uncommon, poorly distributed species (e.g., flax, willow). Insect-distributed pollen is typically deposited within a few feet of the source plant. Pollen analysis consists of species identification and an estimation of the percentage that each plant species represents in an evidentiary sample. A similar pollen composition from shoeprints and from the shoes that made the prints indicates a strong match correlation.

Pollen evidence collected from a burglary entrance and a suspect's shoes, for example, could provide a linkage in a case. A case that exemplifies the use of pollen in criminal casework is described by Horrocks et al. In Auckland, New Zealand, a prostitute alleged that the defendant had raped her in an alleyway approximately seven meters from his car after failing to pay her in advance for her services. The defendant claimed that he had never been more than one meter away from the car and had not entered the alleyway. Furthermore, he claimed that he had not had sex with the victim and the soil on his clothing was from the driveway area. An examination of the crime scene and the evidence showed no footprints and no seminal fluid stains. A soil sample was collected from the defendant's clothing, the disturbed area of ground in the alleyway, and from the driveway area near the defendant's car. All the soil samples were prepared for pollen analysis by deflocculation with potassium hydroxide, acetylation to remove cellulose and organic matter, and a silicate removal step using hydrofluoric acid. Samples were bleached to remove additional organic matter and analyzed under a microscope for pollen identification and counting. The types of pollens were similar between the two locations, but the amounts of each type were different in each sample. The alleyway contained 76% *Coprosma* (an evergreen shrub) pollen, but the driveway sample contained only 8%.The defendant's clothing contained approximately 80% *Coprosma* and only small amounts of other pollen species. These results support the victim's account of the sexual assault taking place in the alleyway. Pollen analysis has also been utilized to establish time of death.

In Magdeburg, Germany, a mass grave containing 32 male skeletons was discovered in February of 1994. The identities of both the victims and the murderers was unknown. Two hypotheses were proposed: (1) the victims were killed in the spring of 1945 by the Gestapo at the end of World War II, or (2) the victims were Soviet soldiers killed by the secret police after the German Democratic Republic revolt in June of 1953. The ability to differentiate between the spring and summer was critical to solving the case. Pollen analysis was performed on 21 skulls. Seven of the skull nasal cavities contained high amounts of pollen from plantain, lime tree, and rye. All of
these plant species release pollen during the months of June and July. Pollen analysis supported the hypothesis that the remains were of Soviet soldiers killed by the Soviet secret police after the June 1953 revolt.

Plant Ecology

Plant ecology involves studying **the growth patterns of vegetation in areas that have been disturbed**. These patterns and the vegetative (non-flowering) portion of plants can be useful in estimating time of death.

- For example, when a body is discovered lying on top of a weed plant with a broken top, useful information can be obtained to define **time windows for when the death occurred**. A certain amount of shading will eventually kill a plant, so if the weed plant is **lacking chlorophyll, a minimum amount of time must have already elapsed**.
- If **new shoots are present at the base of the plant**, this may establish a **second time window**. Agricultural research on many plant species has defined the time for new shoot initiation after the top of a plant has been removed.
- The **length of the new shoot** can sometimes establish a **third time window**.

In one case, the brain cavity of a skull was filled with plant roots. The anatomy and developmental stage of the roots indicated that the plant was approximately one year old, and the plant was putatively identified as *Ranunculus ficaria* L (buttercup family). The predictable stages of plant development were useful in estimating the time that the skeletal remains had been in their present location. The investigators were able to determine that the skeleton had been there for at least one year; however, a maximum time could not be established. The plant could have developed secondarily sometime after the body had lain in its present location, so a maximum time estimate was not possible.

Limnology

Limnology is the study of freshwater ecology and can be applied to a subset of forensic cases. In particular, aquatic plants (e.g., algae, diatoms) have been useful to link suspects to a crime scene or to establish that drowning occurred in freshwater

Limnology is the study of freshwater ecology

and can be applied to a subset of forensic cases. In particular, aquatic plants (e.g., algae, diatoms) have been useful to link suspects to a crime scene or to establish that drowning occurred in freshwater

In 1942 Incze demonstrated that, during drowning, diatoms could enter the systemic circulation via the lungs. Their presence can be demonstrated in tissues like liver, brain and bone marrow.

Properties for which diatom is used as clue:

a: Diatom populations vary seasonally in lakes, rivers, and ponds.

- In early spring, diatom populations expand in freshwater.
- Following this expansion, the live diatoms decline but a large number of dead diatoms remain in summer water.
- In the fall, a second diatom expansion occurs and then progressively declines through the winter months.

b: Each species has a characteristic shape and refractive pattern from the silica in the cell wall which can be used for identification.

c: Diatoms do not occur naturally in the body.

When a person drowns in freshwater, diatoms are taken in along with water into the lungs. The diatoms are dispersed to the internal organs of the body.

In a study of 771 cases, the diatom test was positive for 28% of presumed freshwater drowning cases but was rarely positive for domestic water drowning. The low rate of diatoms observed in domestic drowning could be traced back to cleaning agents containing crushed diatoms for abrasives.

In 1991, two young boys were brutally attacked by teenage assailants while fishing at a suburban pond in Connecticut. The boys were held at knifepoint, bound with duct tape, and savagely beaten and dragged into the pond to drown. One boy managed to get free, save himself, and rescue his friend. After many hours of criminal investigation, three suspects were apprehended. To link the suspects to the crime scene, investigators seized the sedimentencrusted sneakers of both the victims and the assailants and analyzed them for algal and diatom species. microscopic analysis of samples from each pair of sneakers plus reference samples from the pond showed the same species and distribution pattern of each species. These results supported the position that the samples all originated from a common freshwater location.

Plant Molecular Biology and DNA

The previously discussed cases have relied on traditional botanical methods for species identification. In the age of DNA analysis, forensic botany is using molecular biology to aid in criminal and civil investigations. The first criminal case to gain legal acceptance using plant DNA typing was a homicide that occurred in 1992 in Arizona's Maricopa County. A woman's body was found under a paloverde tree in the Arizona desert. Near the body was a beeper eventually traced to a suspect, Mark Bogan. A few seed pods from a paloverde tree were found in the back of Bogan's truck. Officials wanted to know if DNA could match those seed pods to the tree where the body was discovered. Dr. Timothy Helentjaris from the University of Arizona used a technique called randomly amplified polymorphic DNA (RAPD) analysis to generate a band pattern from the evidence in question. He also surveyed a small population of other paloverde trees to determine if the band patterns were unique to each individual. His convincing testimony on plant evidence helped convict Mark Bogan of murder. RAPD marker analysis has also been utilized in civil court cases to identify patent infringements. In Italy, RAPD analysis of a patented strawberry variety "Marmolada" helped settle a lawsuit involving the unauthorized commercialization of the plant.

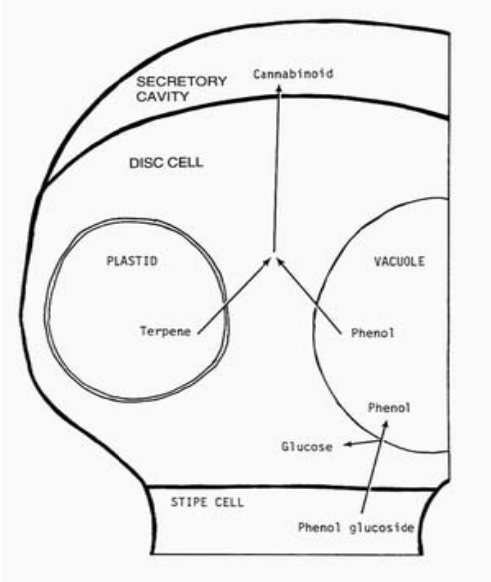
Molecular methods can be used to identify a plant species from minute leaf fragments and pollen grains. Forensic botanists have utilized DNA technology because often botanical trace evidence does not contain the necessary morphological or histological features that would allow one to identify a plant at the genus or species level. This is particularly true for fragmented and deteriorated plant material. The Bode Technology Group Inc. (Dr. Robert Bever; Springfield, VA) is developing and utilizing molecular methods to analyze botanical trace evidence. This type of analysis is a valuable tool for potentially linking an individual to a crime scene or physical evidence to a geographic location. One useful application for the molecular analysis of botanical trace evidence is the identification of a geographic region where a kidnapped individual may be located. Based on flowering times and the plant species represented in the trace pollen evidence found with a ransom note, a geographic region may be identified and would provide the police with an investigative lead. Plant systematists have characterized many loci that are useful for the identification of plants, including several nuclear (18S, ITS1, ITS2) and chloroplast (rbcL, atpB, ndhF) genes.

Bode Technology Group has identified a DNA extraction, cloning, and sequencing procedure to identify plants using some of those genes. Using these methods, they have identified numerous species of plants from physical evidence. These include species of algae, evergreens, and many flowering herbs, shrubs, and trees. Many plants have a limited geographic distribution or grow in specific habitats. Some of these locations will be general areas, such as roadsides or areas of new construction. Other locations will be more specific, like the Mohave Desert or southern Florida, for

plant species that have a severely restricted geographic range. Linking botanical trace evidence to a geographic region could provide law enforcement and investigators with valuable information.

Drug Enforcement and DNA

Often in drug seizures, identification of the seized substance is a problem, especially if the plant material is fragmented and dried. A variety of methods are currently employed to identify *Cannabis sativa* L (marijuana).



- Marijuana can be identified by **classical botanical characterization**, especially if the **type of cystolith** found in an individual's vehicle back to a plant from a growing area near the suspect's home, for example.
- The Connecticut State Forensic Science Laboratory is developing a **molecular strategy for creating unique band patterns from marijuana samples, which uses a technique called amplified fragment length polymorphism (AFLP) analysis**.

AFLP analysis is based on the selective PCR amplification of restriction fragments from a total digest of plant DNA to generate a fluorescent band pattern. Validation of the AFLP technique on marijuana samples and the construction of a marijuana AFLP database for comparative purposes were developed at the Connecticut State Forensic Science Laboratory.

Cannabis has special trichomes. The resin within these trichomes has encouraged our ancestors to work with this plant for millennia, spreading the seeds world wide. **Cannabis resin contains strong smelling medicinal compounds that reflect in the sun to call extra attention**. Trichomes, the resin gland heads, protect the cannabis plant from insects and predation by being sticky and intoxicating. Trichomes also protect growing seeds from the sun and wind by reflecting solar radiation and creating a physical barrier.

The Cannabinoids and Terpenoids in Resin:

Cannabis resin includes: cannabinoids (THC, CBD, CBN, THC-V, over 90 discovered so far!), terpenoids (smell and "high" modulation), and plant waxes/oils. One group of cannabinoids, the THC group, is primarily responsible for the psychoactive properties of cannabis.

Forensic botany uses plant DNA to trace crimes

Date: **February 29, 2016**

Source: **Sam Houston State University**

Summary: **The field of forensic botany is being advanced with the publication of two recent studies that use marijuana DNA to link drug supplies and pollen DNA to aid in forensic investigations.**

Publication in International Journal of Legal Medicine

A test was developed to individualize samples of marijuana that could be used to link drugs across cases. The study examined 11 cases containing a total of 199 samples from U.S. Customs and Border Protection, which included four matching genotypes indicating drugs came from the same source.

"The use of a DNA-based method for identification will allow federal law enforcement agencies (e.g., U.S. Customs and Border Protection (CBP) and Drug Enforcement Administration (DEA)) to form links between cases involving the cross-border trafficking of *Cannabis*," said Dr. David Gangitano, one of the authors on the study.

Publication in Science and Justice

Researchers found that pine pollen could provide a viable source of DNA for criminal investigations. Pine pollen remains viable for DNA testing for at least two weeks on cotton clothing and can help link a suspect or victim to a location. The study examined a new collection device, a high-throughput method for DNA extraction and amplification, and a newly-developed system for genotyping.

This study has shown that pollen can be a stable source of forensic DNA evidence, as a proof-of-principle, and that may persist on cotton clothing for at least 14 days of wear. This method can be applied in forensic cases where pollen grains larger than 10 μm (e.g., from herbs or trees) may be transferred to clothing (worn by suspect or victim) by primary contact.

12. Intellectual Property Rights

Meaning and forms of Intellectual Properety Rights

Intellectual property Right (IPR) is a term used for various legal entitlements which attach to certain types of information, ideas, or other intangibles in their expressed form. The holder of this legal entitlement is generally entitled to exercise various exclusive rights in relation to the subject matter of the Intellectual Property. The term intellectual property reflects the idea that this subject matter is the product of the mind or the intellect, and that Intellectual Property rights may be protected at law in the same way as any other form of property. Intellectual property laws vary from jurisdiction to jurisdiction, such that the acquisition, registration or enforcement of IP rights must be pursued or obtained separately in each territory of interest.

Intellectual property rights (IPR) can be defined as the rights given to people over the creation of their minds. They usually give the creator an exclusive right over the use of his/her creations for a certain period of time.

What is Intellectual Property?

Intellectual property is an intangible creation of the human mind, usually expressed or translated into a tangible form that is assigned certain rights of property. Examples of intellectual property include an author's copyright on a book or article, a distinctive logo design representing a soft drink company and its products, unique design elements of a web site, or a patent on the process to manufacture chewing gum.

What is Intellectual Property Rights?

Intellectual property rights (IPR) can be defined as the rights given to people over the creation of their minds. They usually give the creator an exclusive right over the use of his/her creations for a certain period of time.

Intellectual property (IP) refers to creations of the mind: inventions, literary and artistic works, and symbols, names, images, and designs used in commerce.

Categories of Intellectual Property

One can broadly classify the various forms of IPRs into two categories:

• IPRs that stimulate inventive and creative activities (patents, utility models, industrial designs, copyright, plant breeders' rights and layout designs for integrated circuits) and

• IPRs that offer information to consumers (trademarks and geographical indications).

IPRs in both categories seek to address certain failures of private markets to provide for an efficient allocation of resources. IP is divided into two categories for ease of understanding:

1. Industrial Property

2. Copyright

Industrial property, which includes inventions (patents), trademarks, industrial designs, and geographic indications of source; and

Copyright, which includes literary and artistic works such as novels, poems and plays, films, musical works, artistic works such as drawings, paintings, photographs and sculptures, and architectural designs. Rights related to copyright include those of performing artists in their performances, producers of phonograms in their recordings, and those of broadcasters in their radio and television programs

Intellectual property shall include the right relating to:

i. Literary, artistic and scientific works;
ii. Performance of performing artists;
iii. Inventions in all fields of human endeavour;
iv. Scientific discoveries;
v. Industrial designs;
vi. Trademarks, service marks and etc;
vii. Protection against unfair competition.

Rights protected under Intellectual Property

The different types of Intellectual Property Rights are:

i. Patents
ii. Copyrights
iii. Trademarks
iv. Industrial designs
v. Protection of Integrated Circuits layout design
vi. Geographical indications of goods
vii. Biological diversity
viii. Plant varieties and farmers rights
ix. Undisclosed information

3.2. International Conventions

The most important Conventions and Treaties at International level include:

1. The Paris Convention for the Protection of Industrial Property
2. The Berne Convention for the Protection of Literary and Artistic Works
3. The WIPO Copyright Treaty (WCT)
4. The Patent Cooperation Treaty (PCT)
5. Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure
6. The Madrid Agreement Concerning the International Registration of Marks and the Protocol Relating to the Madrid Agreement
7. The Hague Agreement Concerning the International Deposit of Industrial Designs
8. The Trademark Law Treaty (TLT)
9. The Patent Law Treaty (PLT)

10. Treaties on Classification
11. Special Conventions in the Field of Related Rights: The International Convention for the Protection of Performers, Producers of Phonograms and Broadcasting Organizations ("the Rome Convention")
12. The WIPO Performances and Phonograms Treaty (WPPT)
13. The International Convention for the Protection of New Varieties of Plants
14. The Agreement on Trade-Related Aspects of Intellectual Property Rights ("TRIPS") and WIPO-WTO Cooperation
15. Progressive Development of International Intellectual Property Law

3.3. World Intellectual Property Organisation

The World Intellectual Property Organization (WIPO) is one of the 17 specialized agencies of the United Nations, located in Geneva, Switzerland. The Organization has External Offices at Rio de Janeiro in Brazil, Tokyo in Japan, Singapore and New York.

The **mission of WIPO** is to promote innovation and creativity for the economic, social and cultural development of all countries, through a balanced and effective international intellectual property system.

The origin of WIPO goes back to 1883 and 1886 when the Paris Convention for the Protection of Industrial Property and the Berne Convention for the Protection of Literary and Artistic Works, respectively, were concluded. Both Conventions provided for the establishment of an international bureau. The two bureaus were united in 1893 and, in 1970, were replaced by the World Intellectual Property Organization, by virtue of the WIPO Convention.

The WIPO Convention, the constituent instrument of the World Intellectual Property Organization (WIPO), was signed at Stockholm on July 14, 1967, entered into force in 1970 and was amended in 1979. WIPO is an intergovernmental organization that became in 1974 one of the specialized agencies of the United Nations system of organizations.

WIPO currently has 185 Member States, and 68 intergovernmental organizations (IGOs) and International non-governmental organizations (NGOs) and 63 National NGOs that are accredited as observers at WIPO meetings.

The core tasks of WIPO are:

- working with Member States to support a balanced evolution of international IP law
- administering treaties
- assisting governments and organizations in developing the policies, structures and skills needed to harness the potential of IP for economic development
- servicing global registration systems for trademarks, industrial designs and appellations of origin and a global filing system for patents
- delivering arbitration, mediation and other dispute resolution services
- promoting respect for IP
- providing a forum for informed debate and for the sharing of IP knowledge
- identifying IP-based solutions that can help confront global challenges and maximize the benefits of the IP system for all

How WIPO works?

WIPO's Member States determine the strategic direction and activities of the Organization. They meet in the Assemblies, committees and working groups. The WIPO Secretariat, or International Bureau, is based in Geneva. WIPO staff, drawn from more than 90 countries, includes experts in diverse areas of IP law and practice, as well as specialists in public policy, economics, administration and IT. The respective divisions of the Secretariat are responsible for coordinating the meetings of

Member States and implementing their decisions; for administering the international IP registration systems; for developing and executing the programs designed to achieve WIPO's goals; and for providing a repository of IP expertise to assist its members.

It has to present a program and a budget every 2 years about performance measures, budget planning for all the events of the organization which requires member state approval. t is a self financing organization and its funds are majorly used for organizing events.

WIPO Arbitration and Mediation Centre was created in 1994 for the settlement of international commercial disputes arising between private parties located in Geneva, Switzerland and it has an office in Singapore

WIPO's Goals

The strategic goals defined in WIPO's revised Program and Budget for 2008/09 are:

- A balanced evolution of the international normative framework for IP
- Provision of premier global IP services
- Facilitating the use of IP for development
- Coordination and development of global IP infrastructure
- World reference source for IP information and analysis
- International cooperation on building respect for IP
- Addressing IP in relation to global policy issues
- A responsive communications interface between WIPO, its Member States and all stakeholders
- An efficient administrative and financial support structure to enable WIPO to deliver its programs

Indian Scenario

History of Copyright Law in India

Modern copyright law developed in India gradually, in a span of more than 150 years. Copyright law entered India in 1847 through an enactment during the East India Company's regime. According to the 1847 enactment, the term of copyright was for the lifetime of the author plus seven years post-mortem. But in no case could the total term of copyright exceed a period of forty-two years. The government could grant a compulsory licence to publish a book if the owner of copyright, upon the death of the author, refused to allow its publication. The act of infringement comprised in a person's unauthorized printing of a copyright work for (or as a part of attempt of) "sale hire, or exportation", or "for selling, publishing or exposing to sale or hire".

Suit or action for infringement was to be instituted in the "highest local court exercising original civil jurisdiction." The Act provided specifically that under a contract of service copyright in "any encyclopedia, review, magazine, periodical work or work published in a series of books or parts" shall vest in the "proprietor, projector, publisher or conductor." Infringing copies were deemed to be copies of the proprietor of copyrighted work. Importantly, unlike today, copyright in a work was not automatic. Registration of copyright with the Home Office was mandatory for the enforcement of rights under the Act. However, the Act also specifically reserved the subsistence of copyright in the author, and his right to sue for its infringement to the extent available in law other than the 1847 Act. At the time of its introduction in India, copyright law had already been under development in Britain for over a century and the provisions of the 1847 enactment reflected the learnings from deliberations during this period.

In 1914, the then Indian legislature enacted a new Copyright Act which merely extended most portions of the United Kingdom Copyright Act of 1911 to India. It did, however, make a few minor modifications. First, it introduced criminal sanctions for copyright infringement (sections 7 to 12).

Second, it modified the scope of the term of copyright; under section 4 the "sole right" of the author to "produce, reproduce, perform or publish a translation of the work shall subsist only for a period of ten years from the date of the first publication of the work." The author, however, retained her "sole rights" if within the period of ten years she published or authorised publication of her work a translation in any language in respect of that language. The 1914 Act was continued with minor adaptations and modifications till the 1957 Act was brought into force on 24th January, 1958.

History of Patent Law in India

The first legislation in India relating to patents was the Act VI of 1856. The objective of this legislation was to encourage inventions of new and useful manufactures and to induce inventors to disclose secret of their inventions. The Act was subsequently repealed by Act IX of 1857 since it had been enacted without the approval of the sovereign. Fresh legislation for granting 'exclusive privileges' was introduced in 1859 as Act XV of 1859. This legislation contained certain modifications of the earlier legislation, namely, grant of exclusive privileges to useful inventions only and extension of priority period from 6 to 12 months. The Act excluded importers from the definition of inventor. The 1856 Act was based on the United Kingdom Act of 1852 with certain departures including allowing assignees to make application in India and also taking prior public use or publication in India or United Kingdom for the purpose of ascertaining novelty.

The Act of 1859 provided protection for invention only and not for designs whereas United Kingdom had been protecting designs from 1842 onwards. To remove this lacuna, the 'Patterns and Designs Protection Act' (Act XIII) was passed in 1872. This Act amended the 1859 Act to include any new and original pattern or design or the application of such pattern to any substance

or article of manufacture within the meaning of 'new manufacture'. The Act XV of 1859 was further amended in 1883 by XVI of 1883 to introduce a provision to protect novelty of the invention, which prior to making application for their protection were disclosed in the Exhibitions of India. A grace period of 6 months was provided for filing such applications after the date of the opening of such Exhibition.

In 1888, new legislation was introduced to consolidate and amend the law relating to invention and designs in conformity with the amendments made in the UK law.

In 1911, the Indian Patents and Designs Act, 1911, (Act II of 1911) was brought in replacing all the previous legislations on patents and designs. This Act brought patent administration under the management of Controller of Patents for the first time. This Act was amended in 1920 to provide for entering into reciprocal arrangements with UK and other countries for securing priority. In 1930, further amendments were made to incorporate, *inter-alia*, provisions relating to grant of secret patents, patent of addition, use of invention by Government, powers of the Controller to rectify register of patent and increase of term of the patent from 14 years to 16 years. In 1945, another amendment was made to provide for filing of provisional specification and submission of complete specification within nine months. After Independence, it was felt that the Indian Patents & Designs Act, 1911 was not fulfilling its objective. It was found desirable to enact comprehensive patent law owing to substantial changes in political and economic conditions in the country. Accordingly, the Government of India constituted a committee under the Chairmanship of Justice (Dr.) Bakshi Tek Chand, a retired Judge of Lahore High Court, in 1949, to review the patent law in India in order to ensure that the patent system is conducive to the national interest.

The Committee submitted its interim report on 4th August, 1949 with recommendations for

prevention of misuse or abuse of patent right in India and for amendments to sections 22, 23 & 23A of the Patents & Designs Act, 1911 on the lines of the United Kingdom Acts of 1919 and 1949.

Based on the recommendations of the Committee, the 1911 Act was amended in 1950 (Act XXXII of 1950) in relation to working of inventions and compulsory licence/revocation. In 1952, an amendment

was made to provide compulsory licence in relation to patents in respect of food and medicines, insecticide, germicide or fungicide and a process for producing substance or any invention relating to surgical or curative devices, through Act LXX of 1952. The compulsory licence was also available on notification by the Central Government. Based on the recommendations of the Committee, a bill was introduced in the Parliament in 1953 (Bill No.59 of 1953). However, the bill lapsed on dissolution of the Lok Sabha.

In 1957, the Government of India appointed Justice N. Rajagopala Ayyangar Committee to examine the question of revision of the Patent Law and advise government accordingly. The report of the Committee, which comprised of two parts, was submitted in September, 1959. The first part dealt with general aspects of the patent law and the second part gave detailed note on the several clauses of the lapsed bill of 1953. The first part also dealt with evils of the patent system and solution with recommendations in regard to the law. The committee recommended retention of the patent system, despite its shortcomings. This report recommended major changes in the law which formed the basis of the introduction of the Patents Bill, 1965. This bill was introduced in the Lok Sabha on 21st September, 1965, which, however, lapsed.

In 1967, an amended bill was introduced which was referred to a Joint Parliamentary Committee and on the final recommendation of the Committee, the Patents Act, 1970 was passed. This Act repealed and replaced the 1911 Act so far as the patents law was concerned. However, the 1911 Act continued to be applicable to designs. Most of the provisions of the 1970 Act were brought into force on 20th April, 1972 with the publication of the Patents Rules, 1972.

This Act remained in force for about 24 years till December 1994 without any change. An ordinance effecting certain changes in the Act was issued on 31st December 1994, which ceased to operate after six months. Subsequently, another ordinance was issued in 1999. This ordinance was later replaced by the Patents (Amendment) Act, 1999 that was brought into force retrospectively from 1st January, 1995. The amended Act provided for filing of applications for product patents in the areas of drugs, pharmaceuticals and agro chemicals though such patents were not allowed. However, such applications were to be examined only after 31st December, 2004. Meanwhile, the applicants could be allowed Exclusive Marketing Rights (EMRs) to sell or distribute these products in India, subject to fulfillment of certain conditions.

The second amendment to the 1970 Act was made through the Patents (Amendment) Act, 2002 (Act 38 0f 2002). This Act came into force on 20th May, 2003 with the introduction of the new Patents Rules, 2003 by replacing the earlier Patents Rules, 1972.

The third amendment to the Patents Act, 1970 was introduced through the Patents (Amendment) Ordinance, 2004 with effect from 1st January, 2005. This Ordinance was later replaced by the Patents (Amendment) Act, 2005 (Act 15 Of 2005) on 4th April, 2005 which was brought into force from 1st January, 2005.

Intellectual Property Trends – India

During 2009-10, 34,287 patent applications were filed, 6069 examined and 6168 patents granted. The number of applications filed by the Indian applicants was 7044. Out of the applications filed by the Indian applicants, Maharashtra accounted for the maximum number followed by Delhi, Tamil Nadu, Karnataka, Andhra Pradesh, West Bengal etc.

During 2009-10, 6092 design applications were filed, 6266 examined and 6025 registered. The number of applications filed by the Indian applicants was 4267. The number of registered designs in force at the end of 2009-10 was 39008.

During 2009-10, 1,41,943 trademark applications were filed, 25875 examined and 67,490 registered. The number of applications filed by the Indian applicants was 1,34,403. The total number of registered trademarks as of 31st March, 2010 is 8,22,825.

During 2009-10, 40 Geographical indications applications were filed and 14 were registered. A total of 120 Geographical Indications have been registered till 31st March, 2010.

During 2009-10, 142 applications were received for access of bio-resources for research/commercial use, transfer of research results, intellectual property rights and third party transfer. Totally, 13 agreements have been signed. So far, 11 patents have been granted on the applications cleared by NBA. The NBA has also received a royalty amount of Rs.37.89 lakhs from the applicants who have exported bio-resources.

In 2010-11, a total of 642 applications representing 28 crops were received by the Authority for seeking plant variety protection under the Act. The applications belong to new (395), extant (216), farmers' varieties (30) and essentially derived variety (1) categories.

Initiatives of Government of India towards protection of IPR

1. The Government has brought out A Handbook of Copyright Law to create awareness of copyright laws amongst the stakeholders, enforcement agencies, professional users like the scientific and academic communities and members of the public.

2. National Police Academy, Hyderabad and National Academy of Customs, Excise and Narcotics conducted several training programs on copyright laws for the police and customs officers.

3. The Department of Education, Ministry of Human Resource Development, Government of India has initiated several measures in the past for strengthening the enforcement of copyrights that include constitution of a Copyright Enforcement Advisory Council (CEAC), creation of separate cells in state police headquarters, encouraging setting up of collective administration societies and organization of seminars and workshops to create greater awareness of copyright laws among the enforcement personnel and the general public.

4. Special cells for copyright enforcement have so far been set up in 23 States and Union Territories, i.e. Andhra Pradesh, Assam, Andaman & Nicobar Islands, Chandigarh, Dadra & Nagar Haveli, Daman & Diu, Delhi, Goa, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir, Karnataka, Kerala, Madhya Pradesh, Meghalaya, Orissa, Pondicherry, Punjab, Sikkim, Tamil Nadu, Tripura and West Bengal.

5. The Government also initiates a number of seminars/workshops on copyright issues. The participants in these seminars include enforcement personnel as well as representatives of industry organizations.

Copyright:

Copyright is the set of exclusive rights granted to the author or creator of an original work, including the right to copy, distribute and adapt the work. Copyright lasts for a certain time period after which the work is said to enter the public domain. Copyright gives protection for the expression of an idea and not for the idea itself. For example, many authors write textbooks on physics covering various aspects like mechanics, heat, optics etc. Even though these topics are covered in several books by different authors, each author will have a copyright on the book written by him / her, provided the book is not a copy of some other book published earlier.

Content and Substance

According to Section 14 of the Act, "copyright" means the exclusive right subject to the provisions of this Act, to do or authorise the doing of any of the following acts in respect of a
work or any substantial part thereof, namely:-

(a) in the case of a literary, dramatic or musical work, not being a computer programme, -

(i) to reproduce the work in any material form including the storing of it in any medium by electronic means;

(ii) to issue copies of the work to the public not being copies already in circulation;

(iii) to perform the work in public, or communicate it to the public;

(iv) to make any cinematograph film or sound recording in respect of the work;
(v) to make any translation of the work;
(vi) to make any adaptation of the work;
(vii) to do, in relation to a translation or an adaptation of the work, any of the acts specified in relation to the work in sub-clauses (i) to (vi);
(b) in the case of a computer programme,-
(i) to do any of the acts specified in clause (a);
(ii) to sell or give on commercial rental or offer for sale or for commercial rental any copy of the computer programme:
Provided that such commercial rental does not apply in respect of computer programmes where the programme itself is not the essential object of the rental.
(c) in the case of an artistic work,-
(i) to reproduce the work in any material form including depiction in three dimensions of a two dimensional work or in two dimensions of a three dimensional work;
(ii) to communicate the work to the public;
(iii) to issue copies of the work to the public not being copies already in circulation;
(iv) to include the work in any cinematograph film;
(v) to make any adaptation of the work;
(vi) to do in relation to an adaptation of the work any of the acts specified in relation to the work in sub-clauses (i) to (iv);
(d) In the case of cinematograph film, -
(i) to make a copy of the film, including a photograph of any image forming part thereof;
(ii) to sell or give on hire, or offer for sale or hire, any copy of the film, regardless of whether such copy has been sold or given on hire on earlier occasions;
(iii) to communicate the film to the public;
(e) In the case of sound recording, -
(i) to make any other sound recording embodying it;
(ii) to sell or give on hire, or offer for sale or hire, any copy of the sound recording regardless of whether such copy has been sold or given on hire on earlier occasions;
(iii) to communicate the sound recording to the public.
Explanation : For the purposes of this section, a copy which has been sold once shall be deemed to be a copy already in circulation.

Period and Assignment of Copyright

Sec.18 of the Copyright Act, 1957 deals with assignment of copyright. The owner of the copyright in an existing work or the prospective owner of the copyright in a future work may assign to any person the copyright either wholly or partially and either generally or subject to limitations and either for the whole term of the copyright or any part thereof.
The mode of assignment should be in the following manner:
• Assignment should be given in writing and signed by the assignor or by his duly authorized agent.
• The assignment should indentify the work and specify the rights assigned and the duration and territorial extent of such assignment.
• The assignment should also specify the amount of royalty payable, if any, to the author or his legal heirs during the currency of the assignment and the assignment may be subject to revision, extension or termination on terms mutually agreed upon by the parties.
• Where the assignee does not exercise the rights assigned to him within a period of one year from the date of assignment, the assignment in respect of such rights will be deemed to have lapsed after the expiry of the said period unless otherwise specified in the assignment.

The period of assignment will be deemed to be 5 years from the date of assignment unless specifically mentioned. If the territorial extent of assignment of the rights is not specified, it will be presumed to extend within India.

Infringement and Remedies

Some of the commonly known acts involving infringement of copyright:

Making infringing copies for sale or hire or selling or letting them for hire;

Permitting any place for the performance of works in public where such performance constitutes infringement of copyright;

Distributing infringing copies for the purpose of trade or to such an extent so as to affect prejudicially the interest of the owner of copyright ;

Public exhibition of infringing copies by way of trade; and

Importation of infringing copies into India.

Civil Remedies for Copyright Infringement

A copyright owner can take legal action against any person who infringes the copyright in the work. The copyright owner is entitled to remedies by way of injunctions, damages and accounts.

Penalties

Any person who knowingly infringes or abets the infringement of the copyright in any work commits criminal offence under Section 63 of the Copyright Act. The minimum punishment for infringement of copyright is imprisonment for six months with the minimum fine of Rs. 50,000/-. In the case of a second and subsequent conviction the minimum punishment is imprisonment for one year and fine of Rs. one lakh.

Purpose, Policy and Meaning of Patent

Patent is a grant for an invention by the Government to the inventor in exchange for full disclosure of the invention. A patent is an exclusive right granted by law to applicants / assignees to make use of and exploit their inventions for a limited period of time (generally 20 years from filing). The patent holder has the legal right to exclude others from commercially exploiting his invention for the duration of this period. In return for exclusive rights, the applicant is obliged to disclose the invention to the public in a manner that enables others, skilled in the art, to replicate the invention. The patent system is designed to balance the interests of applicants / assignees (exclusive rights) and the interests of society (disclosure of invention).

Meaning of 'Invention' under Patent Law

Sec.2(1)(J) - Invention" means a new product or process involving an inventive step and capable of industrial application

What is meant by 'New"?

The invention to be patented must not be published in India or elsewhere, or in prior public knowledge or prior public use with in India or claimed before in any specification in India A feature of an invention that involves technical advance as compared to the existing knowledge or have economic significance or both and makes the invention not obvious to a person skilled in the art.

What can be patented?

Any invention concerning with composition, construction or manufacture of a substance, of an article or of an apparatus or an industrial type of process.

What cannot be patented?

Inventions falling within Section 20(1) of the Atomic Energy Act, 1962

Who are the beneficiaries of the patent grant?

1. The inventor is secure from competition and can exploit the invention for his gain.

2. For the public the invention becomes public knowledge. The technology is freely available after expiry of patent and cheaper and better products become available.

Objectives and Patentability

What is meant by patentable invention?

A new product or process, involving an inventive step and capable of being made or used in an industry. It means the invention to be patentable should be technical in nature and should meet the following criteria –

• **Novelty:** The matter disclosed in the specification is not published in India or elsewhere before the date of filing of the patent application in India.

• **Inventive Step:** The invention is not obvious to a person skilled in the art in the light of the prior publication/knowledge/ document.

• **Industrially applicable:** Invention should possess utility, so that it can be made or used in an industry.

What is not an 'Invention'?

According to Sec 3 of the Patent Act, 1970

• Frivolous inventions

• Inventions contrary to well established natural laws

• Commercial exploitation or primary use of inventions, which is contrary to public order or morality which causes serious prejudice to health or human, animal, plant life or to the environment

• Mere Discovery of a Scientific Principle or

• Formulation of an Abstract Theory or

• Discovery of any living thing or

• Discovery of non–living substance occurring in nature

Mere discovery of any new property or new use for a known substance or of the mere use of a known process, machine or apparatus, unless such known process results in a new product or employs at least one new reactant.

• Substance obtained by mere admixture resulting only in the aggregation of the properties of the components thereof or a process for producing such substance

• Mere arrangement or re-arrangement or duplication of known devices, each functioning independently of one another in a known way

• Method of Agriculture or Horticulture

• Any process for medicinal, surgical, curative, prophylactic, diagnostic, therapeutic or other treatment of human beings or a similar treatment of animals to render them free of disease or to increase their economic value or that of their products

• Plants & animals in whole or any part thereof other than micro- organisms, but including seeds, varieties an d species and essentially biological process for production or propagation of plants & animals

• mathematical method or

• business method or

• algorithms or

• computer programme per se

• A literary,dramatic, musical or artistic work or any other aesthetic creation including cinematographic work and television productions

• Presentation of information

• Topography of integrated circuits.
• Inventions which are Traditional Knowledge or an aggregation or duplication of known properties of traditionally known component or components

Procedure

Application is required to be filed according to the territorial limits where the applicant or the first mentioned applicant in case of joint applicants, for a patent normally resides or has domicile or has a place of business or the place from where the invention actually originated. If the applicant for the patent or party in a proceeding having no business place or domicile in India, the appropriate office will be according to the address for service in India given by the applicant or party in a proceeding . The appropriate office once decided in respect of any proceedings under the Act shall not ordinarily be changed. The four patent offices are located at Kolkata, Mumbai, Delhi & Chennai.

Publication:

All the applications for patent, except the applications prejudicial to the defence of India or abandoned due to non-filing of complete specification within 12 months after filing the provisional or withdrawn within 15 months of filing the application, are published in the Patent Office Journal just after 18 months from the date of filing of the application or the date of priority whichever is earlier. The publication includes the particulars of the date of the application, application number, name and address of the applicant along with the abstract. The applications for patent are not open for public inspection before publication. After the date of publication of the application, as stated above, the complete specification along with provisional and drawing, if any, abstract , application on any form or on plain paper and any correspondence between the office and applicant may be inspected at the appropriate office by making a written request to the Controller in the prescribed manner and on the payment of prescribed fee.

Request for examination

An application for patent will not be examined if no request is made by the applicant or by any other interested person in Form-18 with prescribed fee of Rs.2,500/- or Rs.10,000/- for natural person and other than natural person respectively, within a period of 48 months from the date of priority of the application or from the date of filing of the application, whichever is earlier.

Where no request for examination of the application for patent has been filed within the prescribed period, the aforesaid application will be treated as withdrawn and, thereafter, application cannot be revived.

Examination

Application for patent, where request has been made by the applicant or by any other interested person, will be taken up for examination, according to the serial number of the requests received on Form 18. A First Examination Report (FER) stating the objections/requirements is communicated to the applicant or his agent according to the address for service ordinarily within six (06) months from the date of request for examination or date of publication whichever is later. Application or complete specification should be amended in order to meet the objections/requirements within a period of 12 months from the date of First Examination Report (FER). No further extension of time is available in this regard. If all the objections are not complied with within the period of 12 months, the application shall be deemed to have been abandoned. When all the requirements are met the patent is granted, after 6 months from the date of publication, the letter patent is issued, entry is made in the register of patents and it is notified in the Patent Office, Journal.

Withdrawal of patent application

The application for patent can be withdrawn at least 3 (Three) months before the first publication which will be 18 (Eighteen) months from the date of filing or date of priority whichever is earlier. The application can also be withdrawn at any time before the grant of the patent. The application

withdrawn after the date of publication cannot be filed again as it is already laid open for public inspection. However, application withdrawn before the publication can be filed again provided it is not opened to public otherwise.

Opposition proceedings to grant of patents

Where an application for a patent has been published but a patent has not been granted, any person may, in writing represent by way of opposition to the Controller against the grant of any Patent. The representation shall be filed at the appropriate office and shall include a statement and evidence, if any, in support of the representation and a request for hearing if so desired.

Grant of Patent

When all the requirements are met or in case of opposition under section 25(1), if the opposition is decided in favour of the applicant, the patent is granted, after 6 months from the date of publication under section 11 A, the letter patent is issued, entry is made in the register of patents and it is notified in the Patent Office, Journal, thereafter opening the application, specification and other related documents for public inspection on payment of prescribed fee.

Term and Date of Patent

Term of every patent will be 20 years from the date of filing of patent application, irrespective of whether it is filed with provisional or complete specification. Date of patent is the date on which the application for patent is filed. The term of patent in case of International applications filed under the Patent Cooperation Treaty designating India, will be 20 years from the International filing date accorded under the Patent Cooperation Treaty. A patent will have cease to effect on the expiration of the period prescribed for the payment of any renewal fee, if that fee is not paid within the prescribed period.

Rights and Obligations of Patent Holder

Where a patent covers a product, the grant of patent gives the patentee the exclusive right to prevent others from performing, without authorisation, the act of making, using, offering for sale, selling or importing that product for the above purpose. Where a patent covers a process, the patentee has the exclusive right to exclude others from performing, without his authorisation, the act of using that process, using and offering for sale, selling or importing for those purposes, the product obtained directly by that process in India. Where a patent is granted to two or more persons, each of those persons will be entitled to an equal undivided share in the patent unless there is an agreement to the contrary.

Infringement and Remedies

Infringement of a patent consists of the unauthorized making, importing, using, offering for sale or selling any patented invention within the India.

Remedies against infringement of a patented invention

1. Interlocutory Injunction

A patent owner at the start of a trial can request for an interim injunction to restrain the defendant from committing the acts complained of until the hearing of the action or further orders. Permanent injunction is given based on the merits of the case at the end of the trial.

2. Relief of damages: An award of damages focuses on the losses sustained by the claimant. A patent owner is entitled to the relief of damages as compensation to the patentee and not punishment to the infringer.

3. Account of profits: Account of profits focuses on the profits made by the defendant, without reference to the damage suffered by the claimant at the hands of the defendant. The purpose of the account is to prevent the unjust enrichment of the defendant by the use of the claimant’s invention.

The patent owner may also opt for the account of profits where he has to prove use of invention and the amount of profit derived from such illegal use.

Penalties

1) Contravention of secrecy provisions relating to certain inventions (Sec.118) - If any person fails to comply with any directions given under section 35 or makes or causes to be made an application in contravention of section 39 he shall be punishable with imprisonment up to 2 years or with fine or with both. (Section 35 deals with secrecy directions relating to inventions relevant for defence purposes and Section 39 deals with residents not to apply for patents outside India without prior permission.

2) Falsification of entries in register etc (Sec.119) - If any person makes, or causes to be made, a false entry in any register kept under this Act, he shall be punishable with imprisonment for a term that may extend to 2 years or with fine or with both.

3) Unauthorized claim of patent rights (Sec.120) - If any person falsely represents that any article sold by him is patented in India or is the subject of an application for a patent in India, he will be punishable with fine that may extend to Rs.1,00,000. The use of words 'patent', Patented', 'Patent applied for', 'Patent pending', 'Patent registered' without mentioning the name of the country means they are patented in India or patent applied for in India.

4) Wrongful use of words, "patent office" (Sec.121) - If any person uses on his place of business or any document issued by him or otherwise the words “patent office” or any other words which reasonably lead to the belief that his place of business is, or is officially connected with, the patent office, he will be punishable with imprisonment for a term that may extend to 6 months, or with fine, or with both.

5) Refusal or failure to supply information (Sec.122) - If any person refuses or fails to furnish information as required under section 100(5) and 146 he shall be punishable with fine, which may go up to Rs 10,00,000/-. If he furnishes false information knowingly he shall be punishable with imprisonment that may extend to 6 months or with fine or with both.

6) Practice by non-registered patent agents (Sec.123) - Any person practicing as patent agent without registering is liable to be punished with a fine of Rs 1,00,000/- in the first offence and Rs.5,00,000/- for subsequent offence.

7) Deals with offences by companies (Sec.124) - When offence is committed by a company as well as every person in charge of and responsible to the company for the conducts of its business at the time of the commission of the offence will be deemed to be guilty and will be liable to be preceded against and punished accordingly. Provided that nothing contained in this sub-section shall render any such person liable to any punishment if he proves that the offence was committed without his knowledge or that he exercised all due diligence to prevent the commission of such offence.

Protection of Plant Varieties & Farmers’ Rights

Meaning and Content

A plant variety represents a more precisely defined group of plants, selected from within a species, with a common set of characteristics.

The Protection of Plant Varieties and Farmers' Right Act, 2001 has been enacted to provide for the establishment of an effective system for protection of plant varieties, the rights of farmers and plant breeders and to encourage the development of new varieties of plants.

The objectives of the Act are:

• to establish an effective system for protection of plant varieties, the rights of farmers and plant breeders and to encourage the development of new varieties of plants;

• to recognize and protect the rights of the farmers in respect of their contribution made at any time in conserving, improving and making available plant genetic resources for the development of new plant varieties;
• to protect plant breeders' rights to stimulate investment for research and development both in the public and private sector for development of new plant varieties;
• to facilitate the growth of seed industry in the country that will ensure the availability of high quality seeds and planting material to the farmers.

Procedure

The application for protection under the Act can be made by any of the following persons:
• Any person claiming to be the breeder of the variety;
• Any successor of the breeder of the variety;
• Any person being the assignee or the breeder of the variety in respect of the right to make such application;
• Any farmer or group of farmers or community of farmers claiming to be breeder of the variety;
• Any person authorized to apply on behalf of farmers; or
• Any university or publicly funded agricultural institution claiming to be breeder of the variety.

Criteria for registration of new variety

Novelty - A new variety is deemed to be novel if, at the date of filing of the application for registration for protection, the propagating and harvested material of such variety has not been sold or otherwise disposed of by or with the consent of its breeder or his successor for the purposes of exploitation of such variety for a certain period of time before the date of filing of the application. For sale or disposal of a new variety in India, this time period is earlier than one year. Outside of India, in the case of trees and vines, the time period is earlier than six years. In any other case in India, it is earlier than four years.

Distinctiveness - A new variety is deemed distinct if it is clearly distinguishable by at least one essential characteristic from any other variety whose existence is a matter of common knowledge in any country at the time of filing of the application.

Uniformity - A new variety is deemed uniform if subject to the variation that may be expected from the particular features of its propagation it is sufficiently uniform in its essential characteristics.

Stability - A new variety is deemed stable if it's essential characteristics remain unchanged after repeated propagation or, in case of a particular cycle of propagation, at the end of each such cycle.

Period of Validity

The duration of protection of registered varieties is different for different crops which are as below:

1. For trees and vines - 18 years.
2. For other crops - 15 years.
3. For extant varieties - 15 years from the date of notification of that variety by the Central Government under section 5 of the Seeds Act, 1966.

Biodiversity and Environment

Documentation

India has been a party to the Convention on Biological Diversity since 5th June 1992 and ratified the Convention on 18th February 1994. The Convention on Biological Diversity is one of the most broadly subscribed international environmental treaties in the world. Opened for signature at the Earth Summit in Rio de Janeiro Brazil in 1992, it currently has 189 Parties - 188 States and the European Community - who have committed themselves to its three main goals: the conservation of biodiversity, sustainable use of its components and the equitable sharing of the benefits arising out of the utilization of genetic resources. The Secretariat of the Convention is located in Montreal, Canada.

India is also a signatory to Cartagena Protocol on Biosafety signed on 23rd January 2001 and ratified on 11th September 2003.

IPR issues in biodiversity conservation

India enacted The Biological Diversity Act, 2002 and The Biological Diversity Rules, 2004 to fulfill its commitments in the Convention on Biological Diversity and in the Cartagena Protocol on Biosafety. Various states have also enacted state specific Biological diversity rules namely – Kerala Biological Diversity Rules, 2005; Sikkim State Biological Diversity Rules, 2006; Nagaland Biological Diversity Rules, 2010; Rajasthan Biological Diversity Rules, 2010; A.P. State Biological Diversity Rules, 2009, West Bengal Biological Diversity Rules, 2005; U.P. State Biodiversity Rules, 2010; Maharashtra Biological Diversity Rules, 2008 etc. The Biodiversity Act - 2002 primarily addresses access to genetic resources and associated knowledge by foreign individuals, institutions or companies, to ensure equitable sharing of benefits arising out of the use of these resources and knowledge to the country and the people.

The Act has specific provisions about ownership of intellectual property rights associated with exploitation of biodiversity. Industries have to obtain prior consent of the National Biodiversity Authority before exploring the biodiversity in India. In the event of R&D based on exploitation of biodiversity and associated local knowledge, there is a provision for sharing of benefits of such work with the local community. No direct flow of funds is expected to the community. Instead the Union Government will reach the benefits through State Governments to the community.

The Biological diversity Act of 2002 contains 65 sections under 12 chapters while the Biological Diversity Rules of 2004 consists of 24 rules and one schedule. According to Section 2(b) of the Biological Diversity Act, 2002 “Biological Diversity means the variability among living organisms from all sources and the ecological complexes of which they are part and includes diversity within species or between species and of eco-systems.”

Access to Plant Genetic Resources and Benefit Sharing

Chapter II of the Biological Diversity Act, 2000 lays down certain regulations with reference to access to Biological Diversity. The following regulations have been placed in Section 3 to 7 of the said act. Section 3 of the above referred act, requires the following categories of persons to seek previous permission of the National Biodiversity Authority, to obtain any biological resource occurring in India or knowledge associated thereto for research or commercial utilization or for bio-survey and bio-utilization –

- A person who is not a citizen of India.
- A citizen of India who is a non-resident as per section 2(30) of the Income-tax Act, 1961.
- A body corporate, association, organization –

o Not incorporated or registered in India; or

o Incorporated or registered in India under any law for the time being in force which has any non-Indian participation in its share capital or management.

Rule 14 of the Biological Diversity Rules, 2004 mentions the procedure for access to biological resources and associated traditional knowledge.

Section 4 of the above referred act requires that every person shall seek the previous approval of the national bio-diversity authority before transferring the results of any research related to any biological recourses occurring in, or obtained from India to any person as referred to in section 3 above. Rule 17 of the Biological Diversity Rules, 2004 mentions the procedure for seeking approval for transferring results of research.

Bioprospecting & Biopiracy

Biodiversity prospecting is the exploration, extraction and screening of biological diversity and indigenous knowledge for commercially valuable genetic and biochemical resources. While it is true that biodiversity prospecting does not always involve the use of indigenous knowledge, it is clear that valuable chemical compounds derived from plants, animals and micro-organisms are more easily identified and are of greatest commercial value when collected with indigenous knowledge and/or found in territories traditionally inhabited by indigenous peoples.

Between 1956 and 1976 the U.S. National Cancer Institute screened over 35,000 plants and animals for anti-cancer compounds. The program was terminated in 1981 because of its failure to identify a greater number of new anti-cancer agents. A retrospective study conducted on the project concluded that the success rate in finding valuable species could have been doubled if medicinal folk knowledge had been the only information used to target species. Similarly in another instance scientists found that 86 percent of the plants used by Samoan healers displayed significant biological activity when tested in the laboratory.

Biopiracy can be defined as the stealing of knowledge from traditional and indigenous communities or individuals. The term can also be used to suggest a breach of a contractual agreement on the access and use of traditional knowledge to the detriment of the provider and bioprospecting without the consent of the local communities. The Action Group on Erosion, Technology and Concentration [ETC group, Canada (former RAFI)] defines it as “the appropriation of the knowledge and genetic resources of farming and indigenous communities by individuals or institutions seeking exclusive monopoly control (usually patents or plant breeders’ rights) over these resources and knowledge”.

There is a distinct difference between biopiracy and bioprospecting. The term ‘biopiracy’ describes the unauthorized and uncompensated taking and use of biological resources. In contrast, bioprospecting refers to the search for valuable active chemical compounds in nature, and involves accessing natural resources through legal means, securing prior informed consent from the custodians of the relevant natural resources and promoting equitable benefit sharing agreements with appropriate parties. Biopiracy deprives not only the custodians of biological resources but also the country concerned.

The modus operandi of the MNCs has been to collect the plant varieties and their germplasms from poor countries in order to cross- breed them with other varieties, and claim that they had invented something novel, non-obvious and of practical use (which are the requirements for acquiring patent rights), and then to patent them in their own countries or in any other country of their choice. Thus even though India is rich in biodiversity and has a rich biodiversity related intellectual heritage, biopiracy directs this wealth away from India and denies us our rights to use our resources and knowledge, for our needs and our economic benefits.

IPR in environmental sustainability

Creating a world that is sustainable for future generations requires transitions towards novel technologies which are environmentally friendly and socially sustainable. While intellectual property rights (IPR) can incentivise technological innovations, the debate on positive and negative effects of IPR over the last decades has identified a number of issues that hamper the diffusion of innovation. Amongst these issues are the increase of costs for negotiating licensing deals when necessary IPRs are distributed amongst several owners, and deterrent effects of the uncertainty whether existing IPRs are infringed and whether the owners are going to sue. Accordingly, increasing IPR protection is discussed as hindering market competition and for its negative impacts on costs of new technologies, most importantly those of high societal. Whereas the list of theoretical issues associated with IPR and diffusion of sustainable technologies is long, we lack empirical evidence about the extent of these potential problems.

IP issues in Biotechnology

Biotechnology is generally defined as “the application of science and technology to living organisms, as well parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services”1 . This definition is deliberately broad and covers all modern biotechnology but also many other traditional or borderline activities. Taken all together, these activities belong to what is commonly called “Life Sciences”. Biotechnology is a field where technology advances rapidly but returns on investments may be slow. For this reason, it is important for public research organisations and enterprises to protect the innovation that they generate with Intellectual Property Rights (IPR), which provide a basis for return on investment in research and development, by granting monopoly rights for a certain period of time to their owners.

Biotechnology is usually subdivided into three sectors that may overlap, namely:

Healthcare biotechnology or red biotechnology which plays an important role in drug discovery (insulin, erythropoietin, etc.) and today is improving outcomes for patients and addressing unmet medical needs for the future;

Agriculture biotechnology or green biotechnology that is used to enhance plants in order to improve their resistance to disease, tolerance for herbicides or difficult environment conditions, or to achieve higher yields with less inputs (water, fertilizers, etc.);

Industrial biotechnology or white technology, representing the “third wave” in biotechnology, because it follows innovation in the health and agricultural areas; this sector encompasses the application of biotechnology-based tools to traditional industrial processes (“bioprocessing”) and the manufacturing of bio-based products (biofuels, bio-plastics and bio-based chemicals). In this technology enzymes and/or micro-organisms, such as fungi, yeast, bacteria (also referred as “biocatalysts”), are used to make intermediate and endproducts more efficiently, reduce environmental impacts of processes and products and/or enable the creation of new products from renewable resources.

It is obvious that management of IP and IPR is a multidimensional task and calls for many different actions and strategies which need to be aligned with national laws and international treaties and practices. It is no longer driven purely by a national perspective. IP and its associated rights are seriously influenced by the market needs, market response, cost involved in translating IP into commercial venture and so on. In other words, trade and commerce considerations are important in the management of IPR. Different forms of IPR demand different treatment, handling, planning, and strategies and engagement of persons with different domain knowledge such as science, engineering, medicines, law, finance, marketing, and economics. Each industry should evolve its own IP policies, management style, strategies, etc. depending on its area of specialty. Pharmaceutical industry currently has an evolving IP strategy. Since there exists the increased possibility that some IPR are invalid, antitrust law, therefore, needs to step in to ensure that invalid rights are not being unlawfully asserted to establish and maintain illegitimate, albeit limited, monopolies within the pharmaceutical industry. Still many things remain to be resolved in this context.

More than any other technological area, drugs and pharmaceuticals match the description of globalization and need to have a strong IP system most closely. Knowing that the cost of introducing a new drug into the market may cost a company anywhere between $ 300 million to $1000 million along with all the associated risks at the developmental stage, no company will like to risk its IP becoming a public property without adequate returns. Creating, obtaining, protecting, and managing IP must become a corporate activity in the same manner as the raising of resources and funds. The

knowledge revolution, which we are sure to witness, will demand a special pedestal for IP and treatment in the overall decision-making process.

Competition in the global pharmaceutical industry is driven by scientific knowledge rather than manufacturing know-how and a company's success will be largely dependent on its R&D efforts. Therefore, investments in R&D in the drug industry are very high as a percentage of total sales; reports suggest that it could be as much as 15% of the sale. One of the key issues in this industry is the management of innovative risks while one strives to gain a competitive advantage over rival organizations. There is high cost attached to the risk of failure in pharmaceutical R&D with the development of potential medicines that are unable to meet the stringent safety standards, being terminated, sometimes after many years of investment. For those medicines that do clear development hurdles, it takes about 8-10 years from the date when the compound was first synthesized. As product patents emerge as the main tools for protecting IP, the drug companies will have to shift their focus of R&D from development of new processes for producing known drugs towards development of a new drug molecule and new chemical entity (NCE). During the 1980s, after a period of successfully treating many diseases of short-term duration, the R&D focus shifted to long duration (chronic) diseases. While looking for the global market, one has to ensure that requirements different regulatory authorities must be satisfied.

It is understood that the documents to be submitted to regulatory authorities have almost tripled in the last ten years. In addition, regulatory authorities now take much longer to approve a new drug. Consequently, the period of patent protection is reduced, resulting in the need of putting in extra efforts to earn enough profits. The situation may be more severe in the case of drugs developed through the biotechnology route especially those involving utilization of genes. It is likely that the industrialized world would soon start canvassing for longer protection for drugs. It is also possible that many governments would exercise more and more price control to meet public goals. This would on one hand emphasize the need for reduced cost of drug development, production, and marketing, and on the other hand, necessitate planning for lower profit margins so as to recover costs over a longer period. It is thus obvious that the drug industry has to wade through many conflicting requirements. Many different strategies have been evolved during the last 10 to 15 years for cost containment and trade advantage. Some of these are out sourcing of R&D activity, forming R&D partnerships and establishing strategic alliances.

13. Let’s sum up

- Plants are multicellular and mostly photosynthetic organisms which found essentially everywhere, both in water and on land. The aquatic plants include red, brown and green algae and the land plants include mosses, ferns, gymnosperms and angiosperms.
- The microorganisms have always played an essential role in the biosphere with fermented foods and beverages, plant and animal diseases and nutrient cycling foremost.
- Mushrooms are the fruit bodies of edible fungi, commonly belonging to Basidiomycotina (*Agaricus campestris, A. brunnescens, Pleurotus sajor-caju, Volvariella volvacea* etc.) and rarely to Ascomycotina (*Morchella conica, M. esculenta*).

- Most remediation activity still makes use of conventional methods such as excavation and reburial, capping, and soil washing and burning. However, newly emerging biological cleanup methods, such as phytoremediation, are often simpler in design and cheaper to implement.

- Stress is usually defined as an external factor that exerts a disadvantageous influence on the plant. This chapter will concern itself with environmental or abiotic factors that produce stress

in plants, although biotic factors such as weeds, pathogens, and insect predation can also produce stress.

- Genetically modified crops are defined as crops whose genomes have been altered in ways that do not occur naturally. Genetically engineered crops have genes added or removed using genetic engineering techniques, originally including gene guns, electroporation, microinjection and *Agrobacterium*.

- Plants are a vital component of biodiversity and healthy ecosystems. They provide a range of ecosystem services, from production of oxygen and removal of atmospheric carbon dioxide emissions, creation and stabilization of soil, protection of watersheds and provision of natural resources including food, fibre, fuel, shelter and medicine.

- Conservation of biodiversity is protection, upliftment and scientific management of biodiversity so as to maintain it at its threshold level and derive sustainable benefits for the present and future generation.

- The forensic aspects require an understanding of what is necessary for botanical evidence to be accepted as evidence in our judicial system. Forensics requires recognition of pertinent evidence at a crime scene, appropriate collection and preservation of evidentiary material, maintenance of a chain of custody, an understanding of scientific testing methods, validation of new forensic techniques, and admissibility criteria for court.

- Intellectual property Right (IPR) is a term used for various legal entitlements which attach to certain types of information, ideas, or other intangibles in their expressed form. The holder of this legal entitlement is generally entitled to exercise various exclusive rights in relation to the subject matter of the Intellectual Property.

14. Suggested Readings

1. Bhattacharya K., Hait G. & Kumar A. K. (2011) AText Book of Botany Vol I New Central Book Agency
2. Bhattacharya K., Hait G. & Kumar A. K. (2011) AText Book of Botany Vol II New Central Book Agency
3. Ganguli,H.C., Das, K.S.K. & Dutta, C.T. College Botany, Vol. I, latest Ed., New Central Book Agency
4. Ganguli,H.C. and Kar, A.K. College Botany, Vol. II, latest Ed., New Central Book Agency
5. Mukherjee, S. College Botany, Vol. III, latest Ed., New Central Book Agency
6. Lee, R. E. (2008). Phycology.Cambridge University Press.
7. Shaw, A. Jonathan and Goffinet Bernard, Bryophyte Biology, 2009, Cambridge university Press
8. Gifford, E. M. and Foster, A. S. (1998). Morphology & Evolution of Vascular Plants (3rd ed.), Freeman and Co.
9. Dubey, H.C. An Introduction to Fungi (2nd ed.), 1990, Vikas Publishing House 23
10. Coyle. H. M. Forensic_Botany-Principles and Applications to Criminal Casework CRC PRESS Boca Raton London New York Washington, D.C.
11. Taiz, L., & Zeiger, E. Plant Physiology (4th ed.), 2006, Sinauer Associates, Inc. Publishers
12. http://www.biologydiscussion.com/
13. https://en.wikipedia.org/

15. Assignment

1. What is IPR?
2. Give two example of angiosperic plant.
3. Define GMO
4. What are the strategies of ex-situ conservation.
5. Mention two case study associated with plant.
6. Describe cultivation processes of mushroom.
7. What is Golden rice?
8. Write a short note on ecological roles of bryophytes
9. Name two pteridophytes with secondary thickenings.
10. Mention the difference between dictyostelic and solenostelic structures in pteridophytes.
11. What are the differences between fern ally and true fern?
12. Depict the classification system of fern by Sporne (1962) and Smith *et al.* (2006) by highlighting the differences between two systems
13. Distiguish between heterothallism and paraseuality.
14. What is dolipore septum?
15. How Drug enforcement help to solve forensic cases
16. What is typing? Give an overview of DNA typing.
17. Mention the role of pollen in case study. What are the advantages?
18. Name two DNA markers and mention its role in forensic botany.
19. How Diatoms help to solve forensic cases.
20. Distinguish ring porous wood and diffuse porous wood.
21. What botanical evidence can do?
22. How botanical samples are collected?
23. Describe impact of water stress on plants life .
24. Explain structure and function of HSPs in plant.
25. What is chilling injury? How do you overcome it.
26. What is hydroactive closure?
27. Explain the range of thallus structure of algae.
28. Write economic importance of jute.
29. Write the botanical characters of cotton?
30. Give the botanical name of any two fibre yielding plants.
31. Give the botanical name of any two vegetable oil yielding plants
32. What is the origin of center of wheat?
33. Write the botanical name of any two millets.
34. Name two medicinally important plant with their uses.

All the materials are self writing and collected from ebook, journals and websites.

NOTES