

Wollo University

College of Natural Sciences

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Advanced Plant Physiology

(BIOB 511)

By

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Chapter- 1 Plant water relations

Introduction:

The term plant physiology is constructed from the Greek word **physis**= nature and **logos**= discourse. Therefore, physiology is a discourse about the nature of plants.

- Part of biology dealing with the functions and activities of plants.
- Plant physiology is about how plants use the energy of the sun to assimilate carbon.
- How plants convert the carbon to the stuff of which they are made.
- How plants grow/ develop.
- How plants reproduce.

Without water, life could not exist on this planet of the smaller molecules in living organisms; water is the most abundant, typically making up between 80-90% of fresh mass of most herbaceous plants and over 50% of the fresh weight of woody plants. Even dormant seeds and buds contain about 8-10% water. Greek philosopher (Thales) asserted that water was the origin of all things, and it was one of the four basic elements (earth, water, air, fire).

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 to the atmosphere.

Water is the most abundant and arguably the best solvent known. As a solvent, it makes up the medium for the movement of molecules within and between cells and greatly influences the structure of proteins, nucleic acids, polysaccharides, and other cell constituents. Water forms the environment in which most of the biochemical reactions of the cell occur, and it directly participates in many essential chemical reactions.

Importance of water:

In living cells, water plays such a vital role one can not imagine the plant existence without water. Some of the important functions of water have been summarised hereunder-

1. **Major Constituents:** Water constitutes 80-90% of the fresh weight of most herbaceous plants and over 50% of the fresh weight of woody plants. Even dormant seeds and buds contain about 8-10% water. Water is the chief constituent of the protoplasm.
2. **Universal Solvent:** Water acts as universal solvent in which gases, minerals and other solutes enter into plant cells and move from one part of a cell to another and from cell to cell, tissue to tissue and organ to organ.

3. **Metabolic Medium:** Water acts as a medium for many metabolic reactions e.g. in photosynthesis, the hydrogen atom in the water molecules is incorporated into organic compounds and oxygen atoms are released as O₂. Water is a metabolic and product of respiration.
4. **Reactant:** It is reactant of many important processes. The formation of complex carbohydrate from the simple ones also involves the removal of water while the reverse reaction requires water as solvent.
5. **Turgidity:** Water imparts turgidity to growing cells to maintain their form and structure. Turgidity is also essential for stomatal opening, movement of leaves, flower petals and various specialized plant structures.
6. **Growth and Movement:** The elongation phase of cell growth depends on absorption of water. The gain or loss of water from cells and tissues is responsible for a variety of movements of plant parts. These include diurnal swelling and shrinking of stomatal guard cells, the nocturnal folding of leaflets of certain plants and the sensitivity to touch of leaflets of plants, such as the sensitive plant *Mimosa pudica*.

Water Potential (Ψ): The term water potential was coined by Stalyer and Taylor (1960). It is represented by the Greek letter, **Psi (Ψ)**. **Water Potential is a potential energy of water in a particular system under atmospheric pressure and the same temperature.** Water will flow from a region of higher free energy to a region of lower free energy. Free energy is a thermodynamic parameter which determines the direction along with physical and chemical changes should occur. It is measured in terms of **pressure** and the unit of measurement is **pascal**.

Water potential of pure water (solvent) is zero (i.e. maximum). It means that the water potential of a solution has a negative value because the water is less concentrated than in pure water.

Since the water exist in plant tissues can never be pure, its water potential will always be zero. Hence, it always shows negative algebraic sign. Water potential is a powerful tool to diagnose water status of plant cells and tissues. The lower the water potential in a plant cell or tissues, the greater is its ability to absorb water. Conversely, the higher the water potential, the greater is the ability of the tissue to supply water to other more desiccated cells and tissues.

Values of Water Potential under different conditions:

- | | |
|------------------|------------|
| 1. | (Ψ) |
| Fully turgid | 0 |
| Partially turgid | -1.0 |
| Flaccid | -2.0 |
2. Water potential of pure water at atmospheric pressure is **zero (0)**.
 3. In a solution at atmospheric pressure water pressure is **negative**.

The Soil-Plant-Atmosphere Continuum (SPAC):

Soil —————> Plant —————> Atmosphere

The movement of water from the soil through the plant to the atmosphere involves different mechanisms of transport. When water moves from the soil through the plant to the atmosphere, it travels through a widely variable medium (cell wall, membrane, cytoplasm, air spaces), and the mechanisms of water transport also vary with the type of medium.

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.

Soil Water:

Water is essential for life. Water is now said to be the **liquid of life** or **elicitor of life**. Water constitutes about 80-90% of the mass of growing plant tissues. Seeds have only 8-10% water. Root system of the plant is mainly responsible for absorption of the water. Water absorbed by root hairs. A large majority of plants absorb water from soil (except for rootless aquatic plants). Soil plays a dominant role in the life of terrestrial plants by providing them with nutrition and water.

The presence of adequate water in the soil is vital to plant growth, not only because plants need water for their physiological processes but also because the water contains nutrients in solution. Principal source of water is rainfall (precipitation), but rain would do plants little good if the soil could not store water for plant use between rains. Some of the water which falls during rainfall is lost as run-off, which goes back to the source i.e., sea, ocean, and some is lost during evaporation. The capacity of a soil to store water depends on its depth, texture, structure and other properties.

The amount of water that a soil can store in a form available for plant use is known as the available water holding capacity of the soil. Most soil profile can store between 5-25 cm of available water. The difference between high and low available water –holding capacities is often decisive in determining plant growth and crop yield.

When water is added to a dry soil, either by rain or irrigation, it is distributed around the soil particles, where it is held by adhesive and cohesive forces. It displaces air in the pore spaces and eventually fills the pores. When all the pores, large and small, are filled, the soil said to be saturated and or at its maximum retentive capacity. There are following three main classes of water available in the soil.

1. Hygroscopic water: Water held tightly to the surface of soil particles by adsorption forces and is held at a tension greater than 31 atmospheres. It is also called as adsorption water.

2. Capillary water: Water held by forces of surface tension and continuous films around soil particles and in the capillary spaces. It is also known as water cohesion.

3. Gravitational Water: Water that moves freely in response to gravity and drains out of the soil is called gravitational water or free water.

The chief source of soil water is rain fall, besides this, melting of snow also provides water. According to its availability to plants, soil water is described in three different terms-

Holard: Total water present in the soil.

Chesard: Soil water available to the plants.

Echard: Soil water which can not be absorbed by plants.

The water content and rate of water movement in soils depend to a large extent on soil type

Sandy soils: Sandy soils have a relatively low surface area per gram of soil and have large spaces or channels between particles e.g. in sandy soils, the spaces between particles are so large that the water tends to drain from them and remain only on the particle surfaces and at interstices between particles. It has lower moisture holding capacity or field capacity.

Clay soils: Clay soils have much greater surface area and smaller channels between particles. Water in the soil may exist as a film adhering to the surface of the soil particles or may fill the entire channel between particles. In clay soils the channels are the small enough that water does not freely drain from them but is held more tightly. It has large moisture holding capacity. Field capacity refers to the water content of the soil after it has been saturated with water and excess water has been allowed to drain away.

N.B: A large quantity of water is absorbed by plants from the soil in order to perform various biological activities, but one peculiarity is most of this water is evaporated into the atmosphere.

Large quantity of water in soil → plants → evaporation of water from plants into water-vapour turns to condense fall as rain ← moist air cool down → Atmosphere

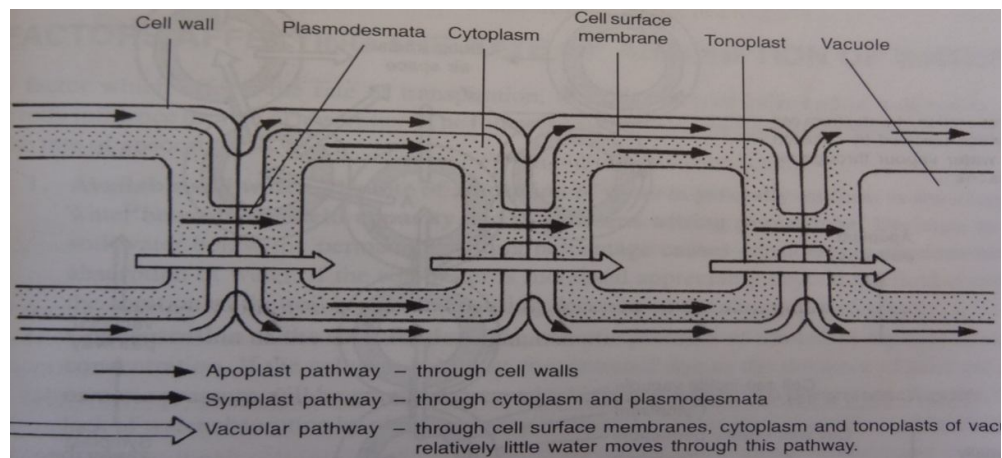
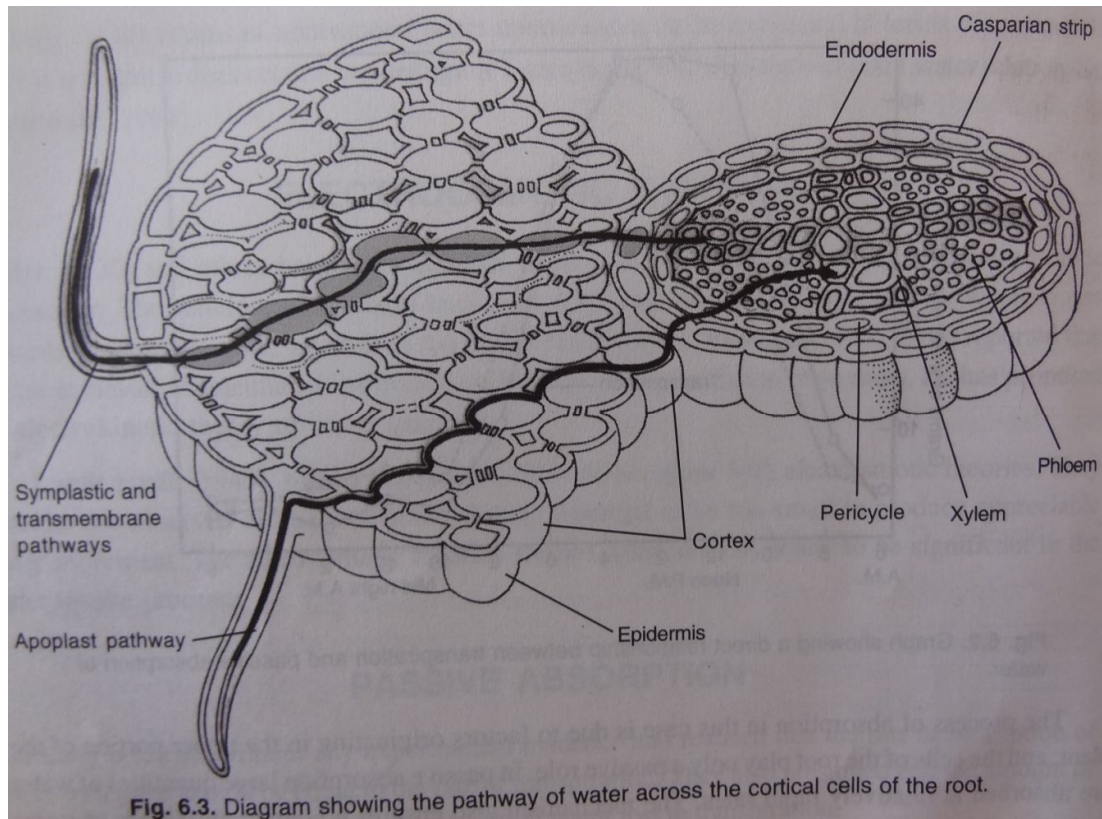
Mechanism of water uptake via Apoplast, Symplast and transmembrane pathway:

If there is an increasing gradient of diffusion pressure deficit between the root hair and soil water, water will enter the root hair and passing through the cortical cells will finally enter the xylem vessel. The adjoining cells of the cortex which still has a higher diffusion pressure deficit absorbs water from the root hair. In this ways water moves from cell to cell in the cortex and reaches the endodermis. There are three components of pathways through which water can enter the endodermis and then into pericycle and the xylem.

1. **Apoplast pathway**
2. **Symplast pathway**
3. **Transmembrane pathway**

1. Apoplast pathway: The apparent free space of the roots and the xylem vessels constitutes the apoplast. Dissolved substances or solutions may diffuse freely through the **apoplast**. In the roots the apoplast is discontinuous due to the presence of a ring of endodermis with thick casparian strips. Water moves exclusively through the cell wall without crossing any membranes. The apoplast is the continuous system of cell walls and intercellular spaces in plant tissues.

2. Symplast pathway: The protoplasm of all living cells of the roots is connected to each other by means of plasmodesmata and therefore, this continuous mass of protoplast is called **symplast**. The major pathway for movement of water is the apoplastic pathway. In the symplastic pathway water moves from cell to cell through their cytoplasm and cytoplasmic strands called plasmodesmata.



3. Transmembrane pathway:

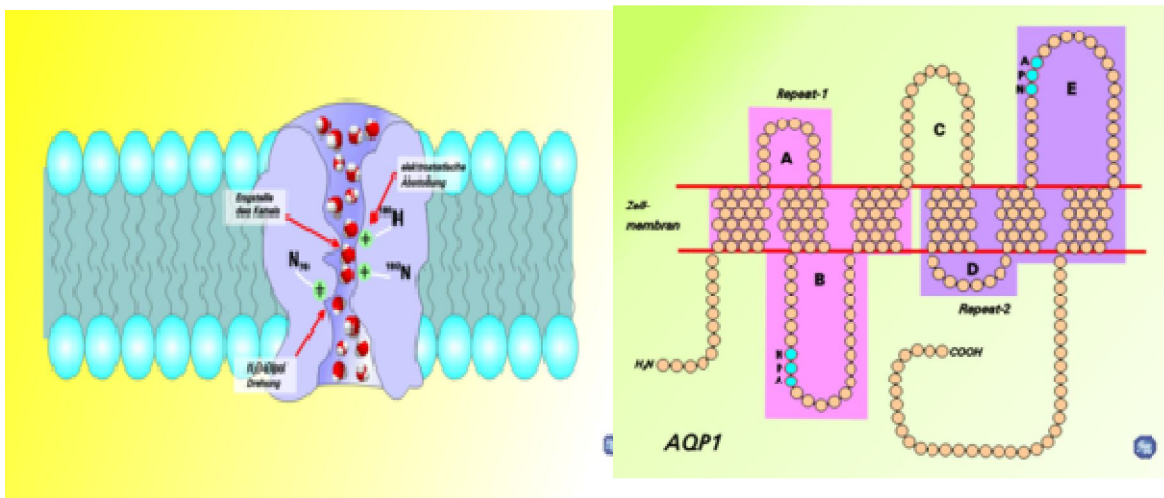
The membrane pathway is the route followed by water that sequentially enters a cell on one side, exits the cell on the other side, enters the next in the series, and so on. In this pathway, water crosses at least two membranes for each cell in its path. Transport across the tonoplast may also be involved.

Aquaporins:

Aquaporins are integral membrane proteins from a larger family of major intrinsic proteins (MIP) that form pores in the membrane of biological cells. It regulates the flow of water. Peter Agre and Roderick MacKinnon (2003) got Nobel Prize Jointly in Chemistry for the discovery and structure of aquaporins. "Aquaporins selectively conduct water molecules in and out of the cell, while preventing the passage of ions and other solutes. The presence of aquaporins in the cell membranes seems to serve to facilitate the transcellular symplastic pathway for water transport. When plant roots are exposed to mercuric chloride, which is known to inhibit aquaporins, the flow of water is greatly reduced while the flow of ions is not, supporting the view that there exists a mechanism for water transport independent of the transport of ions.

Structure of Aquaporins:

Aquaporin proteins are made up of six transmembrane α -helices arranged in a right-handed bundle, with the amino and the carboxyl termini located on the cytoplasmic surface of the membrane. There are also five interhelical loop regions (A – E) that form the extracellular and cytoplasmic vestibules. Loops B and E are hydrophobic loops that contain the highly, although not completely conserved, asparagine–proline–alanine motif, which overlap the middle of the lipid bilayer of the membrane forming a 3-D 'hourglass' structure where the water flows through. This overlap forms one of the two well-known channel constriction sites in the peptide, the asparagine–proline–alanine motif and a second and usually narrower constriction known as 'selectivity filter'. The silencing of plant aquaporins has been linked to poor plant growth and even death of the plant.



Chapter-2. The Ascent of xylem sap:

The water absorbed by the root hairs is translocated upward through the xylem. The upward movement of water from base to tree top is called **ascent of sap**. Mystery of the upward movement of water is yet to be solved in a satisfactory way. The upward transport of water in plants which are 400 feet high has not been satisfactorily explained till date.

A young tomato or balsam seedling was taken and kept in a beaker containing water coloured with eosin. After some time it was seen that streaks of red colour were running up the stem. When a cross section of the stem was taken, it was found that xylem was coloured proving that ascent of sap takes place through the xylem tissues.

Path of Ascent of sap: Though mechanism for upward movement of water or ascent of sap is not clear, it has been proved that ascent of sap takes place through xylem. The girdling experiment was done in a plant with thick stem where the outer layer of phloem was removed. Still it was found that ascent of sap continued to take place proving that, the water travels up along the vascular tissues. Vascular tissues have two components (xylem and phloem). Between these two, which tissue is the path for translocation of water? It is only xylem or phloem or both?

How does water move in plants?

Mechanism of Ascent of sap: A number of theories have been proposed from time to time to explain the mechanism of ascent of sap. These are-

1. Vital theories
2. Root pressure theory
3. Physical force theory

1. Vital theories:

Early investigators believed that the ascent of water in plants was under the control of vital activities of the stem. It was based on the fact that the vessels and tracheids are closely associated with the living cells present in the xylem tissues.

i. Westermaier: Westermaier (1883) proposed that the upward movement of water in the stem takes place through the xylem parenchyma cells. The tracheids and vessels serve as reservoirs where water is held by capillary.

ii. Godlewski: Godlewski (1884) proposed that the living cells of xylem (wood parenchyma and medullary rays) caused the upward movement of water due to their pumping activity,

which results from the periodic changes in their osmotic pressure. When osmotic potential of the living cells decreases, water from the tracheids will diffuse into it, thus osmotic potential will increase and the water will pump into the tracheid above.

iii. Sir J.C. Bose: Bose (1923) experimented to prove that the living cells of the innermost layer of the cortex, just outside the endodermis were in a state of rhythmic pulsations which caused the pumping of water from cell to cell in an upward direction.

Objections to vital theories: Strasburger experimentally discredited the vital theory and proved that the ascent of sap was independent of living cells and water continued to be transported above in the plants even after the living cells had been killed by physical treatments.

2. Root Pressure theory: Stephen Hales (1727) first time coined the term root pressure. If a well watered plant is cut a few inches above the ground level with a sharp knife, the xylem sap is seen flowing out with some forces. This is called **exudation** or **bleeding**. Priestley for the first time observed this phenomenon and explained the root pressure theory. According to him- a sort of hydrostatic pressure develops in the roots due to continuous absorption of water and this is responsible for the upward flow of water.

Stocking (1956) believes the root pressure to be an active process. He has defined the root pressure as a pressure developing in the tracheary elements of the xylem as a result of the metabolic activities of roots.

It is true that root pressure is a dynamic process, but it is not sufficient to translocate water to a distance of 400 feet in the trunk of tall trees. Kramer, Unger, Renner, Dixon, Jolly, Steward etc. have objected very strongly the involvement of root pressure in the ascent of sap.

1. Root pressure is not universal phenomenon, but ascent of sap is. In gymnosperms, there is no or little root pressure observed.
2. Root pressure is commonly observed during favourable periods of growth when the transpiration rate is high, root pressure is not found.
3. The maximum root pressure seen under most favourable condition is about 2 atmospheres which can raise water upto 60-62 feet, while a pressure of about 20 atmospheres is needed to raise water to the tops of tall trees.
4. Water continues to rise up even in the absence of roots.

3. Physical force theory:

i. Capillary force theory: Boehm (1809) proposed this theory. He stated that when a narrow tube is dipped in water, due to surface tension water rises up in the tube upto a certain height. This phenomenon has been observed by everybody by dipping a straw in can of soft drink.

Objections to this theory-

1. The smallest diameter observed in tracheids is about 0.02 mm. With this diameter, water can not travel beyond a height of 150 cm. whereas in larger vessels which have a diameter of 0.5 mm, it will rise only upto 6 cm. Only the capillaries of 1.0 μm diameter can translocate the water upto 29.95 meters.
2. The soil water not directly connected with the vessels; hence this theory can not be functional in vessel bearing plants.
3. Vessels are not found in gymnosperms whereas trees of this group are very long.

ii. Transpiration pull or Cohesion-tension theory:

This theory was propounded by Dixon and Jolly (1894) and supported by Curtis and Clark (1951), Milburn and Johnson (1966) and Levitt (1969). This theory is now popularly known by various names such as- Cohesion hypothesis, Theory of cohesive force, Dixon and Jolly theory of cohesion. It is for the most accepted theory which would be better explained as follows-

Cohesion and Adhesion:

Attraction between water molecules (similar) is called **cohesion**. The water molecules have strong mutual attraction (cohesion) due to which they can not be easily separated from one another. Cohesive force has been measured upto 350 atmosphere (which is excess than the minimum requirement).

The wall of tracheids and vessels which transport water are made up of **lignin** and **cellulose** and have high affinity for water and this is called **adhesion**. Xylem vessels have perforated end walls and form a tubular structure from roots to the shoot tip. This provides a continuous channel for movement of water which can not be pulled away from xylem wall due to cohesive and adhesive properties.

Transpiration pull: transpiration takes place through leaves causes negative pressure or tension in xylem sap which is transmitted to the roots. This is called transpiration pull which is responsible for the movement of water column upward.

Objections and Explanations: Air bubbles may enter the water column due to atmospheric pressure variations. Septa in xylem vessel may check the flow of water. But the suction force or negative tension developed by transpiration pull is sufficient to overcome resistance developed by septa. Gases are dissolved in solutions at night or during rain.

Transpiration (a phenomenon of water loss):

Plants continuously absorb and lose water. Most of the water lost by the plant evaporates from the leaf. 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. **The loss of water in the form of vapour from the aerial parts of the plant is known as transpiration. Almost 70-95% water is evaporated by plants.**

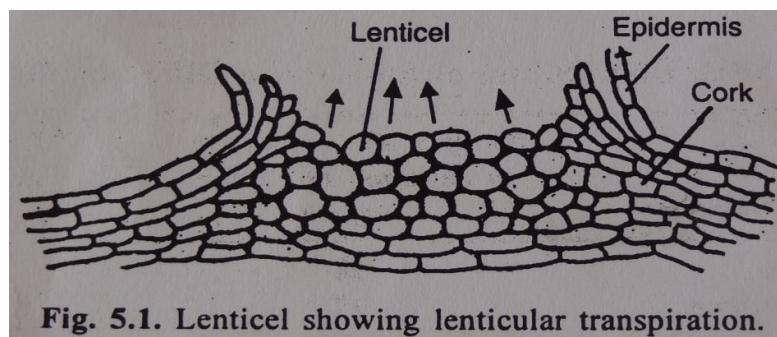
Types of transpiration:

Transpiration in plants is essentially of three types-

- i) Cuticular transpiration
- ii) Lenticular transpiration
- iii) Stomatal transpiration

i) Cuticular transpiration: Cuticular transpiration takes place through outer covering of the epidermis called **cuticle** made up of **cutin**. Only a very little (5%) part of transpiration takes place by this process.

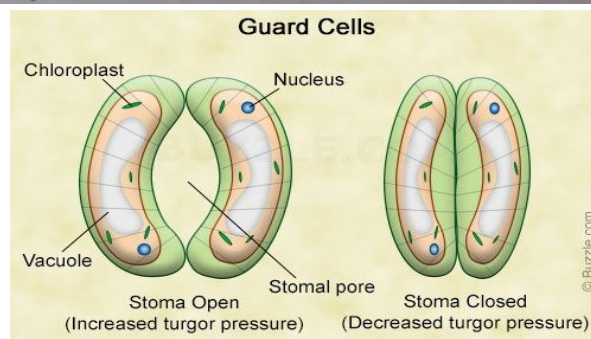
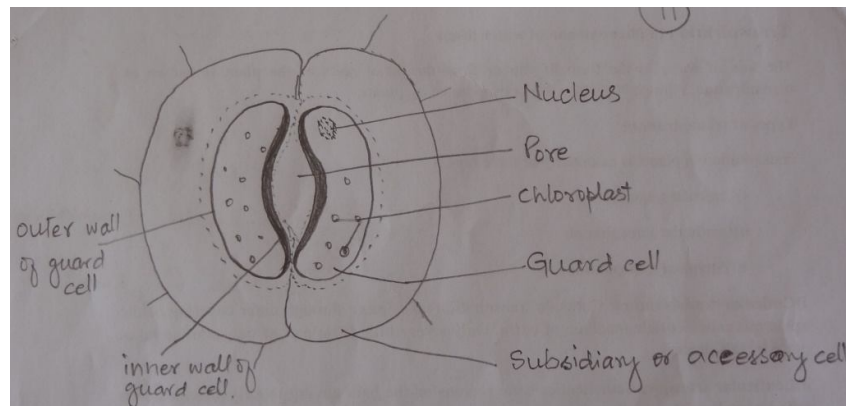
ii) Lenticular transpiration: Lenticels are regions on the bark having loosely arranged cells called **complementary cell**. A very little amount of (0.1%) of water is lost by transpiration through lenticels.



iii) Stomatal transpiration: Stomata are the minute pores in the epidermis of leaves and stems. Most of the water vapours (95%) evaporated by stomata.

Structure of stomata: A stomata is minute pore on the epidermis of aerial parts of plant through which exchange of gases and transpiration takes place. Each stoma is surrounded by a pair of **kidney-shaped guard cells**. Each guard cell is modified epidermal cell showing a prominent nucleus, cytoplasm, vacuole and plastids. The inner wall of guard cell facing the stomata is concave, thick and rigid. The outer wall is convex, permeable, thin and elastic. The

guard cell is surrounded by variable number of epidermal cells called **subsidiary cell** or **accessory cells** support in the movement of guard cells.



Mechanisms of stomatal movement:

Opening and closing of stomata takes place due to changes in turgor pressure of guard cells. Generally stomata open during day and close at night. The turgor changes in the guard cells are due to entry and exit of water into and out of the guard cells. During the day, water from subsidiary cells enters into guard cells making the guard cells fully turgid, the pressure exerted on their walls. As a result, the thin, elastic convex outer walls are bulged out causing the thick and rigid concave inner walls to curve away from each other causing the stomata to open. During night time, water from guard cells enters the subsidiary cells and as a result, the guard cells become flaccid due to decrease in turgor pressure. This causes the inner concave walls to straighten up and the stomata close. The actual mechanism responsible for entry and exit of water to and from the guard cells has been explained by several theories. The most important theories are-

- i) The starch-sugar interconversion theory
- ii) Active Potassium transport
- iii) pH theory of Scarth
- iv) Proton-Potassium pump theory

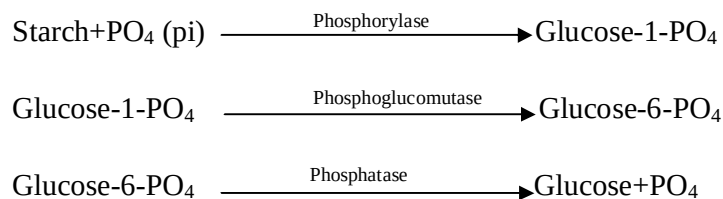
i)The Starch-Sugar conversion theory:

Scarth (1932) proposed the starch-sugar conversion theory as follows-

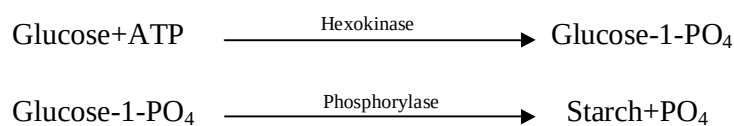
S.No.	Illuminated Guard cells	Dark Guard cells
1.	Respiratory CO ₂ accumulated in intercellular spaces used up by mesophyll cells during photosynthesis.	Respiratory CO ₂ accumulates in the intercellular spaces.
2.	pH value of guard cells rises.	pH of guard cells falls.
3.	The decreasing acidity favours the hydrolysis of starch to sugar. Starch+Pi $\xrightarrow{\text{Phosphorylase}}$ Glucose-1-PO ₄	The increase in acidity favours conversion of sugar into starch. Glucose-1-PO ₄ $\xrightarrow{\text{Phosphorylase}}$ Starch+Pi
4.	Osmotic pressure of guard cells increased due to increment of the concentration of the guard cells.	Osmotic pressure of the sap of guard cells decreased.
5.	Water enters into (endosmosis) guard cells, turgor pressure and volume of guard cells increased.	Water leaves the guard cells and their turgor pressure and volume decrease.
6.	The guard cell bulge out and stomatal aperture widen (open).	The guard cells collapse and apertures are narrowed.

Steward (1964) According to him the osmotic pressure of the guard cells will increase unless glucose-1-PO₄ is further converted to glucose and Pi (inorganic phosphate).

At higher pH opening of the stomata will be caused in following manner-



Closing of stomata takes place when glucose converted first into glucose-1-phosphate in the presence of enzyme **hexokinase** with the help of ATP.

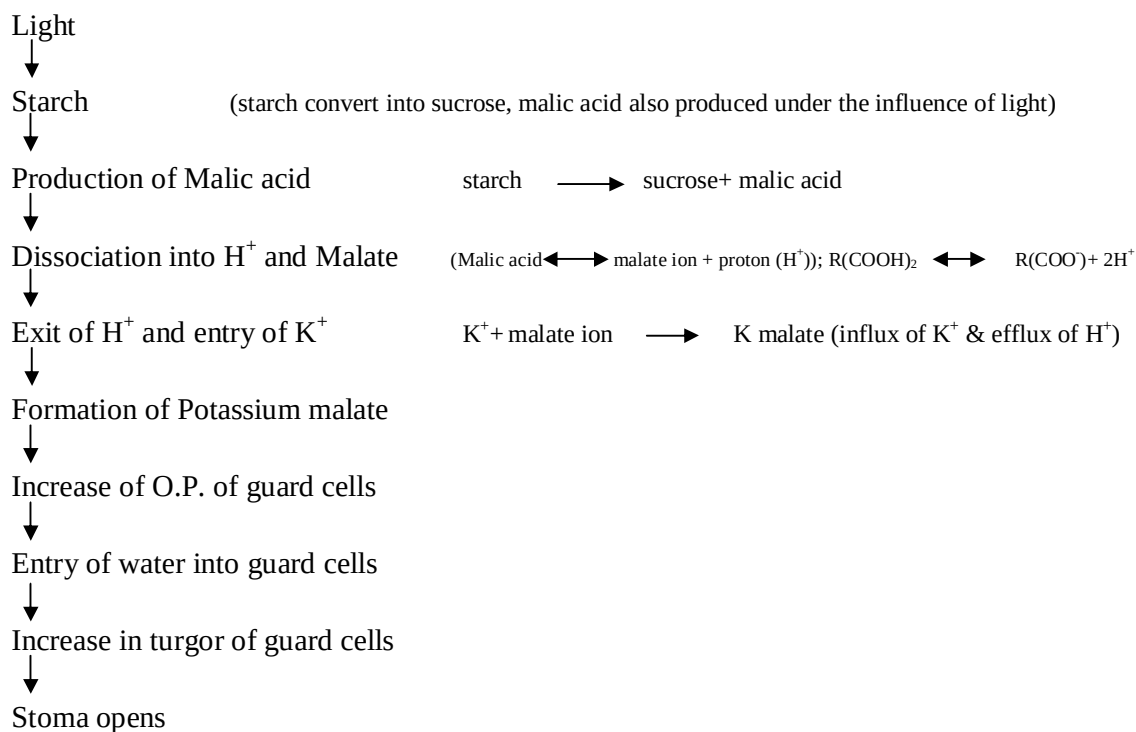


2. Proton-Potassium pump theory:

Levitt (1974) combined the points in Scarth's and Steward's hypothesis and gave a modified version of the mechanism of stomatal movement which was called the **proton-potassium pump hypothesis**. According to this hypothesis K^+ ions are transported into the guard cells in the presence of light. The sequence of events taking place are-

- i) Under the influence of light, protons formed by dissociation of malic acid move from cytoplasm into the chloroplast of guard cells.
- ii) To counter the exit of protons K^+ enters in the guard cells from the surrounding mesophyll cells.
- iii) K^+ ions react with the malate ions present in the guard cells to form potassium malate.
- iv) Potassium malate causes increase in the osmotic potential of guard cells causing entry of water into the guard cells as a result of which the stoma opens.
- v) At night the dissociation of potassium malate takes place and K^+ ions exit out of guard cells causing loss of water from guard cells and so the stoma closes.

Noggle and Fritz (1976) supported this theory and gave a scheme for opening of stomata.



This theory is widely accepted one as Levitt was able to demonstrate rise in K^+ level during the day and the formation of organic acids like Malic acid with the unused CO_2 present in the guard cells.

Factors affecting the rate of transpiration (stomatal movement):

There are a number of factors which influence stomatal movements. These include light, temperature, potassium chloride, organic acid, carbon dioxide concentration, water and abscisic acid.

Light: Light greatly influences the opening and closing of stomata as it stimulates production of malic acid due to conversion of starch to sugar. Stomata do not open in UV light and green light but remain opened in the blue and red regions of the spectrum.

Temperature: Stomata open in rise in temperature and close at lower temperature as light and temperature are directly related. But higher temperatures also cause stomatal closure.

Potassium chloride: Accumulation of potassium chloride causes opening of stomata.

Organic acid: The increase of organic acid content in the guard cells causes the stomata to open.

Carbon dioxide concentration: Stomatal movement is influenced by the concentration of CO₂. At low concentration of CO₂, the stomata open. With increase in the concentration of cells is higher than its concentration in the air, the stomata completely close. Stomatal movement is always influenced by the CO₂ concentration of the intercellular spaces of the leaf and not the concentration of the air.

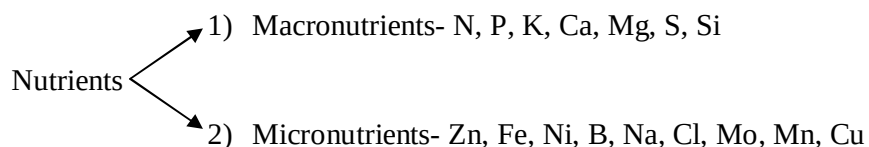
Water: Water is responsible for causing changes in the turgor of the guard cells. Guard cells become flaccid on losing water and so the stomata close. Similarly the guard cells become fully turgid on gaining water and the stomata open. Under the conditions of water scarcity also, the stomata close.

The Role of Transpiration and Water use Efficiency:

1. Transpiration is necessary evil. It is vital unavoidable phenomenon of plants.
2. Supposed role in the movement of water (ascent of sap).
3. Creates suction force and help in the ascent of sap.
4. Transpiration brings about rapid translocation of water in the plant.
5. It affects the absorption of water and mineral salts by plant root.
6. Rapid ascent of sap would not occur if there was no loss of water in transpiration.
7. It helps in the evaporating excess amount of water.
8. Role in the absorption and translocation of mineral salts.
9. Role in the regulation of temperature (leaves absorb radiant energy and convert it into heat energy. Transpiration prevents the heating of the leaf.
10. Stomatal openings are essential for gas exchange, photosynthesis, respiration.

Chapter-3 Plant Nutrition

Nutrients in the soil system: To complete the life cycle normally, a living organism requires the supply of a large number of nutrients from outside are called **nutrients**. Almost all the nutrients are present in the soil except than C, H and O. Soil is the main source of mineral salts. There are two types of nutrients-



1. Macronutrients: The macronutrients are needed in concentrations of more than 100 mg kg⁻¹ of dry matter. Nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, carbon, hydrogen and oxygen are essential macronutrients.

2. Micronutrients: The micronutrients or trace elements are needed in tissues concentrations equal to or less than 100 mg kg⁻¹ of dry matter. The Cl, Fe, B, Mn, Zn, Mo, Na, Ni, and Cu come in this group.

Essential nutrients: Those elements which are necessary to complete the vegetative and reproductive life cycle of a plant are called essential nutrients. It means it is impossible to complete the life cycle of a plant without these nutrients e.g. N, P, K, Ca, Mg, S, C, H and O.

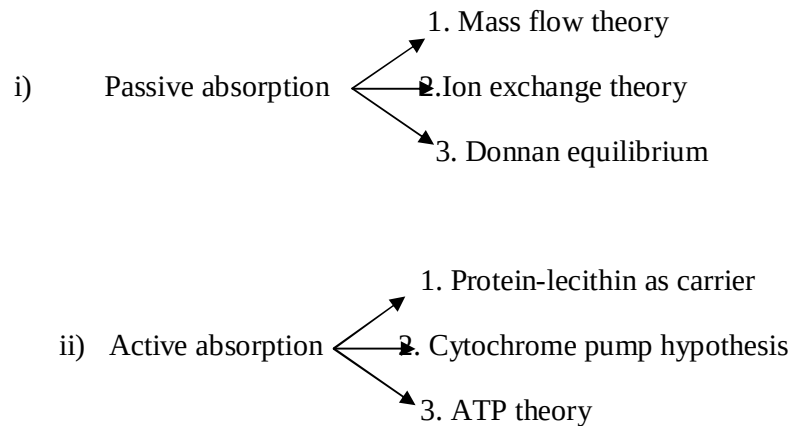
Beneficial Nutrients: Beneficial nutrients are nutrients that are not for the plant to complete its life cycle, but may provide other benefits such as disease resistant etc eg. Si, Co and Na.

Essentiality criteria: The term essential mineral element (or mineral nutrient) was proposed by Arnon and Stout (1939) who concluded that, for an element to be considered essential, it must fulfil following criteria.

1. A given plant must be unable to complete its life in the absence of that mineral element.
2. The function of the element must not be replaced by another mineral element.
3. The element must be directly involved in plant metabolism. Those elements which are required for the normal growth of the plants.

Plants show physiological abnormalities, chlorosis, premature, falling of leaves, stunting etc. in the absence of essential nutrients.

Uptake and translocation of nutrients: Nutrients or inorganic substances are absorbed by root hairs and translocated by tracheary elements (xylem cells). Various theories proposed to explain the mechanism of mineral salt absorption can be placed under two broad categories-



i) Passive absorption: In passive absorption, the ions move into a cell along their electrochemical potential gradient without any expenditure of energy by cells. At equilibrium, no further net movement of solute can occur without any application of a driving force. When a plant cell or tissue is transferred from a medium of low salt concentration to a medium of relatively high salt concentration, there is an initial rapid uptake of ions, followed by a slow steady uptake that is under metabolic control. The temperature or metabolic inhibitors did not affect the initial rapid uptake. It indicates that metabolic energy is not involved in the process. The direction of initial uptake gets reversed if the tissue is transferred back to a low concentration. It means a part of the cell or tissue immersed into salt solution is opened to free diffusion of ions.

1. Mass Flow theory: According to this theory ions are taken up by roots along with mass flow of water under the influence of transpiration.

2. Ion Exchange theory: According to this theory ions from the external solution in which tissue is immersed may exchange with the ions absorbed on the surface of the cell wall of the tissue. **Cations** may exchange with hydrogen ions and **anions** with hydroxyl ions adsorbed on the surface of tissue.

3. Donnan Equilibrium: This theory assumes that certain fixed ions are present in cell sap. In order to maintain an internal balance, such ions would require ions of other charges.

ii) Active absorption: In active absorption, the movement may occur against the electrochemical potential gradient. It is not spontaneous and it requires that work be done on

the system by the application of cellular energy. The transfer of one ion or particle across the membrane has been found to require 1340 cal/mol.

1. ATP theories: Ion uptake into the cell is energised by ATP. The energy from hydrolysis of ATP molecules can be made available to energise ion pump through the action of enzymes.

3.3 Factors affecting the uptake of nutrients (or factors affecting the salt absorption): There are several factors which affect the uptake of nutrients. Some of those are as follows-

1. Temperature: When temperature increase passive and active salt absorption also increased.

2. Light: When stomata open allow more transpiration and increased mass flow and photosynthesis provides energy and oxygen for salt uptake. Light affects the rate of salt absorption by affecting the opening and closing of stomata and the process of photosynthesis.

3. pH: Low pH damage the plant tissue and affect the rate of absorption. Some nutrients dissolved at low pH.

4. Oxygen tension: Deficiency of oxygen decreases salt absorption as the active phase of salt absorption is inhibited by the absence of oxygen.

5. Interaction: Absorption of one ion may be influenced by the presence of another ion. If enough binding sites on the carriers are present, the interaction will not be apparent.

6. Growth: Increase in surface area, number of cells, synthesis of new binding sites or carriers and volumes of water uptake stimulate salt absorption. Vegetative growth and increased metabolic activity with more water uptake enhances salt absorption.

3.4. Role of Nutrients and their Deficiency Symptoms:

Essential Elements	Functions	Deficiency Symptoms
1.Nitrogen NO_3^- , NH_4^+	Proteins, amino acids, purine, pyrimidine, DNA, RNA, chlorophyll, cytochromes, vitamins, hormones, co-enzymes, and ATP	Suppressed shoot growth, older leaves completely pale yellow, <u>flowering and fruiting reduced</u> , <u>leaf purple or red due to anthocyanin</u> , stunt growth, dormancy of lateral bud, symptoms appear first in older leaves then in younger leaves.
2.Phosphorus H_2PO_4^- , H_3PO_4^- , HPO_4^{--}	Nucleic acid, nucleoproteins, phospholipid of membranes, sugar phosphates, healthy root growth, fruit ripenning, ATP, ADP, AMP	Stunted plant, dark blue-green colour leaves, premature falling of leaves, <u>brown necrotic</u> (dead cells) patches on leaves
3.Potassium K^+	Maintenance of cell organization, stomatal movement, chlorophyll and nucleic acid synthesis.	Decolouration of leaves, chlorosis of tips and margins (scorch), <u>shoots may die-back</u> , shorter internodes, plastid disintegration.
4.Calcium Ca^{++}	Cell wall structure, membrane structure, ion transport, cell elongation, turgor of cells	Death of root, stem, leaf tips, <u>chlorosis in younger leaves along the margins</u> , margins curled backward or forward or rolled.
5.Magnesium Mg^{++}	Component of chlorophyll, combines the subunits of ribosomes, synthesis of DNA and RNA	Symptoms develop on older leaves systematically towards younger leaves, <u>mottled chlorosis</u> , <u>necrosis</u> , premature leaf abscission.
6.Sulphur SO_4^{--}	Constituents of amino acids (cysteine, cystene, methionine), vitamin B (thiamine, biotin), chlorophyll synthesis, lipid synthesis and CoA.	Necrosis of young leaf tip and margins, <u>shoot die-back</u> , leaf fall is rapid.
7.Zinc Zn^{++}	Chlorophyll synthesis, RNA synthesis	Seed production and fruit size reduced, leaves show <u>interveinal chlorosis and necrosis</u> , leaf margins distorted.
8.Iron Fe^{+++} Fe^{++}	Chlorophyll synthesis, constituents of cytochrome, leghaemoglobin, ferredoxin, hematin	<u>Chlorosis with green veins</u> , young leaves becomes yellow and white.
9.Manganese Mn^{++}	Chlorophyll formation, maintenance of chloroplast membrane structure	<u>Chlorosis with mottled appearance of leaf</u> , necrotic on younger leaf, <u>chloroplast disintegrate</u> , solubility increases with increasing acid it means crop failure.
10.Copper Cu^{++}	Oxidase enzyme: tyrosinase, ascorbic acid oxidase	<u>Die-back in citrus</u> , necrosis of leaf tips and margins.

Mechanism of Phloem conduction

Phloem Anatomy:

Phloem is a complex tissue and consists of the following elements-

1. Sieve elements
2. Companion cells
3. Phloem fibres
4. Phloem parenchyma

1. Sieve elements: It is conducting element of the phloem. It conducts **protein** and other **carbohydrate**. Sieve tube elements do not have nucleus, golgibody, ribosomes, tonoplast, microtubules, and microfilaments. Sieve elements are the chief food conducting tissues. Sieve elements have made up of two types of cells-

- i) Sieve cells- cells are long slender, tapering.
- ii) Sieve tube- bearing sieve plates.

2. Companion cells: Companion cells closely associated in origin, position, and function with sieve tube elements. Companion cells have prominent nucleus, chloroplast, mitochondria, endoplasmic reticulum and several plasmodesmata connections with the adjoining sieve tube elements. It translocate many **soluble food materials** e.g. **sugar**. Companion cells regulate the activity of the sieve tube element; provide ATP with the help of mitochondria. Companion cells transfer prepared food from the mesophyll to the sieve tube elements.

Companion cells have three types of cells-

- i) ordinary cells
- ii) transfer cells
- iii) intermediary cells

In ordinary and transfer cells, food materials (sugars) translocate through apoplastic pathway. Intermediary cells diffuse sugars from mesophyll cells to sieve elements through symplastic pathway.

i) Ordinary companion cells

- i. have chloroplasts
- ii. few plasmodesmata between companion cell and surrounding cells, except for own sieve elements
- iii. symplast of sieve element and its companion cell is relatively isolated from surrounding cells

ii) Transfer cells

- i. similar to ordinary companion cells
- ii. develop fingerlike wall ingrowths, particularly on walls that face away from sieve element
- iii. away from sieve element
- iv. wall ingrowths increase surface area of plasma membrane (increases potential for solute transfer across membrane)

iii) Intermediary cells

- i. have numerous plasmodesmata connecting them to bundle sheath cells
- ii. have many small vacuoles
- iii. poorly developed thylakoids and lack of starch grains

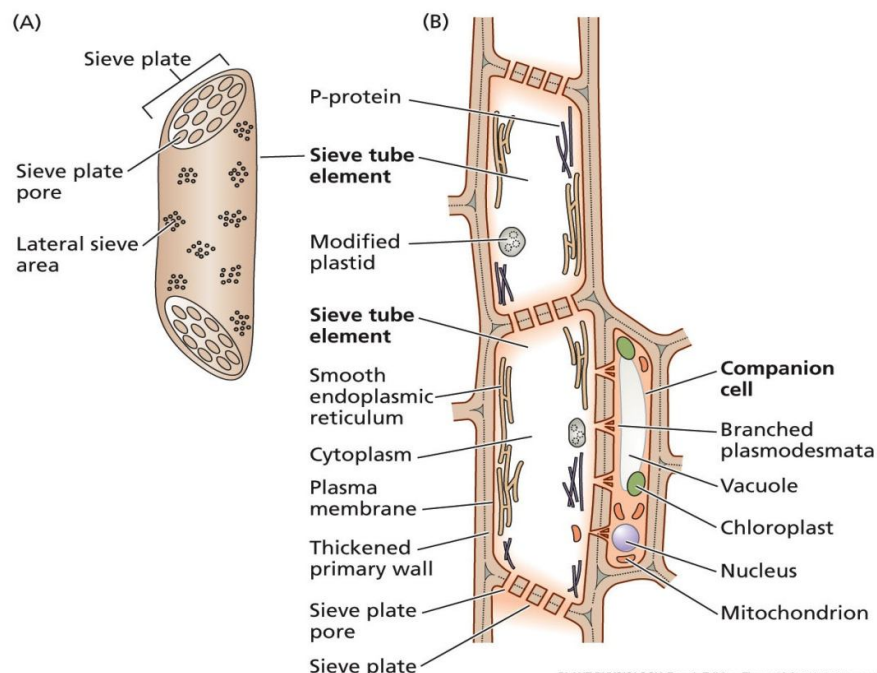


Fig.3.1. Structure of phloem.

3. Phloem fibres: simple pits are found on the walls of phloem fibres, strong rope, cords, cloths, mats, linen cloth, and thread.

4. Phloem parenchyma: It is storage of starch, fat and other organic substances. Tannins and resins are also found in these cells. Phloem parenchyma conducts **amines, amino acid, and soluble carbohydrate**. All phloem elements are living and made up of cellulose.

Pattern of Translocation: Source to Sink:

Sources: sources are **green leaves (photosynthetic cells)** where food is prepared in the form of sugar.

Sinks: Sinks are growing point of root and shoot, storage organs (fruits and seeds), stems, rhizomes, tubers etc.

The regions which supply sugar to phloem is referred to as source and that where it is utilized as sink. Translocation of food material is always from a point of higher concentration (supply end) to a point of lower concentrations (consumption end).

Phloem loading:

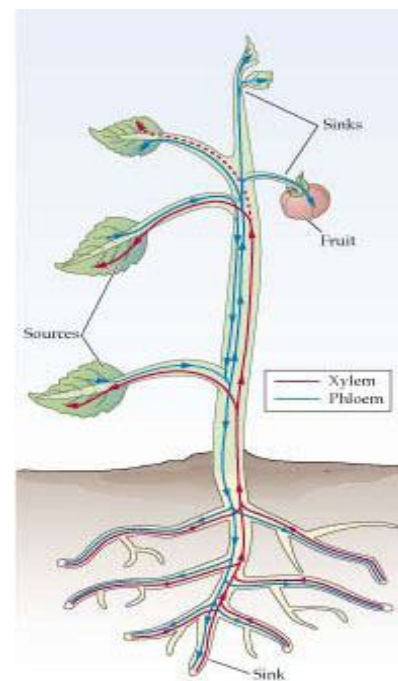
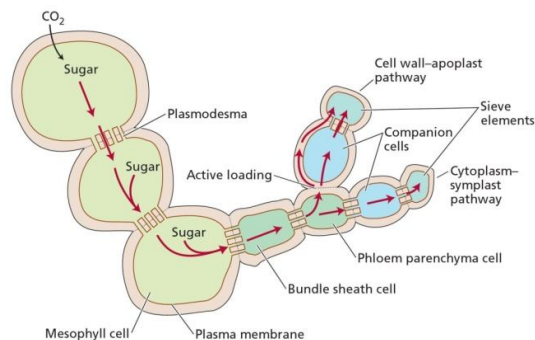
According to **Oparka** and **Van Bel (1992)** there are 4 steps in the phloem loading.

- i) Diffusion of triose phosphates from the matrix of the chloroplasts into the cytosol where it is finally converted into sucrose.
- ii) Sugars (sucrose) travel a few cells from the mesophyll cells to near the sieve elements (short distance transport)
- iii) Sugars enter the sieve elements and companion cell complex.
- iv) Sugars are then transported to the sink (long distance transport) through the vascular system (export).

Phloem Unloading: The transport of sugar from the sieve elements into the cells of roots, tubers, underground rhizomes, corms etc where they are either stored or metabolised is generally symplastic and to some extent apoplastic.

The phloem unloading (import) into storage tissues of underground rhizomes, corms, tubers, roots etc. occurs in the following steps:

- i) Transport of sugar out of sieve elements.
- ii) The sugars diffuse into the storage tissues (sink).
- iii) Sugar hydrolysed and converted into starch for the purpose of storage.



A series of biochemical changes taking place inside or outside the plant body which results in the construction of complex nitrogenous food from its simpler derivatives and the destruction of complex nitrogenous food into its complex. It means nitrogenous metabolism include both anabolic (constructive) and catabolic (destructive) processes.

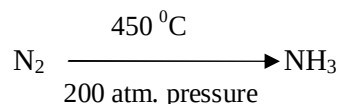
Nitrogen Fixation:

“The phenomenon of conversion of free nitrogen into nitrogenous salts to make it available for absorption by plants”. $N \equiv N$ is inert (inactive). N_2 is present 78% in atmosphere. Nitrogen can be absorbed by plants in the form of NO_3^- , NH_4^+ and organic nitrogenous compounds.

Nitrogen fixation classified into two types:

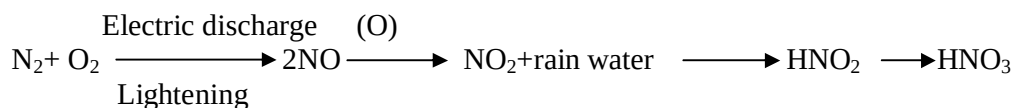
1. Industrial N-fixation
2. Natural N-fixation

1. Industrial N-fixation: Nitrogen is fixed at high temperature and pressure.



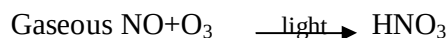
2. Natural N-fixation: Naturally, nitrogen can be fixed by three ways-

i) Lightening and Electric discharge: almost **8%** nitrogen is fixed by lightening and electric discharge.



$HNO_3 + Ca$ or K salts in soil as $CaNO_3$ or KNO_3 .

ii) Photochemical Reaction: The gaseous nitric oxide (NO) and ozone (O_3) react together in the presence of light to produce nitric acid (HNO_3). This accounts for **2%** of the total nitrogen fixation.



iv) Biological Nitrogen fixation: Biological nitrogen fixation is brought about by either non-symbiotic or by symbiotic association with several bacteria or cyanobacteria which are provided with **nif** gene and **nitrogenase** enzyme. **90%** nitrogen is fixed by this method.

a) Non-Symbiotic nitrogen fixation:

Free-living microorganisms fix atmospheric nitrogen e.g. *Clostridium*, *Azotobacter*, *Rhodopseudomonas*, *Rhodospirillum*, *Rhodomicrobium*, *Thiobacillus*, and *Bacillus polymyxa*.

b) Symbiotic nitrogen fixation:

Association of *Rhizobium* in the root of leguminous plants (Fabaceae) produce nodules and participate in the symbiotic nitrogen fixation. *Rhizobium* is gram negative bacteria, non spore forming, and aerobic rods e.g.

Rhizobium leguminosarum, *R. japonicum*, *R. phaseoli*, *Bradyrhizobium*, *Frankia* spp.

Symbiotic cyanobacteria- *Nostoc*, *Anabaena*- in *Cycas* and *Anthoceros*.

Non-legume Symbiosis: *Rhizobium meliloti*- in *Medicago sativa* (Alfalfa), *Casuarina equisetifolia*, *Alnus*.

Biochemistry of nitrogen fixation:

The nitrogen fixing bacteria have a set of nitrogen fixing genes (**Nif** genes), which produce a special enzyme complex called **nitrogenase**. The enzyme is versatile in action. Besides nitrogen it can reduce hydrogen (H_2), nitrous oxide (N_2O), carbon monoxide (CO), acetylene (C_2H_2), and methyl isocyanide (CH_3NC) etc. they all, therefore act as competitive inhibitors during nitrogen fixation.

This enzyme system is a complex of two enzymes, one is a molybdenum-iron protein (Mo-Fe protein) and the other is an iron protein (Fe-protein). Cobalt is also needed in small amounts in nitrogen fixation.

This enzyme nitrogenase has two components known as components I and II. They are proteins and are irreversibly inactivated by oxygen. Component I has 28 iron and 2 molybdenum atoms (Fe-Mo). Component II has only 4 atoms of iron associated with protein (Fe- protein). This component becomes a very strong reductant after binding with ATP. The Fe-protein receives electrons from ferredoxin and gets reduced. The electrons are then transferred to component I during which the ATP is hydrolysed to ADP. The electrons are finally transferred to N_2 to produce NH_4^+ .

The nitrogen is reduced to ammonia with the help of six electrons and six hydrogen ions. The electrons are first taken by the Fe- protein part of the enzyme. The reduced Fe- protein which is joined to two Mg ATP molecules transfer electrons singly to the Fe-Mo protein part of the enzyme. In the process Mg ADP and Pi are released. The electrons are then passed on to N_2 and H^+ .

The ATP required for the reduction of N_2 to NH_4^+ is believed to come from oxidative metabolism, perhaps oxidation of pyruvic acid. In photosynthetic nitrogen fixers NADPH as well as ATP may be provided by the light phase.

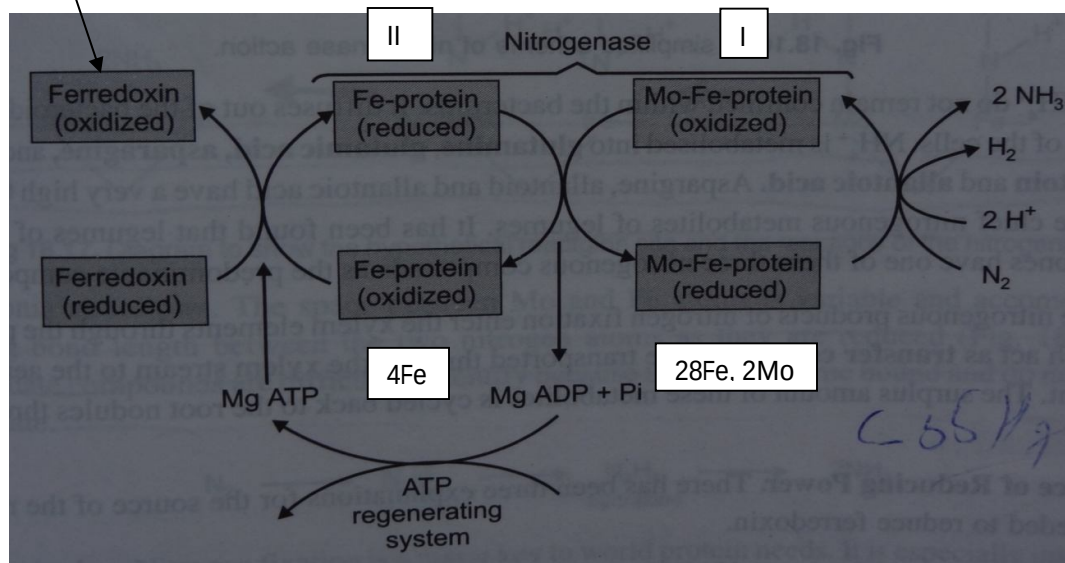
The NH_4^+ do not remain confined within the bacteroids. It diffuses out of the bacteroids into the cytoplasm of the cells. NH_4^+ is metabolised into glutamine, glutamic acid, asparagine and allantoic acid. Asparagines, allantoids and allantoic acid have a very high C/N ratio and are the chief nitrogenous metabolites of legumes. These nitrogenous products of nitrogen fixation enter the xylem elements through the pericycle cells which act as transfer cells. The surplus amount of these metabolites is cycled back to the root nodules through the phloem.

Sources of reducing power: There have been three explanations for the source of the reducing power needed to reduce ferredoxin.

- i) Respiratory process e.g. NADH
- ii) Photosynthetic process e.g. NADPH
- iii) Phosphoroclastic split of pyruvate as follows:



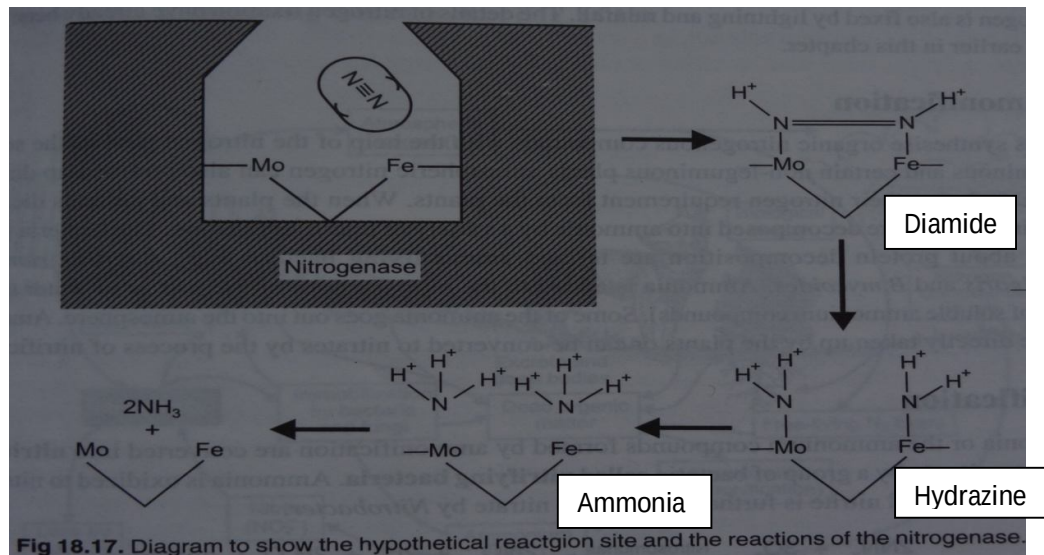
Light
Light reaction in photosynthesis



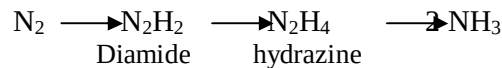
The reactions

The reaction on the Mo-Fe active sites of the enzyme nitrogenase has been explained by Burns and Hardy (1975). According to them the reactive site is in a pocket of the enzyme surface so

that only small molecules can be absorbed in it. Two nitrogen atoms N_2 ($N \equiv N$) are first of all accepted in the active site. The protons and the electrons are added in three steps to reduce nitrogen to ammonia as follows.



The space between Mo and Fe atoms is variable and accommodates the changing bond length between nitrogen atoms as they are reduced. The intermediate compounds are difficult to identify because they are enzyme bound and do not occur in a free state.



Nodule Development:

The whole process of nitrogen fixation can be studied as below-

1. In nitrogen deficiency soil, roots of legumes and *Rhizobium* exchange signals for the process of symbiosis. Several genes of both *Rhizobium* and plants are involved in initiation of symbiosis e.g.

Bacterial genes-

Non-specific gene- nod A, nod B, nod C

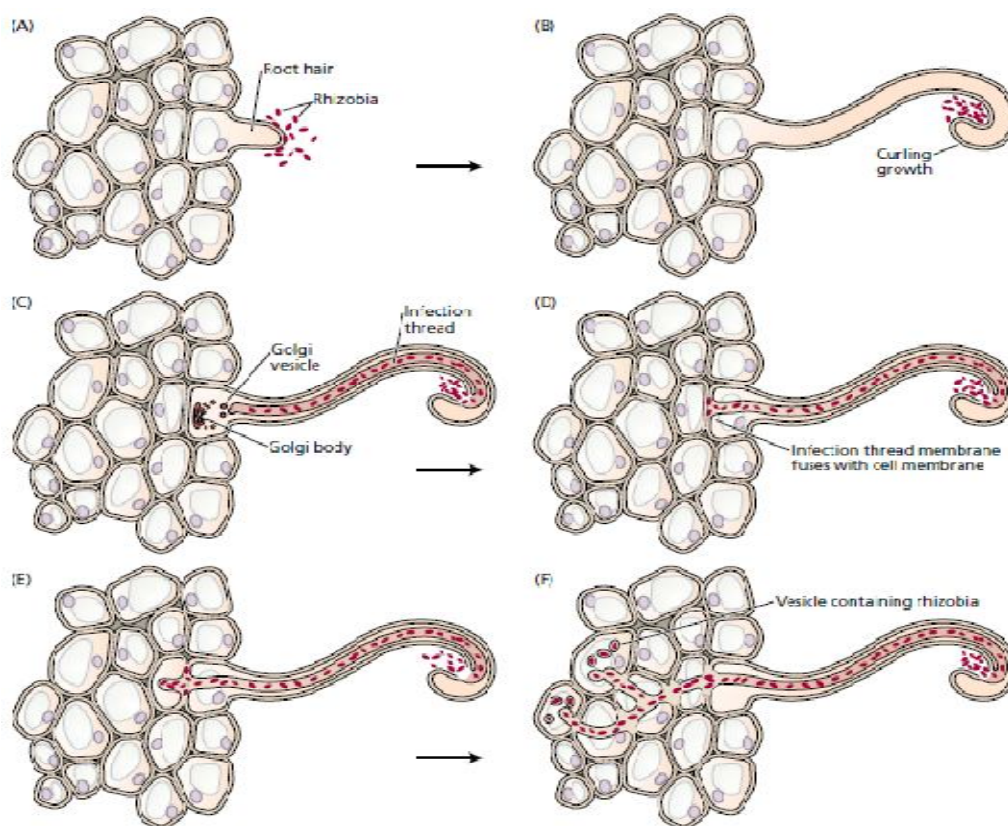
Host- specific gene- such as nod P, nod Q, and nod H; or nod F, nod E, and nod L—differ among rhizobial species and determine the host range.

Plant genes- nodulin (nod) genes.

2. The roots secrete **flavoids** and **betains** which attract the bacteria. Chemotactic movement of bacteria towards root and make contact with root. The legume receptors for Nod factors appear to be special lectins (sugar-binding proteins) produced in the

root hairs. Nod factors activate these lectins. Lectin proteins help and act as binding site. These attractants activate the rhizobial Nod D protein, which then induces transcription of the other nod genes. This lectin activation directs particular rhizobia to appropriate hosts and facilitates attachment of the rhizobia to the cell walls of a root hair. Two processes—infection and nodule organogenesis— occur simultaneously during root nodule formation.

3. Rhizobium attach to the root hair
4. Enzyme secreted by bacterium degrades the cell wall of root hair.
5. Root hair produces a thread like structure infection thread.
6. Root hair curling factor (produced by *Rhizobium*) help in root hair curling to trap the *Rhizobia*. *Rhizobia* enter into the cytoplasm of inner cortex and multiply rapidly.



7. Bacteria multiply and begin to enlarge and differentiate into n-fixing endosymbiotic organelle called bacteroids.
8. A membrane surrounds the bacteroids are called peribacteroids membrane.
9. Bacteroids stimulate polyploidy cells.
10. Bacteroids also induce formation of cortex nodule.

Leghaemoglobin: The nodules which fix nitrogen are usually large and pink in colour. This pink colour of nodule is due to the presence of a pigment protein called leghaemoglobin. This pigment is found in the cytoplasm of the nodule and similar to the haemoglobin of the blood. It is haem protein synthesized by the cooperative action of both partners; haem is synthesized by bacteroids and globin by the host plant.

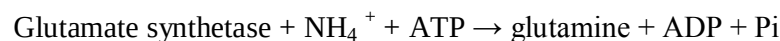
Due to its high affinity for oxygen its function is to transport oxygen to bacteroids in root nodule and at the same time to supply the correct oxygen concentration to the bacteroids surface for synthesis of ATP and nitrogenase activity.

Ammonium assimilation

Plant cells avoid ammonium toxicity by rapidly converting the ammonium generated from nitrate assimilation or photorespiration into amino acids. The primary pathway for this conversion involves the sequential actions of **glutamine synthetase** and **glutamate synthetase**.

Conversion of Ammonium to Amino Acids Requires Two Enzymes:

Glutamine synthetase (GS) combines ammonium with glutamate to form glutamine:

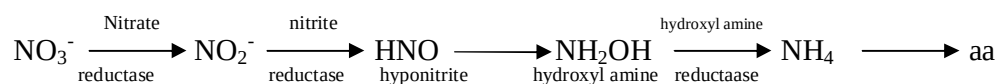


Stored Ammonium or Nitrate Can Be Toxic

Plants can store high levels of nitrate, or they can translocate it from tissue to tissue without deleterious effect. However, if livestock and humans consume plant material that is high in nitrate, they may suffer **methemoglobinemia**, a disease in which the liver reduces nitrate to nitrite, which combines with haemoglobin and renders the haemoglobin unable to bind oxygen. Humans and other animals may also convert nitrate into nitrosamines, which are potent carcinogens. Plants assimilate ammonium near the site of absorption or generation and rapidly store any excess in their vacuoles, thus avoiding toxic effects on membranes and the cytosol.

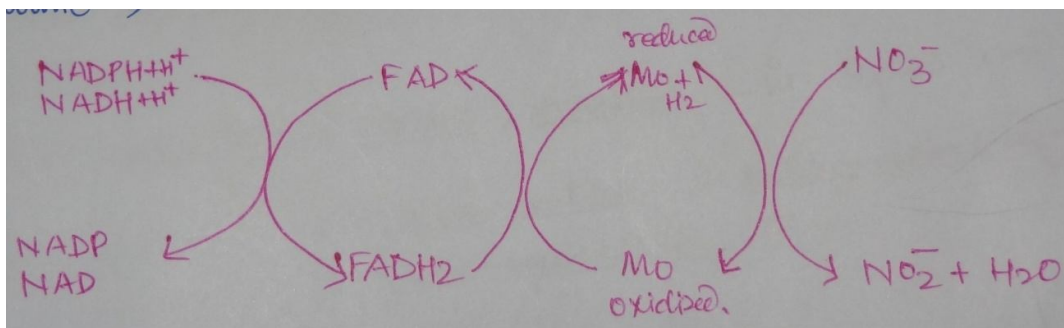
Assimilation of Nitrate:

The atmospheric nitrogen is fixed in the soil in the form of nitrate which are absorbed by the plants and converted into ammonia through a series of steps catalysed by various enzymes.



Nitrate Reduction or Reduction of Nitrate in plant Tissues: Nitrate reduction takes place in roots and green leaves in the presence of nitrate reductase enzyme. The reductase enzymes contain FAD (Flavin Adenine Dinucleotide) and Mo (Molybdenum) serves as electron carrier. Nitrate reduction is closely linked with photosynthesis which obtains energy from light. Blue light which is absorbed by flavin pigment and form NADPH which reduce $\text{NO}_3^- \rightarrow \text{NO}_2^-$. FAD receives hydrogen from reduced NADP or NAD transfer to molybdenum (Mo).

NO_2^- is a highly reactive, potentially toxic ion. Plant cells immediately transport the nitrite generated by nitrate reduction from the cytosol into chloroplasts in leaves and plastids in roots.



Nitrite Reduction: Nitrite is reduced to amino nitrogen by the enzyme nitrite reductase.

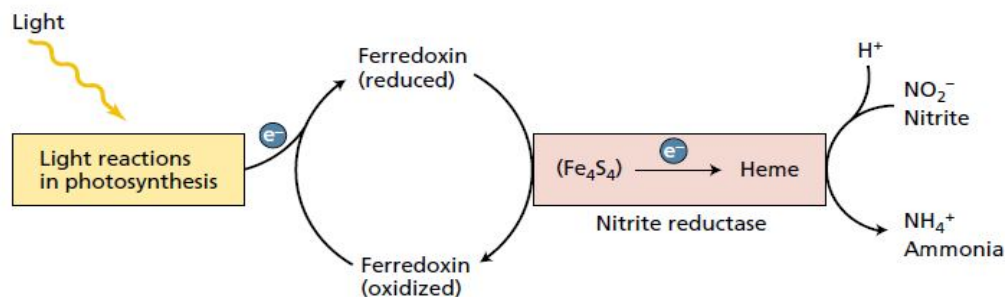
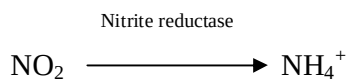


FIGURE 12.5 Model for coupling of photosynthetic electron flow, via ferredoxin, to the reduction of nitrite by nitrite reductase. The enzyme contains two prosthetic groups, Fe₄S₄ and heme, which participate in the reduction of nitrite to ammonium.

Chapter-5

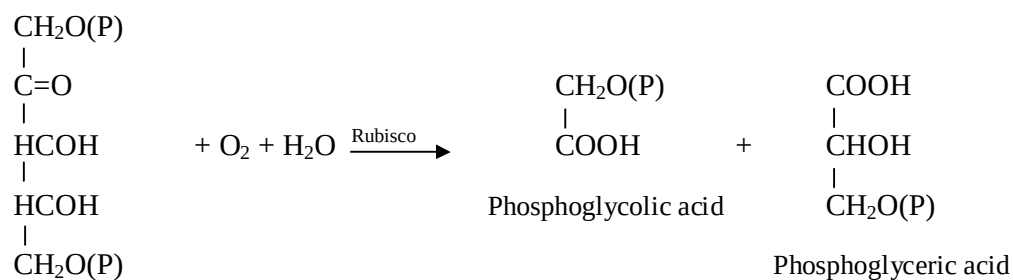
GLYCOLATE CYCLE: Photorespiratory carbon oxidation (PCO) cycle or C₂ Cycle.

Zelitch (1964) working on tobacco leaves concluded that glycolic acid formation is primary to phosphoglyceric acid formation when carbon dioxide concentration is low. The glycolate metabolism has been thoroughly investigated by Tolbert and others. It involves three organelles of the plant cell: chloroplast, peroxysomes and mitochondria which are often closely associated with one another. The enzyme RuBP carboxylase oxygenase can not only react with CO₂ but also with O₂. Lorimer and Andrews (1981) have shown that RuBP oxygenase activity of this enzyme, in the presence of high concentration of oxygen converts RuBP into one molecule of phosphoglyceric acid and one molecule of phosphoglycolic acid. It should be noted that O₂ is a competitive inhibitor of CO₂ on the active sites of the enzyme Rubisco. Warburg (1920) first detected the inhibitory role of oxygen on photosynthesis. It is, therefore known as **Warburg effect**. Glycolate cycle is called **C₂ cycle** because glycolate is a C₂ compound.

Phosphoglycolic acid is dephosphorylated into glycolate by a specific phosphoglycolate phosphatase enzyme present within the chloroplast. Luckily three quarters of the glycolate is conserved and put back into the chloroplast in the form of phosphoglyceric acid for synthesising carbohydrate once again.

The glycolate diffuses out of the chloroplast and enters into the peroxysome where it is oxidized by atmospheric O₂ to glyoxylate and H₂O₂ by the enzyme glycolic acid oxidase. This oxidation of glycolate in light is termed **photorespiration** was named by **Decker**. Since O₂ is used and CO₂ is released it apparently looks like aerobic respiration. The H₂O₂ is completely destroyed by the enzyme catalase.

Glyoxylate is transmitted to glycine by a glyoxylate-glutamate amino-transferase. Two molecules of glycine (one formed from serine) now enter into mitochondria where they are decarboxylated, dehydrogenated and deaminated. Lorimer (1981) has found that only 25% of the carbon is lost and 75% are conserved back in the form of 3-phosphoglycerate to produce serine in the presence of the enzyme glycine decarboxylase and hydroxymethyl transferase. Serine diffuses into the peroxysome where it is converted into glycerate which enters the chloroplast to produce carbohydrate.

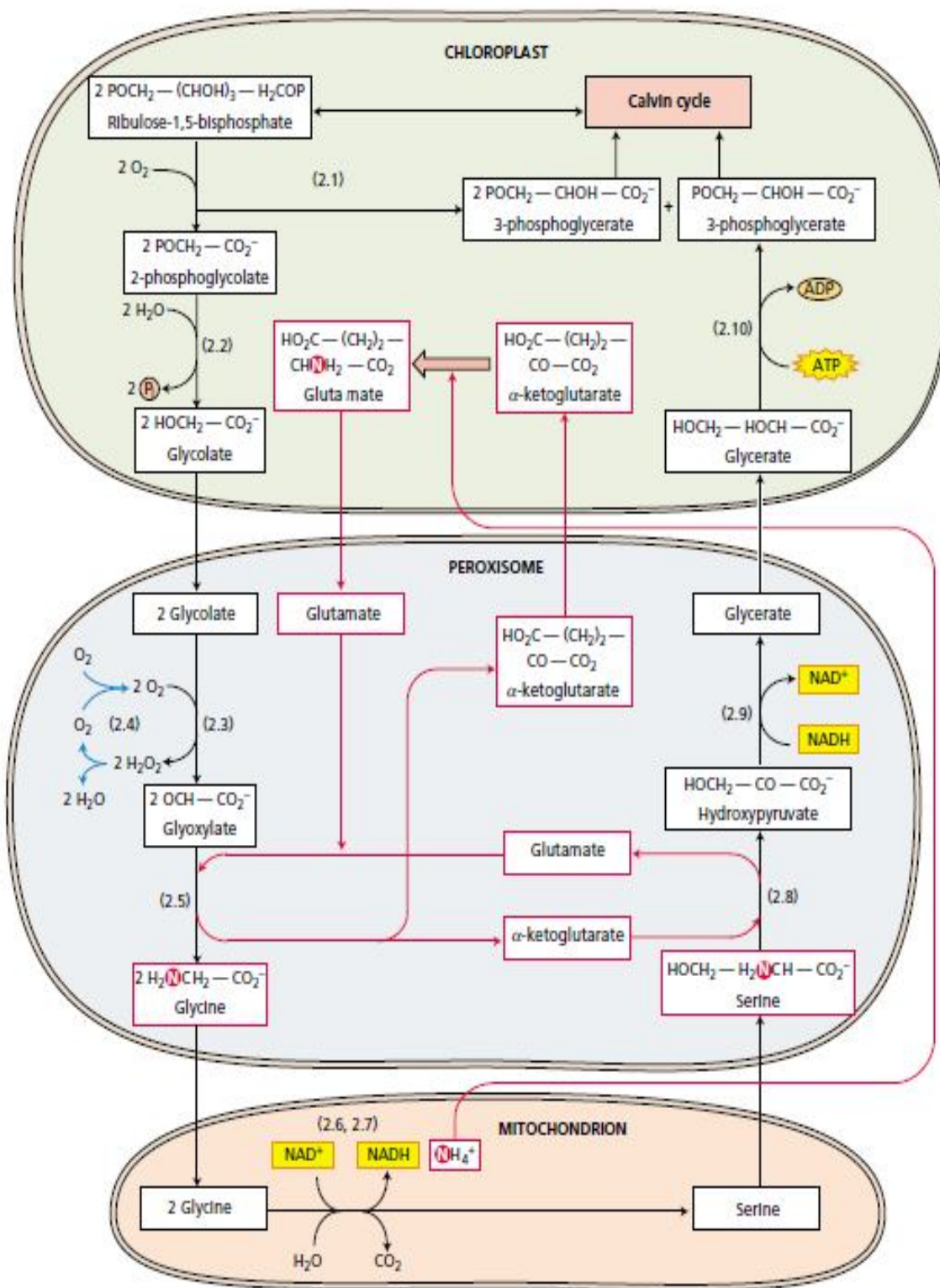


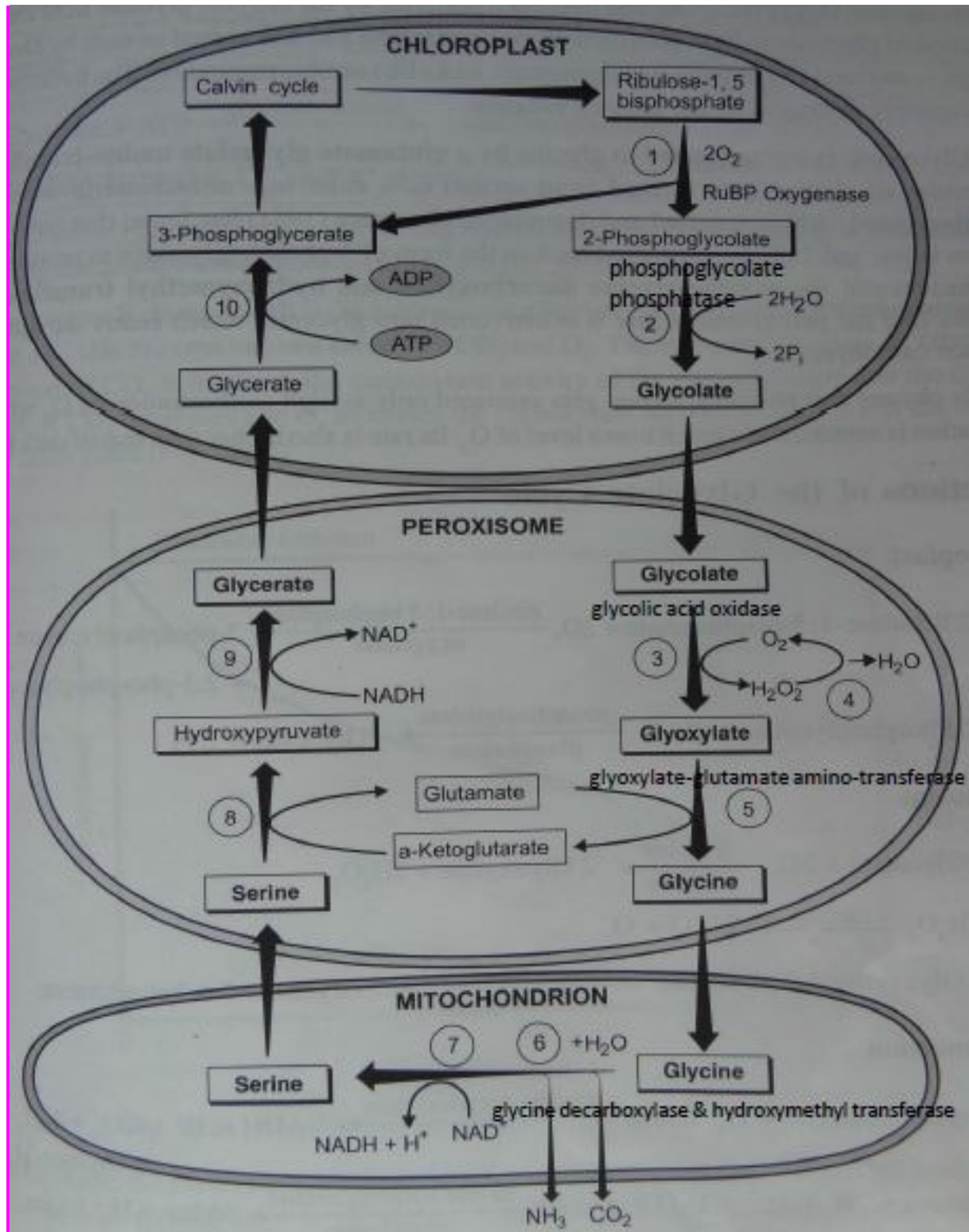
Ribulose-1,5 biphosphate

The significance of Photorespiration:

The C₂ oxidative photosynthetic carbon cycle recovers 75% of the carbon originally lost from the Calvin cycle as 2-phosphoglycolate. It has been suggested that a function of photorespiration is to protect chloroplast against photooxydative destruction of its component in a natural environment of high light and low CO₂ such as often found during day when stomata closure occurs during the period of water stress. By consuming O₂ during water deficit in the process of photorespiration plant reduces the concentration of oxygen free radicals such as superoxide, OH⁻ and H₂O₂ in chloroplast and protect the chloroplast membrane from the deleterious effect of these harmful radicals.

Another function is to provide the glycine and serine pool for the plant through the glycolate pathway. One more proposed role of photorespiration is transfer of reductant from chloroplast to mitochondria via the glycine oxidation reaction.



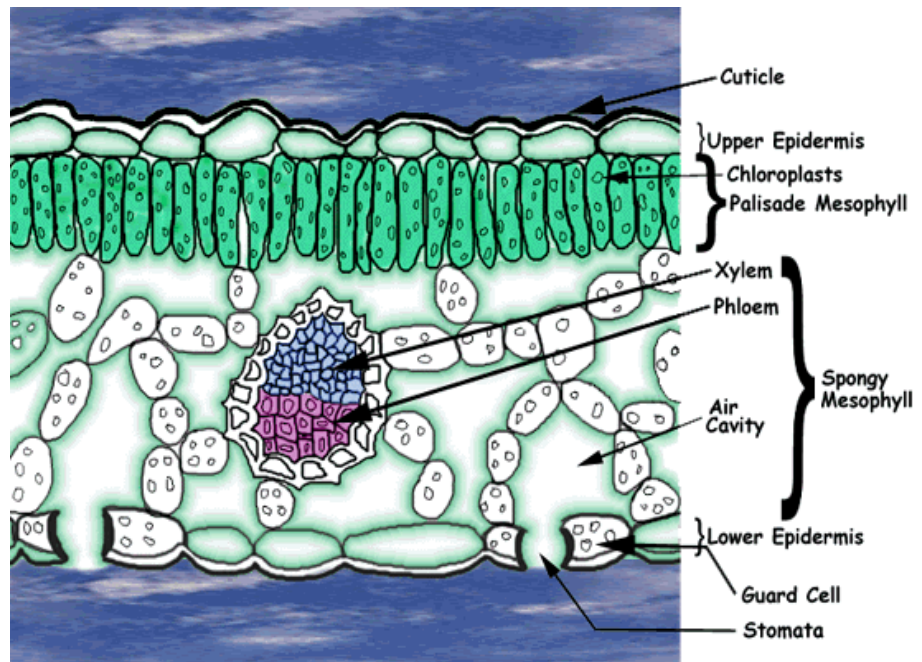
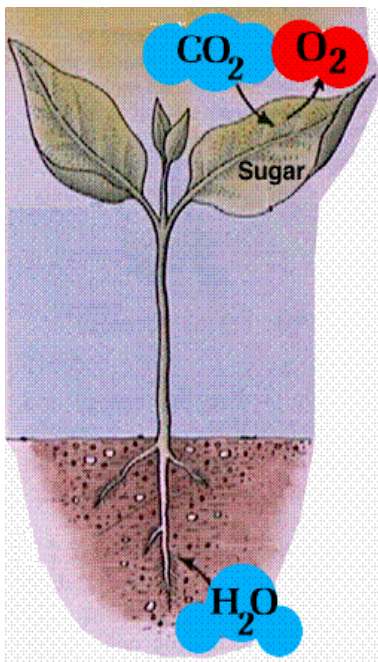
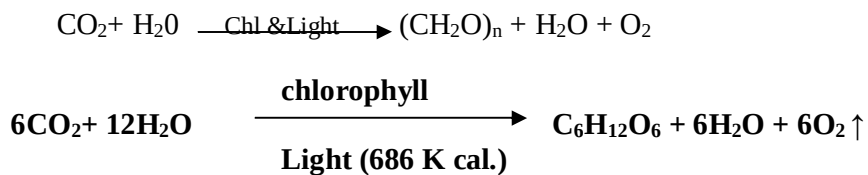


Chapter-6 Photosynthesis

Formation of carbon containing compounds from carbon dioxide and water by illuminated green cells, water and oxygen being the by-products.

Photosynthesis is an oxidation-reduction process in which water is oxidised and carbon dioxide is reduced to carbohydrate level, the water and oxygen being by-products. This reduction of CO_2 to carbohydrate needs assimilatory powers such as ATP and $\text{NADPH}^+ + \text{H}^+$. Reduction of CO_2 occurs in dark but the production of assimilatory power is light dependent. The Photosynthesis is the process by which plants and some bacteria use the energy from sunlight to produce sugar, which cellular respiration converts into ATP, the "fuel" used by all living things. The conversion of unusable sunlight energy into usable chemical energy is associated with the actions of the green pigment chlorophyll.

General formula of photosynthesis is-



Light and light absorption by pigment molecules:

Light is essential to photosynthesis, the plants primary means of energy generation. Without the energy produced by photosynthesis, the plants will not be able to continue the thousands of chemical reactions occurring every second necessary to maintain itself. The process of photosynthesis occurs in an organelle called the chloroplast. Chlorophyll consist two reaction centres namely-

- i) PS-I (P700) which adsorbs light of both shorter and longer wavelengths and other
- ii) PS-II (P680) which absorb only shorter wavelength light and makes the initiation of photosynthesis.

Pigments:

Pigments are "molecules that absorb specific wavelengths (energies) of light and reflect all others." Pigments are coloured: the colour we see is the net effect of all the light reflecting back at us. The colour of the pigment comes from the wavelengths of light reflected (in other words, those not absorbed). Chlorophyll, the green pigment common to all photosynthetic cells, absorbs all wavelengths of visible light except green, which it reflects to be detected by our eyes. Black pigments absorb all of the wavelengths that strike them. White pigments/lighter colours reflect all or almost all of the energy striking them. Pigments have their own characteristic absorption spectra, the absorption pattern of a given pigment.

The Photosynthetic Pigments: Photosynthesis is a photobiological reaction and needs absorption of light by photosynthetic pigments. The most important pigments required in the process are the chlorophylls and in addition to chlorophylls, the carotenoid and phycobilin pigments also actively participate in photosynthesis.

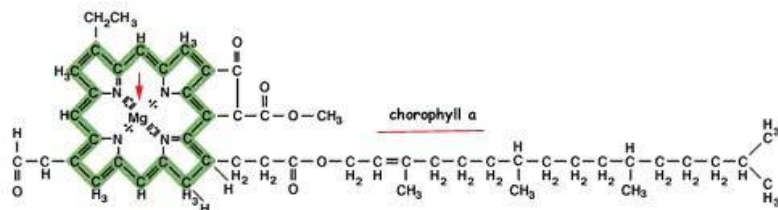
The Pigments involved in Light absorption and Photosynthesis:

Light travel in the form of stream called **quanta** or **photons** which represent a packet of energy. The chlorophyll has got capacity to absorb radiant energy coming from the sun. It can absorb waves of a certain length, all falling within the range of vision (**visible light, 400-700 nm**). Wavelengths longer than red are referred to as infrared, while those shorter than violet are ultraviolet.

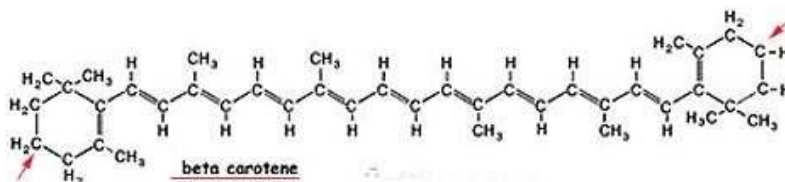
There are at least 10 types of chlorophyll known: chlorophyll a, b, c, d, e bacteriochlorophyll and bacterioviridin.

Name of the pigment	Distribution in plants	Absorption peaks in nm (wavelength)
Chl a	All green plants	435, 670-680
Chl b	All green plants	480, 650
Chl c	Diatoms and red brown algae	645
Chl d	Xanthophyceae(Rhodophyta) red algae	740
Chl e	Golden algae and higher plants	-
Chl f	Cyanobacteria	-
Accessory pigments		
Bacteriochlorophyll	Purple sulphur bacteria	800, 850 & 890
Bacterioviridin	Green sulphur bacteria	750 or 760
Carotenoids (orange) α , β , γ carotene	Many higher plants, plant bacteria, green and red algae	400-500
Xanthophylls (Yellow)	Occurs in all photosynthetic organisms	-
Phycobilins	Blue-green, brown and red algae (green light)	490-654

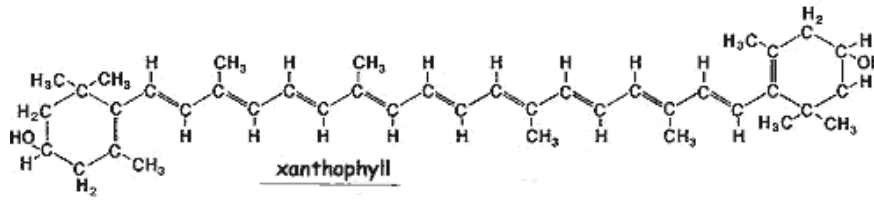
1. **Chlorophyll ‘a’:** This is the most abundant pigment in plants. Chlorophyll ‘a’ absorbs light with wavelengths of 435nm (blue) and 670-680nm (red). It reflects green light strongly so it appears green to us. The molecular formula of chl a is $C_{55}H_{72}O_5N_4Mg$.



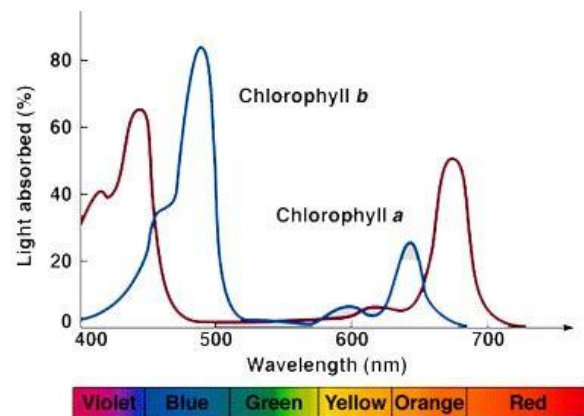
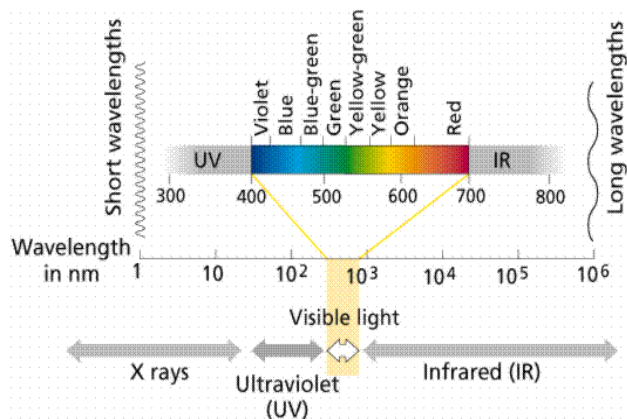
2. **Chlorophyll ‘b’:** It absorbs light of 480nm and 650 nm maximally. It is not as abundant as chlorophyll ‘a’, and probably evolved later. The molecular formula of chl b is – $C_{55}H_{70}O_6N_4Mg$.
3. **Carotenoids:** This is a class of **accessory** pigments that occur in all photosynthetic organisms. Carotenoids absorb light and transfer light energy to chlorophyll ‘a’ and protect photosynthetic apparatus.



4. **Xanthophylls:** They are yellow and do not absorb energy as well as carotenoids. They absorb light energy and transfer it to the chlorophyll a. The molecular formula of xanthophylls is $C_{40}H_{56}$.



5. **Phycobilins:** Englemann found blue green light to be very effective in increasing the rate of photosynthesis of **brown and red algae**.
6. **Anthocyanins:** The colour of leaves is modified in certain plants due to the presence of purple pigment called anthocyanins. This pigment does not take any part in photosynthesis.

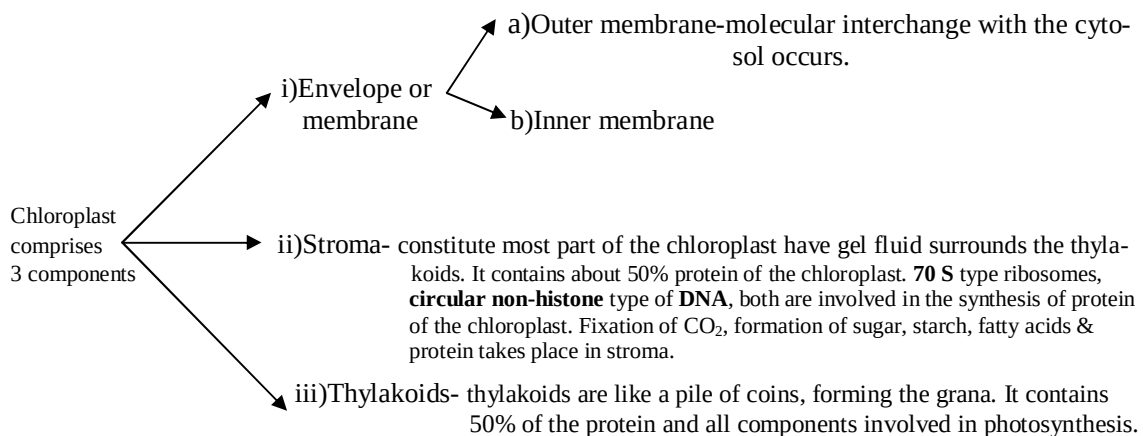
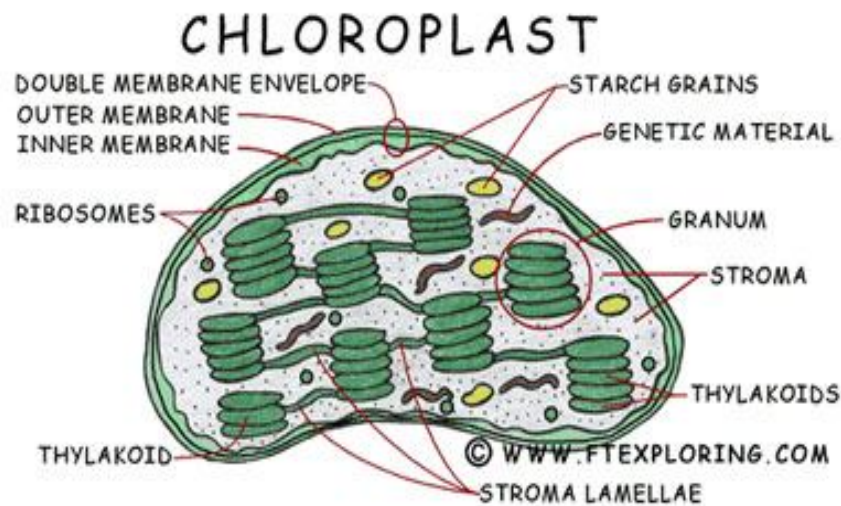


Chlorophyll a, b are the two most abundant chlorophyll. Both are found in autotrophic plants. Chlorophyll a is found in all autotrophic except than photosynthetic bacteria. Chlorophyll b is absent in blue-green, brown and red algae. The molecular formula of bacteriochlorophyll is $C_{55}H_{74}O_6N_4Mg$.

The structure of Photosynthetic Apparatus

The chloroplasts are small, green discoid or ellipsoidal, 5-10 μm in diameter and 2-3 μm thick organelle found in the cytoplasm of green plants. Chloroplast is green instrumental for photosynthesis. The chloroplast occupies more than 90% of the volume of the cytoplasm in leaf. Membranes have a yellow colour due to presence of small amount of carotenoids. These areas however lack chlorophyll and cytochromes and contain only 1- 2% of the protein of the chloroplast.

In higher plants, chloroplasts are mainly found distributed in- palisade cells (36 chloroplast) and spongy cells (20 chloroplast).



Molecular organization of thylakoid:

Modern idea about the molecular organization of thylakoid is based on fluid mosaic model of the membrane proposed by Singer and Nicolson in 1972. Lipid represents about 50% of the thylakoid membrane including those directly involved in the photosynthesis, such as chlorophylls, carotenoids and plastoquinones. There are also structural lipids which are mainly glycolipids.

The protein components of the thylakoid membrane are represented by 30-50% polypeptides disposed in the five major supramolecular complexes, which can be isolated with mild detergent. These protein complexes are photosystem I, PS II, cytochrome b_6f complex, ATP synthetase and light harvesting complex which together comprise 75-100% protein which perform the photosynthetic reactions.

Chlorophyll protein complex:

- a. **Photosystem I:** this complex contains a reactive centre composed of chlorophyll P_{700} , several peptides, a lower chl a/b ratio than found in PS II and β carotene. It acts as light trap and present in unstacked membrane.
- b. **PS II:** This complex is comprised of two intrinsic proteins that bind to reaction centre composed of chl P_{680} . The PS II is associated with light harvesting complex. PS II works as a light trap in photosynthesis and is mainly present in stacked membrane of grana.
- c. **Cytochrome b_6f :** This cytochrome contains one cytochrome f, two cytochromes b 563, one FeS centre and a polypeptide. It is uniformly distributed in grana.
- d. **ATP synthetase:** As in mitochondria, this complex consist of CF_0 hydrophobic protein, a protolipid that makes a proton channel and a CF_1 or coupling factor that synthesize ATP from ADP and P_i using the proton gradient provided by electron transport.
- e. **Light harvesting complex (LHC):** The main function of LHC is to capture solar energy. It contains two main polypeptides and both chl a and b. it is mainly localized in stacked membranes and lacks photochemical activity.

Important metabolic pathways of photosynthesis:

The Light Reactions of Photosynthesis (Light Dependent Reactions) or Hill Reaction:

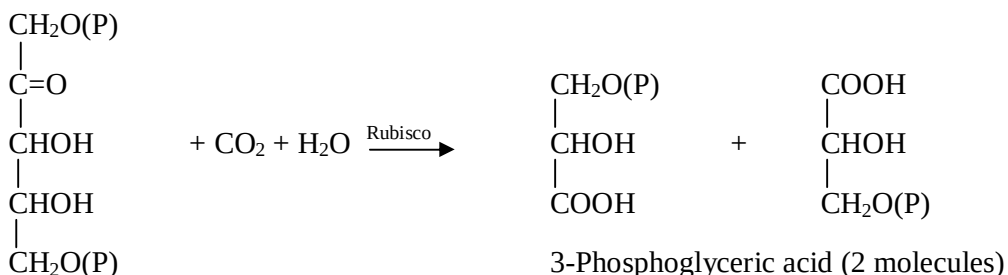
The process in which green plants (chl 'a') capture and store energy from sunlight. In the Light Dependent Processes (Light Reactions) light strikes chlorophyll a in such a way as to excite electrons to a higher energy state. In this process, light energy is converted into chemical energy, in the form of the energy carrying molecules **ATP and NADPH**. Water is split in the process, releasing oxygen as a by-product of the reaction. The ATP and NADPH are used to make C-C bonds in the Light Independent Process (Dark Reactions). Light reactions found in **thylakoids** or **grana**. According to Hill, water is used as source of electron producer for carbon dioxide fixation.

CO₂ Fixation (The Dark Reactions of Photosynthesis) or Light Independent Reactions:

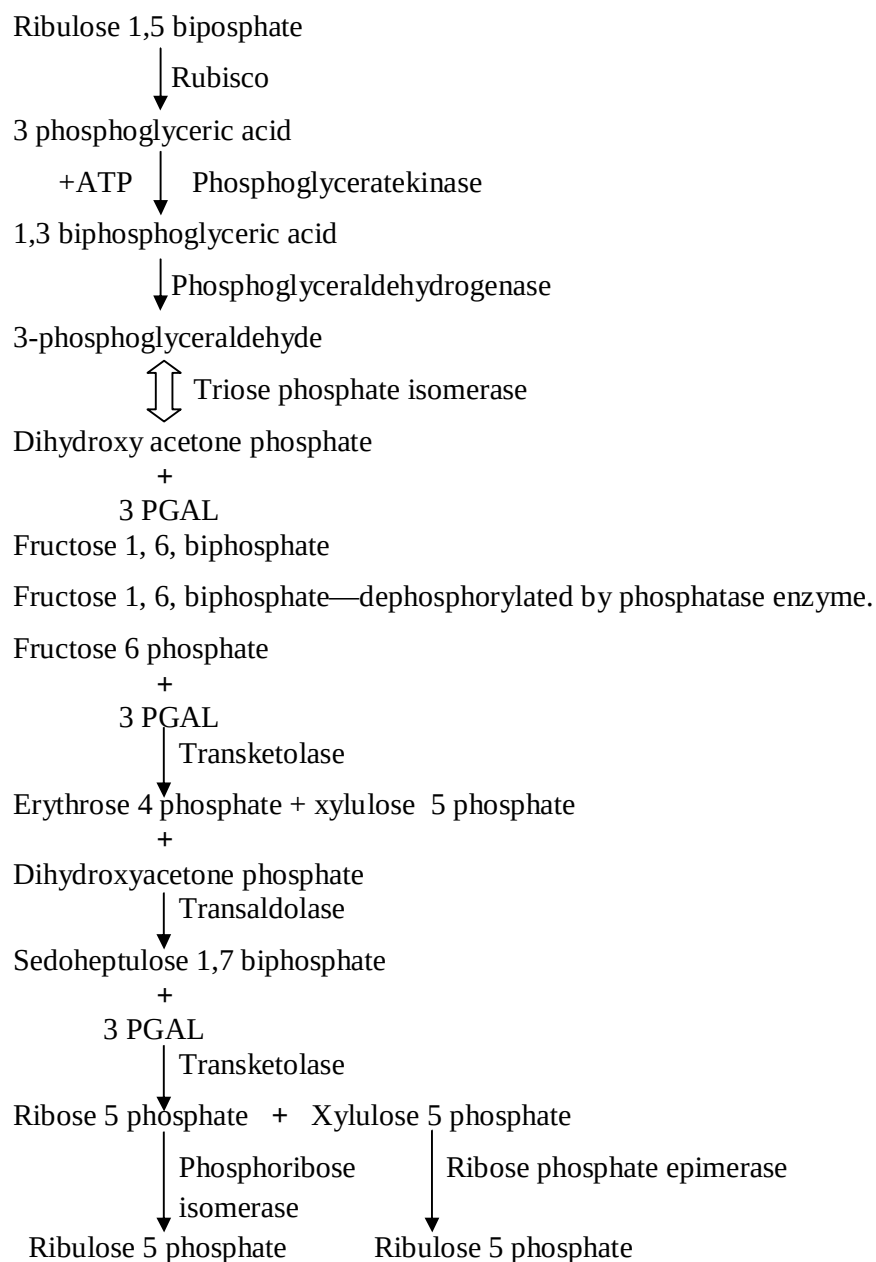
The light independent reactions of photosynthesis are chemical reactions that convert carbon dioxide and other compounds into glucose. **The incorporation of carbon dioxide into organic compounds is known as carbon fixation.** The energy for this comes from the first phase of the photosynthetic process. These reactions are light dependent reactions and perform further chemical process on them. These reactions occur in **stroma** in the fluid filled area of a chloroplast outside the thylakoid membrane. There are more than one pathway of dark fixation of CO₂ leading to synthesis of carbohydrate exists. Following three types are now well established:

1. Calvin Cycle (C₃ plants)
2. Hatch and Slack cycle (C₄ plants)
3. CAM cycle (CAM plants).

C₃ Plants (Calvin Cycle): The first stable product of the Calvin Cycle is phosphoglycerate (PGA), a 3-C chemical. The energy from ATP and NADPH energy carriers generated by the photosystems is used to attach phosphates to the PGA.



Ribulose-1,5 biphosphate



The CO₂ acceptor is ribulose-1, 5biphosphate (RuBP). The first stable product is PGA.

Enzyme catalyzing carboxylation is rubisco. Optimum temperature is 10-25⁰C.

Photorespiration occurs.

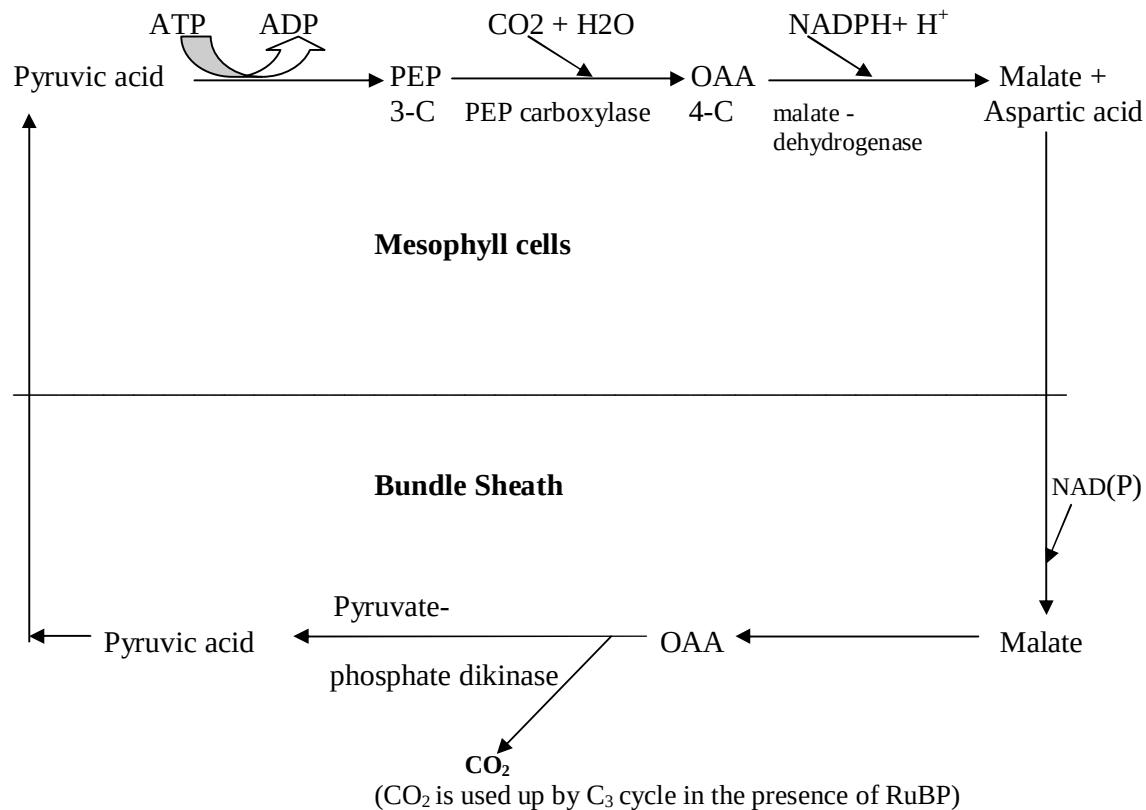
C₄ Plants (Hatch and Slack, 1967): or C₄ Pathway:

This reaction occurs in the mesophyll cells.

There are 4 distinct stages in the C₄ cycle-

- i) CO₂ fixation in the mesophyll by phosphoenol pyruvate (PEP) to form C₄ acids first oxalo acetic acid (OAA) then malic and aspartic acid.
- ii) The transfer of C₄ acids into the bundle sheath through plasmodesmata.

- iii) Decarboxylation of the C₄ acids in the bundle sheath
- iv) The diffusion of pyruvate back into the mesophyll for the regeneration of CO₂ acceptor pyruvate.



C₄ begins with a new molecule, phosphoenolpyruvate (PEP), a 3-C chemical that is converted into oxaloacetic acid (OAA, a 4-C chemical) when carbon dioxide is combined with PEP. The OAA is converted to malic acid, aspartic acid and then transported from the mesophyll cell into the bundle-sheath cell, where malate and aspartate is converted into pyruvic acid plus carbon dioxide. The carbon dioxide then enters the Calvin Cycle, with pyruvic acid returning to the mesophyll cell. The capture of carbon dioxide by PEP is mediated by the enzyme PEP carboxylase, which has a stronger affinity for carbon dioxide than does RuBP carboxylase. The Example of C₄ cycle is crabgrass, maize, sugarcane, sorghum, tropical grasses, *Cyanodon dactylon*, *Atriplex spongiosa*, *Panicum maxicum* etc.

The leave of C₃ plants have one type of chlorophyll containing cells i.e. mesophyll cells while the leaves of C₄ plants have two types of chlorophyll containing cells i.e. mesophyll cells and bundle sheath cells.

The CO₂ acceptor is PEP. The first stable product is OAA. Enzyme catalyzing carboxylation is phosphoenol pyruvate carboxylase. Optimum temperature is 30-40 °C. Photorespiration does not occur.

Differences between C₃ and C₄ plants:

C ₃ plants	C ₄ Plants
1.The first stable product of the cycle is a 3-C compound i.e. phosphoglyceric acid.	The first stable product of the cycle is a 4-C compound i.e. oxaloacetic acid.
2.The CO ₂ is first accepted by ribulose 1, 5 biphosphate.	The CO ₂ is first accepted by phosphoenol pyruvate.
3.The photosynthetic cells have monomorphic type of chloroplast.	The photosynthetic cells show dimorphic type of chloroplast.
4.Calvin cycle enzymes are present in mesophyll chloroplast.	The Calvin cycle enzymes are present in bundle sheath chloroplast.
5.The CO ₂ fixation is catalysed by Rubisco.	The CO ₂ fixation is catalysed by PEP carboxylase
6.The optimum temperature requirement for the process is 10-25.	The optimum temperature requirement for the process is 30-40.
7.For the synthesis of one glucose molecule, 18 ATP are required.	For the synthesis of one glucose molecule, 30 ATP are required.

Crassulacean Acid Metabolism (CAM) Plants:

A third mechanism of concentrating CO₂ by the rubisco is found in certain plants belonging to family Crassulaceae, Cactaceae, Liliaceae, Orchidaceae, Nictaceae, Euphorbiaceae, Bromeliaceae etc show diurnal pattern of organic acid formation. All such plants are called Crassulacean acid Metabolism (CAM) plants. CAM occurs in **mesophyll cells**.

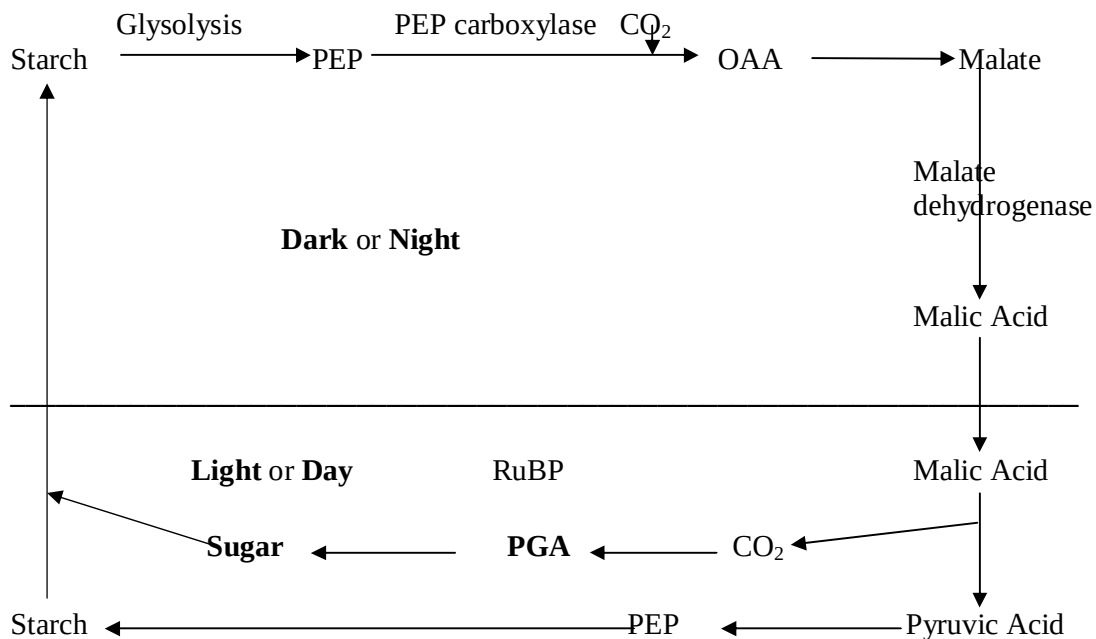
The organic acid metabolism is very common in deserts where soils are dry, nights are cool and the days have very high light intensities and high temperature. It is also occurs where salt concentrations are very high in the soils. Water stress has been found to be mainly responsible for the evolution of CAM. CAM plants like cacti and several other desert plants have to keep their stomata closed during the day to avoid transpiration. The metabolism is, therefore, devised in such a manner that they can photosynthesise during the day with the help of CO₂ released from organic acids. The organic acids are synthesised in plenty during the night with the help of CO₂ released in respiration and the CO₂ available from the atmosphere through the open stomata. This is why the succulents have open stomata and

plenty of organic acids during the night and closed stomata and plenty of carbohydrates during the day.

These plants have adopted organic acid metabolism at night and have the normal photosynthesis during the day. The stomata are kept open during night to trap atmospheric CO_2 and are closed during the day to avoid the adverse effect of transpiration. During night time the phosphoenol pyruvic acid produced in glycolysis reacts with CO_2 in the presence of the enzyme PEP carboxylase to produce oxaloacetic acid. The oxaloacetic acid is then reduced to malate by the enzyme malic acid dehydrogenase. The malate is converted into malic acid by gaining 2H^+ ions. Malic acid enters the vacuole and gets stored there. During the day, the stored malate is transported to chloroplast for decarboxylation by NADP malic enzyme. The released CO_2 is refixed by Calvin cycle.

Characteristic features of CAM plants:

1. The diurnal pattern of organic acid formation, high pH during night and low pH during daytime.
2. Nocturnal stomatal opening.
3. Large vacuoles in cell that contain chloroplast e.g. pineapple, *Agave*, *Kalanchoe*.



Chapter 7: Growth and Development of Plant

Phytohormone or Plant Growth Regulators:

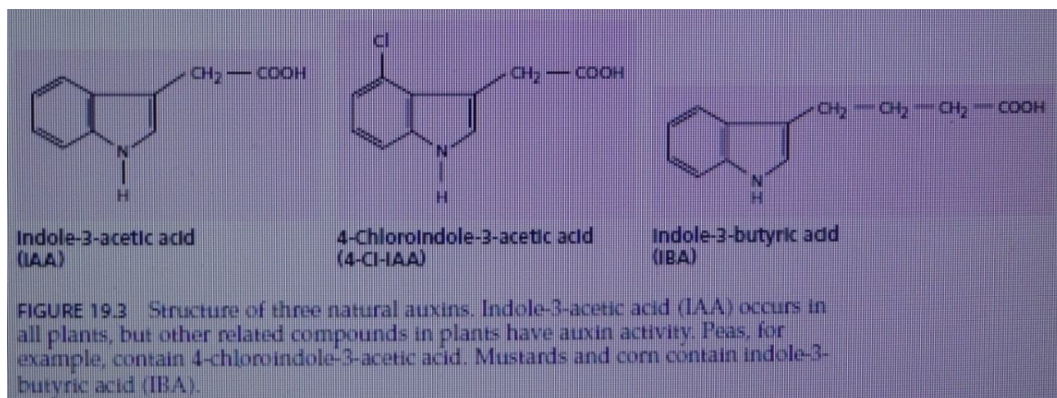
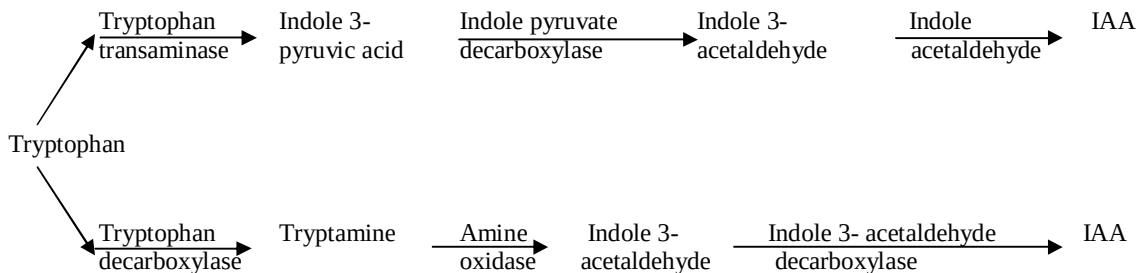
Substances which are synthesized in particular cells and transported to other sites where they produce specific effects on growth and development are called phytohormones, plant growth regulators, growth factors, growth hormones growth substances, and plant hormones.

Auxin:

Frits Went was the first who used the term **auxin** (**Greek auxien** means to **increase**).

Indole -3 acetic acid (IAA- $C_{10}H_9O_2N$), Indole-3-butyric acid (IBA), Indole-3-acetaldehyde, Indole-3-acetonitrile, Indole-3 ethanol, 4-chloro IAA, 2,4-dichlorophenoxy acetic acid (2, 4-D), and Naphthaline acetic acids (NAA).

Biosynthesis of auxins: Auxins are synthesized from tryptophan in apical meristems of the shoot (leaf primordium), root, young expanding leaves, developing fruits and embryo, basipetal (base seeking) movement, acropetal (through stele). There are two major pathways in which tryptophan may be converted to IAA of which one or both function. The first pathway is via the conversion of tryptophan to indole-3-pyruvic acid by the enzyme tryptophan transaminase. It is then decarboxylated by indole pyruvate decarboxylase to indole-3-acetaldehyde dehydrogenase, which is subsequently converted to IAA via indole acetaldehyde dehydrogenase.



Chemistry of auxin:

1. Auxins have an unsaturated ring.
2. It has a carboxyl group (-COOH).
3. There is at least one carbon between the ring and COOH

Boiassay of auxin:

Cress root inhibition test: Roots are comparatively more sensitive to auxin and their growth is inhibited by the concentrations of auxin that normally stimulate stem growth. Low concentration of auxin induces root growth. Seed are surface sterilized with sodium hypochlorite solution and then germinated in moist filter paper in petri dish. When the roots of the seedlings have reached a desired length, they are placed in petri dishes containing 15 ml of test solution. The growth is measured after 48 hours.

Role of Auxin:

1. **Apical bud dominance:** On removal of apical buds, lateral buds begin to sprout and large numbers of lateral buds are formed.
2. **Cell enlargement and cell elongation:** Stimulates cell enlargement and cell elongation.
3. **Acid growth hypothesis:** During elongation of the cell, the external medium becomes acidic.
4. **Cell wall hypothesis:** Dry weight of cell wall increased due to incorporation of sugars and inorganic acid into cell wall during cell expansion.
5. Promote synthesis of RNA.
6. Xylem differentiation from callus.
7. Auxin plays a specific role in seed germination, growth, rooting on stem cutting, flowering, abscission, parthenocarpy, ethylene production, tissue culture etc.
8. **Fruit setting and growth:** Auxins are synthesized in pollen and developing seeds (endosperm and embryo) and are known to involve in the regulation of fruit development.
9. **Stimulation of Fruit set:** When environmental conditions are not suitable to fruit set, the treatment of auxin to flower can promote this process.
10. Prevention of premature fruit drop.
11. Eradication of weeds.

Gibberellins:

Yabuta, Hayashi and Sumiki (1938) isolated **Gibberellins A and B** in the form of crystals from *Gibberella fujikuroi* (*Fusarium moniliforme*), the causal organism of **Foolish disease of rice or Foolish seedling or Bakanae disease of Rice or Ahonae (stupid seedling) disease**. Infected plants were usually taller, seedless and pale in colour. Till date, GA₁-GA₁₂₅ has been discovered. 25 Gibberellins are isolated from *Gibberella Fujikuroi*.

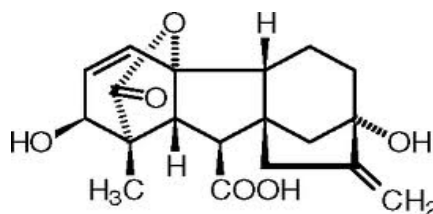
Gibberellins are synthesized by young leaves (major site) root tips and immature seeds. It translocate readily in all directions in tissues via the xylem sap.

Biosynthesis of gibberellins:

Acetate $\rightarrow$ CoA $\xrightarrow{\text{Condensation}}$ Mevalonic acid $\xrightarrow{\text{Phosphorylated}}$ Mevalonic acid pyrophosphate

Chemistry of gibberellins:

1. All are tetracyclic diterpenoid acid.
2. All gibberellins are acid and represented as GA.
3. There are two types of GA, the C₂₀ and C₁₉.
4. All gibberellins have one –COOH group attached to C₇.

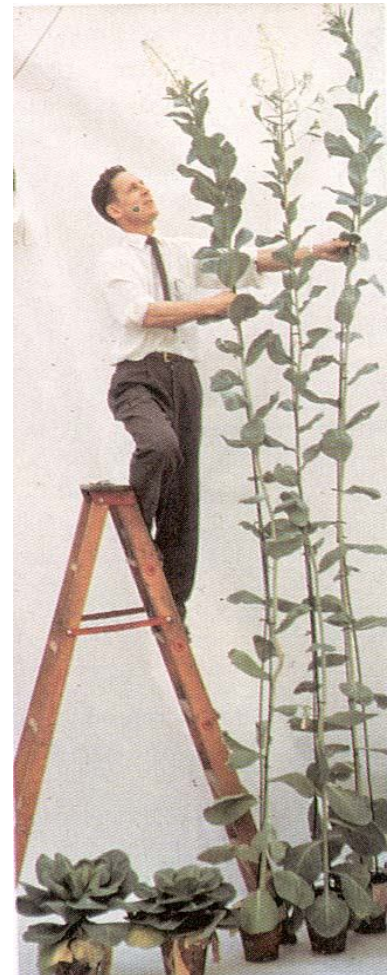
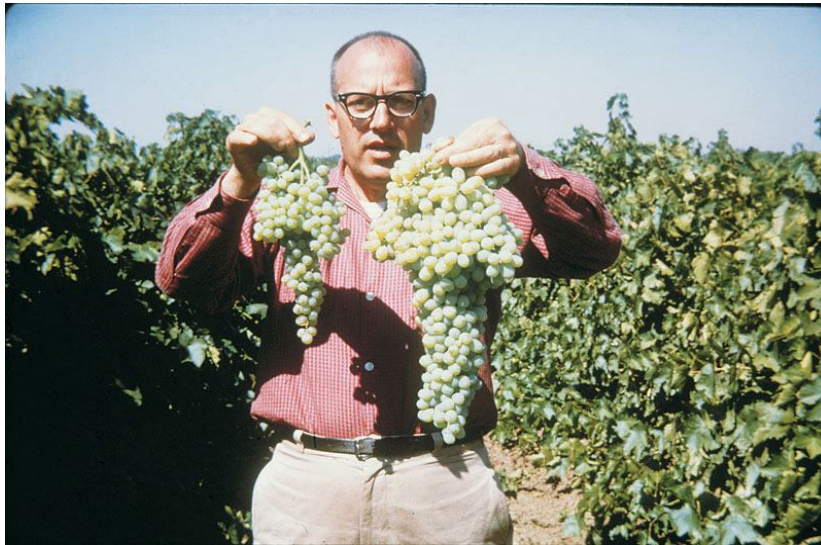


gibberellic acid

Bioassay: Increased growth of dwarf seedling when applied to ligule of the first leaf.

Role of Gibberellins:

1. **Stem elongation:** Internodes increase in length e.g cabbage, lettuce.
2. **Dwarf plants become normal and long** are called **bolting** (cabbage).
3. **Promoting flowering** in long day plants under unfavourable short day conditions.
4. **Parthenocarpic fruits:** It is more effective than auxin in causing parthenocarpic development of fruits in tomato, apples, pears, pome fruit and seedling grape bunch increase in length.
5. **Breaking Dormancy:** in potato tubers and tree buds in winter.
6. Mobilization of foods in seed storage cells.
7. **Fruit production:** Gibberellins application causes the grape bunches to elongate so that they are less tightly packed and less susceptible to fungal infection. It also promotes the elongation of fruit. Usually the grape vines are sprayed twice with 20 ppm gibberellins, once at bloom and again at the fruit set stage.



Cytokinins:

Root tip is an important site of its synthesis. Developing seeds are also the site of cytokinin biosynthesis. The molecular formula of cytokinin is $C_{10}H_9N_5O$. Cytokinin move upward in the xylem stream.

Chemistry of cytokinin: Cytokinins are adenine derivatives characterized by ability to induce cell division in the presence of auxins. The term cytokinin has been given to kinetin just to differentiate from animal biochemistry where the same term, kinetin is used. The most commonly found cytokinin is zeatin.

BAP – 6-benzylaminopurine

BA- benzyl adenine

2iP (IPA) - N⁶-(2-isopentyl) adenine

Kinetin – 6- furfuryl aminopurine

Zeatin – 4-hydroxy 3 methyl Trans 2-butanyl aminopurine

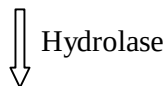
Thidiazuron – 1 phenyl 3- (1, 2, 3- thiadizol 5- yl) urea.

Biosynthesis of cytokinin: A direct cytokinin synthesis is possible via the condensation of dimethylallyl pyrophosphate (DMAPP) and 5` AMP to form isopentyladenosine 5` phosphate. Subsequent dephosphorylation and deribosylation and/ or side chain hydroxylation leads to the production of cytokinin.

Dimethylallyl pyrophosphate



Isopentyl adenosine 5` - monophosphate



Zeatin ribosides 5` monophosphate



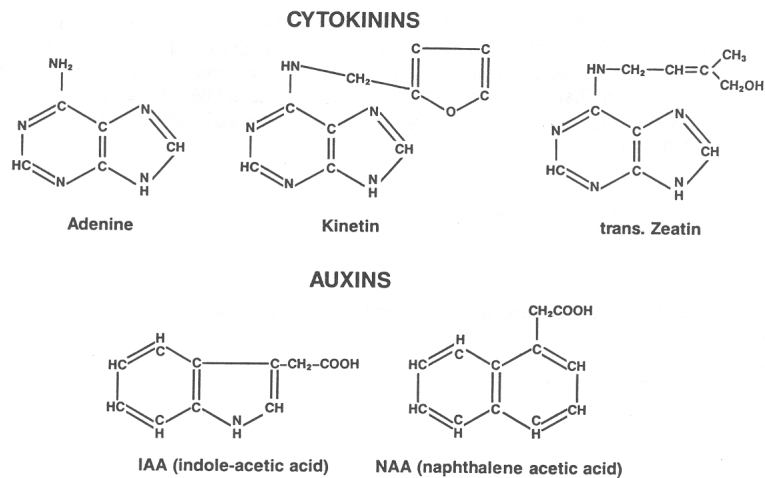
Zeatin riboside



Zeatin

Bioassay

Callus formation: Addition of cytokinin in nutrient medium leads the callus formation.

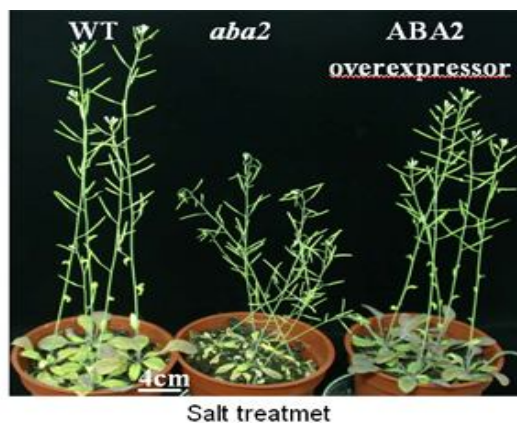
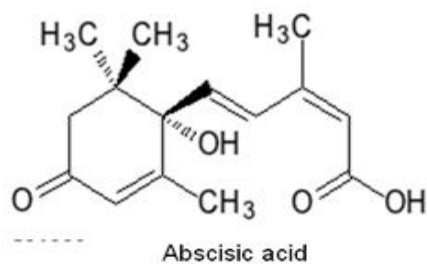


Role of Cytokinins:

1. Cell division
2. Cell enlargement
3. **Morphogenesis:** Help in organ formation in a variety of tissue cultures.
4. Breaking of dormancy: Breaking the dormancy of seed and some other plant organs and promote the germination of seeds.
5. Cytokinin induce lateral bud formation
6. Rate of photosynthesis increased after the application of cytokinin.
7. Cytokinin most likely influence protein synthesis through their involvement in the attachment of t-RNA to the ribosome-mRNA complex during protein synthesis.

Abscisic Acid (ABA):

It has a wide range of physiological effect promoting senescence and abscission, retardation and inhibition of growth. ABA synthesized in mature leaves containing chloroplast and moves upto the shoot apex. ABA is translocated mainly through xylem and phloem.



Biosynthesis of ABA:

Mevalonate $\longrightarrow$ Isopentyl pyrophosphate $\longrightarrow$ Geranyl pyrophosphate $\longrightarrow$ Farnesyl pyrophosphate $\longrightarrow$ ABA

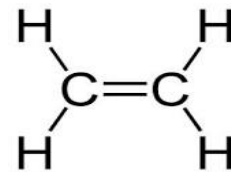
Role of Absciscic Acid:

1. Bud and seed dormancy due to the presence of growth inhibitor.
2. **Abscission:** Due to ABA causes ageing and abscission of leaves.
3. **Senescence:** ABA accelerates the senescence of leaves.
4. It inhibits gibberellins i.e. **antigibberellin**.
5. It promotes root growth but inhibit shoot growth.

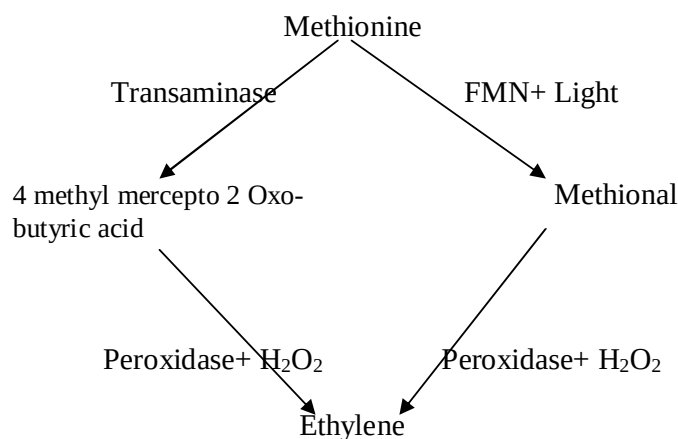
6. ABA accumulates in high concentration in leaves which causes wilting.
7. ABA inhibits RNA and protein synthesis, and biosynthesis of other hormones
8. It stimulates the release of ethylene.
9. **ABA as stress hormone:** ABA level increases when plant is exposed to salt, cools or high temperature stresses.

Ethylene (The ripening hormone):

The meristems and nodes are the chief sites for the production of ethylene. Ethylene can be synthesized by any part of the plants.



Biosynthesis of Ethylene:



Role of Ethylene:

1. Promotion of leaf abscission.
2. Natural fruit ripening: fruit ripening refers to the changes in the fruit that make it ready to eat. A survey of wide range of fruits showed that not all of them respond to ethylene. The apples, bananas, avocados and tomatoes are the examples of **climacteric** fruits.
3. Degreening of citrus and banana.
4. Chlorophyllase activity.
5. Induction of root hair formation.
6. Induction of flowering in pineapple.

7. Ethylene breaks seed and bud dormancy.
8. Ethylene acts as wound hormone: reduce the stress.
9. Ethylene promotes induction of female flowers in cucurbitaceae for earlier and yield increase.
10. Increase the latex flow in *Hevea*.

Photomorphogenesis:

Photo=light; morpho= appearance or development of plants; genesis= to produce.

The control of morphogenesis by light is called photomorphogenesis.

The Role of light in seed germination:

The germination of several seeds affected by light are called **photoblastic**. Germination of seed is stimulated by light are called **positively photoblastic**. Germination of seeds are inhibited by light are called **negatively photoblastic**.

Role of light in vegetative growth:

Formation of leaf primordia, increase in the rate of chlorophyll accumulation, ascorbic acid synthesis, synthesis of anthocyanin, formation of plastids in the mesophyll etc. formation of tracheary elements, differentiation of stomata in the epidermis, spore and oospore germination.

Role of Phytochrome (phyton= plant; chroma = colour/pigment).

It is a protein pigment with a molecular mass of about 125 kDa (kilodaltons) that absorb red (650–680 nm) and far red (710–740 nm) light most strongly. Also absorb blue light. Phytochromes are the most important photoreceptors in vascular plants.

- Red light induced seed germination is the result of an increase in the level of the hormone gibberellins, thus affecting gibberellins biosynthesis.
- Chloroplast (chl a) development and synthesis of photosynthetic enzyme
- Induced flowering – Under long day periods, P730 stimulates flowering in LDP and suppresses it in SDP. Conversely, under short day photoperiod, P660 stimulates flowering in SDP and suppresses it in LDP.
- Enzymatic activity- controls peroxidase and nitrate reductase activity.
- DNA and protein synthesis: Red lights induce DNA and protein synthesis.
- Shade avoidance response: Sense the shading caused by other plants.

Environmental and Genetic Factors Affecting Plant Growth and Development

Plant growth factors control or influence plant characteristics as well as adaptation. In general, there are two factors affecting plant growth and development:

- A. Genetic factor and
- B. Environmental factor.

The genetic factor is also called internal factor because the basis of plant expression (the gene) is located within the cell. The environmental factor is considered external, and refers to all factors, biotic and abiotic, other than the genetic factor. Both plant growth factors interact in various ways. The genetic factor determines the character of a plant, but the extent to which this is expressed is influenced by the environment.

A. Genetic Factor

The genotype of a plant affects its growth and development and crop productivity. For example, selected varieties of rice grow rapidly, maturing within 110 days, whereas others, in the same environmental conditions, grow more slowly and mature within 155 days. A producer has control over the genetic factor by his choice of variety. Genetic factor also determines whether a plant should be a tree, a shrub, a herb, a vine, a liana, either a vascular or a non-vascular plant, a gymnosperm or an angiosperm, and down to the smallest classification of a species, a variety, a line, or a strain. It controls adaptation, resistance to pests and diseases, stress tolerance, and crop yield. Crop yield is controlled by multiple numbers of genes, each of which determines the expression of certain character but contributes to the final yield through additive and/or interactive effect. Phenotype is controlled by a genetic constitution, or genotype.

B. Environmental Factors

With the right gene combination, favourable environment can ensure full expression of crop yielding potential. Conversely, unfavourable conditions can negate or diminish the full expression of genes. Environmental factors which affect plant growth can be classified as abiotic factors and biotic factors.

a) Abiotic Factors

The abiotic factors that affect plant growth and development include topography, soil, and climatic factors. They are the non-living components of the environment which, along with the biotic factors, determine the extent to which the genetic factor is expressed in the plant.

1. Topography

Topography is a nonliving or abiotic factor that refers to the “lay of the land.” It includes the physical features of the earth such as the land elevation, slope, terrain (flat, rolling, hilly, etc.), mountain ranges and bodies of water. The steepness of a slope affects plant growth through differential incidence of solar radiation, wind velocity and soil type. The altitude or elevation of the land with respect to the level of the sea surface influences plant growth and development primarily through temperature effect. The relationship of this abiotic factor to temperature is like that of distance from the equator to the arctic poles. Temperature decreases by 1⁰C for every 100 metre increase in altitude in dry air.

2. Soil

Soil is the outermost layer of the surface of the earth in which plants grow. It is composed of eroded rock, mineral nutrients, decaying plant and animal matter, water and air. This abiotic factor is likewise important in crop farming and is treated under the heading - Soil and Climatic Adaptation or Requirement of crops. Most plants are terrestrial in that they are anchored to the soil through their roots, with which they absorb water and nutrients. But epiphytes and floating hydrophytes do not need soil to live. Variations in the physical, chemical, and biological properties of the soil have distinct effects on plant growth and development, depending on natural adaptation.

There are two properties of the soil having pronounced direct effects on plant growth and crop production: physical and chemical properties. There are also biological factors or living organisms in the soil such as the earthworms, insects, nematodes and micro organisms like bacteria, fungi, actinomycetes, algae, and protozoa. These organisms help in improving soil structure, tilth (breaking and powdering of soil lumps), aeration, water permeability and soil nutrient availability.

The physical and chemical properties of the soil are referred to as edaphic factors of the plant environment. The physical properties include the soil texture, soil structure, and bulk density which affect the capacity of the soil to retain and supply water, while the chemical properties consist of the soil pH and Cation Exchange Capacity (CEC) which determines its capacity to supply nutrients. It is now known that this abiotic factor (soil) is not essential as such for plant growth. Rather, it is the nutrients that are present in the soil that make plants grow and enable them to complete their life cycle.

3. Climate

The climatic factors which affect plant growth include:

- Humidity
- Aeration
- Light
- Temperature
- Moisture

In nature, there is an interaction between these factors and they all affect each other. In a controlled environment, such as a nursery or open field seed bed, temperature is the most influential factor in this interaction. A plant has the natural ability to regulate its level of activity according to environmental conditions, such as at specific levels of temperature and humidity. At extreme temperatures and humidity, such as when it is extremely dry or humid or extremely hot or cold, growth will stop, which may lead to the plant dying if the conditions persist. Environmental conditions therefore play an important role in the ability of a plant to grow and in general plant health. Effectively controlling these factors enables one to propagate and grow healthy plants.

4. Humidity

Humidity, also referred to as relative humidity, is the amount of water vapour in the air at a given temperature, and is expressed as a percentage. This means that at 20% relative humidity, 20% of any given volume of air will consist of suspended water molecules. Humidity levels are especially important in allowing the plant to carry on with its metabolic processes at desired rates. The ideal relative humidity for propagation ranges between 80% and 95% for seeds and cuttings, and in the region of 60% outdoors for budding, grafting and seedbed methods. Seed germination is faster at higher humidity levels, as is the case of cuttings. In warm and dry areas, the level of humidity often falls below 55% on hot summer days, making budding and grafting more delicate and requiring close monitoring.

5. Aeration

Plants can only grow and survive in a balanced environment, where both oxygen (O₂) and Carbon dioxide (CO₂) are sufficient. The processes of respiration and photosynthesis make use of both O₂ and CO₂ to sustain the growth and development of the plant. In the open, such as under shade-cloth or in seedbeds, the ambient air movement is sufficient to aerate plants. In structures such as tunnels, ventilation becomes important. Ventilation extracts warm air

containing the CO₂ produced by plants from tunnels, thereby maintaining a balanced environment.

6. Light

All green plants require light for growth to take place. Some plants (most species) prefer growing in direct sunlight, while others prefer growing in the shade where they are subjected to indirect sunlight. Light is essential for photosynthesis, while light quality, which is determined by the wavelength of the light, also influences germination and flowering.

Plants grown under protection such as greenhouses and shade-houses, require adequate light for the process of photosynthesis. If the plant does not receive enough light, which may be due to shading or over-crowding, it displays symptoms of retarded growth.

In seed germination, red light, with a wavelength of 660 nanometre (nm), is used in chambers to stimulate germination of certain kinds of seeds. Incandescent globes are commonly used as an artificial source for red light for this purpose, while fluorescent tubes provide the blue light required for photosynthesis after germination. These lights are used extensively and kept on for as long as possible. It is not uncommon to have lights on 24 hours a day week round.

The depth of sowing light sensitive seed also determines the time seeds take to germinate, because light cannot penetrate deeply into the soil. Therefore light sensitive seeds should be planted shallower than non-sensitive seeds.

With no or inadequate light, weak seedlings of poor quality are produced. These seedlings display an excessive elongation, referred to as etiolation.

7. Temperature

If heat and light, which cause an increase in temperature, is not controlled properly, plants may suffer from heat injury. The ideal temperature for propagation is 29°C, and it must be monitored closely. In propagation chambers, the temperature can often be maintained at this ideal level by heating and cooling systems. The heat is also used for increasing the humidity in the chambers, by drenching the trays and dampening the floor.

8. Moisture

- i. Moisture is essential for germination and healthy plant growth.
- ii. Too much water suffocates the plant roots, and can cause diseases such as root rot, damping off, and collar rot. The other extreme is insufficient water supply, or drought, and is detrimental to all plants, but even more so to cuttings and young

seedlings. A uniform and constant water supply is required for seed germination to produce healthy and vigorous seedlings, and for seedlings to grow into healthy plants.

- iii. In all propagation methods, the properties of the growth-medium determine the quality and quantity of water that will be available for uptake by the plant. A good medium is one that has a low salinity level, sufficient water holding capacity (50-60 %), make water available to the plant easily, and the ability to allow lateral water movement.
- iv. In the case of germination, the seed, and the later seedling stage, has to be kept in media wetted to field capacity, being the maximum amount of water that a particular soil can hold.

b) Biotic Factors

The effect of these biotic factors on plant expression may be advantageous or disadvantageous, depending on how they interact with the plant. These interactions include mutualism, herbivory, parasitism, and allelopathy.

1. Mutualism

Mutualism is a species-to-species interaction in which both the biotic factor and the plant are benefited by the relationship. Examples of beneficial influence of biotic factors on plant growth and development through mutualism are:

- i. The symbiotic relationship of the Rhizobium bacteria and leguminous plants.

The Rhizobia live in the roots of the legume and obtain their supply of energy from the host plant. In exchange, the Rhizobia fix atmospheric nitrogen and supply it to the plant in absorbable form.

Nitrogen is an essential macronutrient for plant growth and development. It is a component of enzymes, DNA, and the chlorophyll molecule.

N fixing nodules formed by Rhizobium in legumes

- ii. Forest and fruit trees and other plants including annual crops associate with micorrhizal fungi which aid in the absorption of water and nutrients, such as phosphorus and zinc, from the soil.
- iii. Birds, insects and bats serve as vectors (agents) of pollination, the transfer of pollen from an anther to a stigma, which is a precondition for the development of fruits and seeds from flowers in the angiosperms. As a reward, the pollinators feed on the nectar that the flower secretes or obtain some other benefit from the plant.

2. Herbivory

In herbivory, plant-eating organisms called herbivore, such as ruminant animals, rodents (eg. Rats, squirrels), insects, and molluscs feed on plant parts. Herbivores with significant deleterious effects on crop growth and yield are called „pests“. Damage caused by these biotic factors are varied such as death of the entire plant or organs, reduced root, stem, leaf or inflorescence mass, total defoliation, bores and holes on plant parts, and other marks of feeding.

3. Parasitism

Parasitism is an interaction between two organisms in which one organism, called parasite, is benefited, but causes harm to another, called host. The parasite steals its food from the host. Micro organisms such as fungi, bacteria, nematodes and virus injure crops by causing diseases and are called pathogens. Examples of parasitic plants are the loranthus, cuscuta, and some orchids. Loranthus on mango tree.

4. Plant Interaction and Allelopathy

The word allelopathy derives from two separate words. They are allelon which means "of each other", and pathos which means "to suffer". Allelopathy refers to the chemical inhibition of one species by another. The "inhibitory" chemical is released into the environment where it affects the development and growth of neighboring plants.

Allelopathic chemicals can be present in any part of the plant. They can be found in leaves, flowers, roots, fruits, or stems. They can also be found in the surrounding soil. Target species are affected by these toxins in many different ways. The toxic chemicals may inhibit shoot/root growth; they may inhibit nutrient uptake; or they may attack a naturally occurring symbiotic relationship thereby destroying the plant's usable source of a nutrient.

Growth:

Growth is a vital process brings about a permanent change in any plant or its part in respect to its size, form weight and volume. Growth regions in plants are the shoot and root apex is called apical meristems or primary growth. Apical meristems are responsible for the increase in length, differentiation and formation of plant tissues. There are three types of growing points in plants:

1. Apical meristems
2. Intercalary meristems
3. Lateral meristems- responsible for secondary growth to increase the thickness of plant in girth.

Phases of growth:

Growth is not a simple process. It occurs in meristematic regions where before the completion of this process, a meristematic cell has to pass through the following three phases:

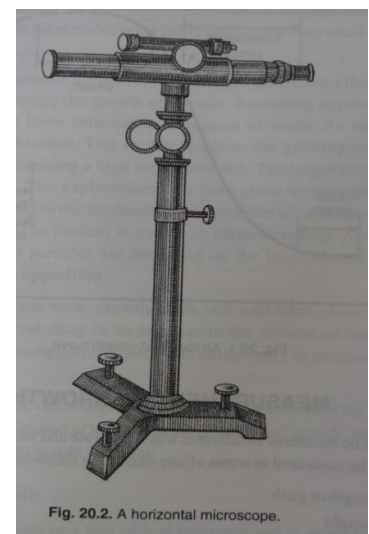
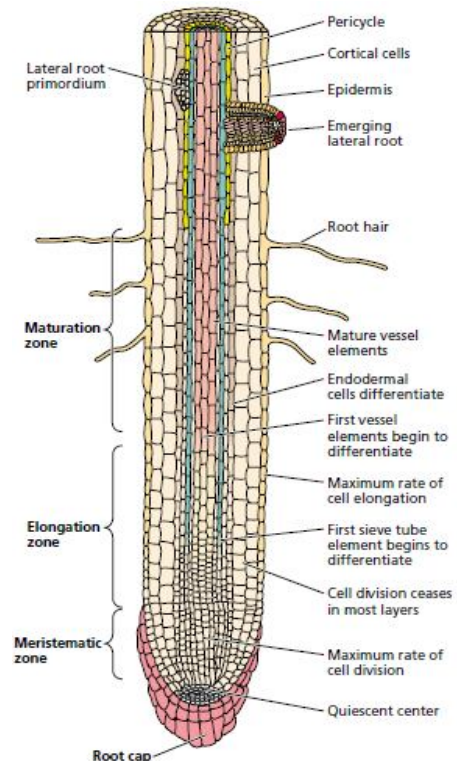
- i) Cell formation phase
- ii) Cell elongation phase
- iii) Cell differentiation (cell maturation)

Cell formation is represented by meristematic zone and the cell elongement by cell elomgation zone. The dividing meristematic cells are thin walled, dense protoplasm with a large nucleus without or very small vacuole and lack of intercellular spaces.

Measurement of Growth: The rate of growth can be measured in different ways:

- a) Increase in length or girth
- b) Increase in girth
- c) Increase in volume or area

1. Horizontal Microscope: It can measure the increase in length with the help of a microscope which slides on vertical scale. A particular point marked on the stem tip is focussed. After a gap of time the microscope is moved to focus it again and the distance covered by the



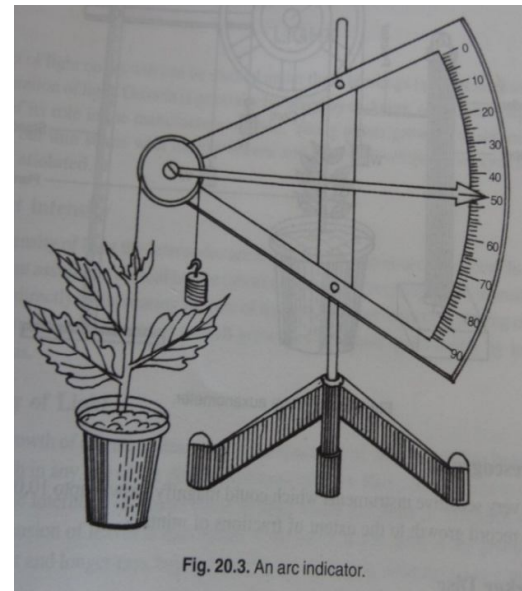
microscope is equivalent to increase in the length of the plant.

2. Direct Method: One of the simplest methods to measure increase in the length of a plant organ is to measure it with scale in the beginning and then after a particular period of time to find out the change in length.

3. Auxanometer: Two types of auxanometers are in use now for measuring growth.

- i) Arc auxanometer
- ii) Pfeffer's automatic auxanometer

i) Arc auxanometer: It consists of a vertical stand with a pulley connected to a pointer on arc scale. A thread can be passed over pulley with one end tied to the growing point of the plant and the other end carrying a weight to keep the thread stretched. With the growth, the pulley moves resulting in the movement of the pointer to indicate the rate of growth on arc scale. The growth rate can be easily calculated.

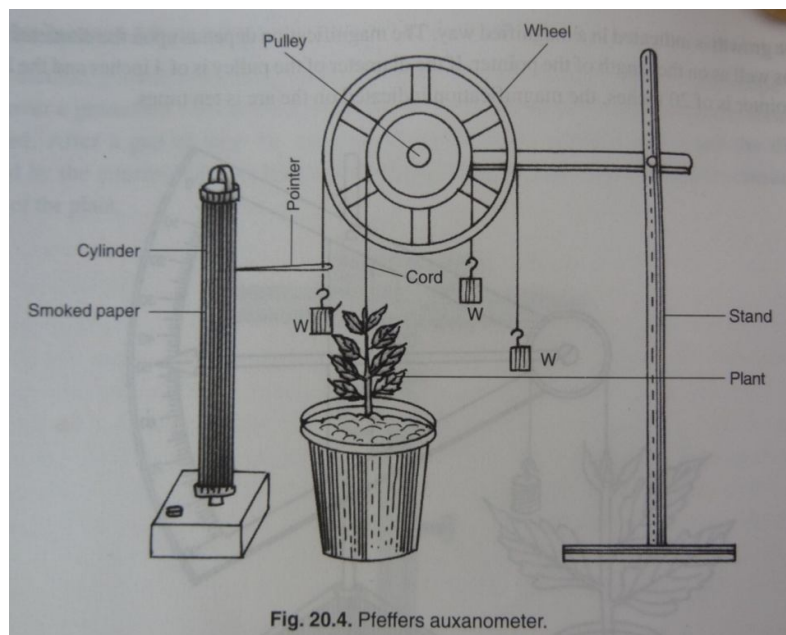


ii) Pfeffer's Automatic Auxanometer: It consists of a revolving drum and two pulleys and two wheels: small and large, the smaller one being attached to the central axis of the large one on one side. A thread tied to the stem apex is hung around the small wheel and its other end is tied to a weight.

Similarly a thread is hung on the large wheel, with both the ends tied with weights. An indicator is attached to the weight of the thread on one end to trace a line on the smoked paper pasted around the drum, which is kept in rotation (once every hour) by a clock work.

Growth in stem tip lowers the weight to which the indicator is attached resulting in a special

marking going downwards on the smoked paper. The perpendicular distance between two



adjoining marking on the paper indicates the hourly growth. The actual rate of growth can be calculated by taking into consideration the rotation of the drum and the radii of the small and the large wheels.

4. Space marker disc: By space marker disc the area of a leaf is measured and marked at regular intervals and recorded to calculate the rate of growth.

Factors Affecting Growth:

Growth is affected by a large number of factors both environmental and physiological.

Physiological factors: Physiological factors such as absorption of water, minerals, photosynthesis, respiration etc.

Environmental factors: Environmental factors including climatic and edaphic both.

1. Light: Light affects variously e.g. light intensity, quality of light and periodicity (duration) of light.

a) Intensity of Light: High light intensities induce dwarfening of the plant, reduce the rate of growth indirectly by increasing the rate of the loss of water from the growing cells. Plants at the hill tops are short but in valley are quite tall. In weak light intensity, photosynthesis and growth of the plants reduced. Short internodes and etiolation (yellow colour) developed due to low intensity of light. Very low light intensity reduces the growth of plants by lowering the rate of photosynthesis.

b) Quality of Light: Plant growth has been found to be better in the spectrum of visible light (400-700 nm). Red colour seems to be most favourable for growth. Infra-red and ultraviolet are detrimental to growth. UV rays are necessary for the development of anthocyanin pigments in the flowers.

c) Duration of light: There is a remarkable effect of the duration of light on the growth of vegetative as well as reproductive structures. The induction and suppression of flowering are dependent on duration of light.

2. Temperature: Temperatures for growth vary plant to plant and habitat to habitat. The minimum (5°C), optimum ($25-30^{\circ}\text{C}$) and maximum ($35-40^{\circ}\text{C}$) temperatures are for the growth of plants of temperate zone. These values will be lower for arctic and higher for the tropical zone than those of the temperate zone.

Low temperature during nights reduces the rate of respiration and high temperature during day time increases photosynthesis and accumulates photosynthates also increase growth of tubers and other vegetables, fruits growing on hills are much larger than those of the plains.

3. Growth Hormones: Growth of plant is controlled by certain organic compounds (Hormones) which are synthesized by the plants in very small quantities in one tissue and translocated to other sites where they produce specific effects on growth and development.

4. Food Supply: The food supply is directly proportional to the rate of growth because it provides growth materials and potential energy to the growing regions.

5. Water Supply: Water is necessary for all metabolic activities of the protoplasm and increasing the turgidity of the cell for cell enlargement.

6. Oxygen Supply: Oxygen increasing growth by converting potential energy into kinetic energy needed for vital activities of the plants.

Plant Response to Environments:

Plants are affected by their environment. Plants respond to a wide variety of stimuli other than light because of their immobility. Plants may display a bending movement called tropism (trope: turn). It is often caused by hormones. Kinds of tropism are –

1. Phototropism: Plants respond to light e.g. bending of stems towards light.

a) Positive Phototropism: Stems move towards the light.

b) Negative Phototropism: Roots move away from the light.

2. Geotropism (gravitropism): Bending of plant parts due to gravity e.g. roots are positive geotropism and stem is negative geotropism.

3. Hydrotropism: Bending of plants toward the source of water e.g. only roots. Roots are always positive.

4. Thigmotropism: Plant response to touch e.g. Makahiya plant (*Acacia, Mimosa pudica*).

5. Response to day length: Short day plant bloom e.g. *Poinsettia*.



J. Levitt (1972) considered biological stress to be any change in environmental conditions which may cause negative effect or adversely change a normal plant growth and development are called **stress**. Biological species show a range of tolerance to environmental factors e.g. low temperature, unavailability of water, extreme cold, excess of salt, high temperature etc also causes stress in plant. The plants flourish best in the optimum tolerance range.

What happens if you do not supply water to a potted plant for four to five days? You may observe that the leaves bend down and the plant wilts. Here due to lack of water the usual life processes of plant are disturbed. We can say that the plant is facing stress in its life. This may be called as biological stress. Not only water, there are a number of factors responsible for causing stress in plants like temperature, salt, shade, light, pollutants, etc.

The effect that is produced in plant as a result of stress is called strain. In the above example bending of leaves and wilting of plant are strains. So the reaction of plant facing the stress is called **strain**.

Stress factors and plant responses to stressors

There is a diversity of types of stressors that may affect plants. Stressors or stress factors are defined as extreme environmental conditions that induce functional changes in plants to such an extent that stress of the organism develops, resulting in inhibited growth, reduced bioproduction, physiological acclimatisation, and adaptation of species or some combination of these changes.

Stress Factors:

1. Biotic: Competition, allelopathy, herbivory, diseases, pathogens, viruses etc.

2. Abiotic:

a) Physical

- i) **Temperature:** high and low (chilling & frost)
- ii) **Water:** drought and flooding
- iii) **Radiation:** infrared ultraviolet, ionizing radiation etc.
- iv) **Mechanical stress:** Wind, soil movement, submergence,
- v) **Other stresses:** Magnetic field etc.

b) Chemical: Air pollution, Pesticides, Toxins, Soil and water pH, Salinity, Mineral salts (Deficiency, over-supply) Pollutants (heavy metals, pesticides) and Gaseous toxins.

Abiotic stressors may be of physical or chemical character. Physical attributes of the environment can affect physiological patterns in a positive or a negative manner. For example, a rise in temperature increases enzyme function within a certain range, above which strong inhibition results. Many chemical attributes of the environment can act as stressors if they are not normally in the environment (pesticides) or if their levels are relatively high (pH). Young tree seedlings are sensitive towards the water content in the upper layers of the soil, and they may die as a result of flood or drought, while adults of the same species need not have any sensitivity towards the water content of the upper soil layers.

Changes in the environmental factors that cause stress in plants may occur within different time-scales, varying in duration and intensity during the life-span of an organism. With the increase of longevity of an organism, the chance of exposure to stress will increase, formerly only because of natural changes of climatic and edaphic factors, but recently because of anthropogenic changes. Air pollution affects plants directly, damaging their above-ground organs, or indirectly, accumulating in the growth substrate and affecting plant metabolism through soil and water. Under the influence of biotic stress factors plants develop specific protection mechanisms, for example products of phenolic metabolism to check further development of fungal pathogens in host or monoterpenes against bacteria. The initial stages of responses involve damage in cell membranes, changes in enzymatic processes, and subsequent deviations in cell structures and metabolism.

The sensitivity of the plant species towards stressors may cause its extinction or changes in the plant community.

Anthropogenic stress symptoms can be grouped into the following categories and used in bio indication systems:

1. Invisible injuries, physiological and biochemical responses (e.g., changes in bio membranes, enzyme activities etc.);
2. Visible injury of plant parts (necrosis, chlorosis etc.);
3. Retarded growth, development and reproduction;
4. Floristical changes on the level of population;
5. Coenological changes in ecosystems

Water stress or Moisture Stress:

Flooding or excess amount of water in soil reduces root and shoot growth, and causes blackening of root tips and yellowing of leaves, etc. Shortage of water results in drought injury and excess of water causes flooding injury. Water deficit causes the following-

1. Leaf abscission
2. Decreased leaf area
3. Extensive root growth
4. Decreased photosynthesis
5. Increase in wax deposition on the leaf surface
6. Stomatal closure
7. Increase in the concentration of salts solutes which damage the enzymes present in the cells.
8. Lowering the osmotic potential by accumulating some organic compounds such as proline sucrose etc.
9. Decrease in the synthesis of protein, cell wall materials, nitrate reductase stomata closed with quick decrease in the rate of photosynthesis and transpiration.
10. Increase in the production of ABA which causes decrease in the growth of the shoot.
11. Decrease in the translocation of metabolites and transport of ions.
12. Changes in membrane. Cell enlargement is checked, cell size is deformed and cell rigidity lost due to deficiency of water.

Note: Excess of water results in lack of aeration. Flooding results in lack of root growth and root hairs. The absorption of water and the plant is wilted.

Heat Stress:

Heat stress is often defined as the rise in temperature beyond a threshold level for a period of a time sufficient to cause irreversible damage to plant growth and development. In general a transient elevation in temperature, usually 10-15 °C above optimal, is considered heat shock or heat stress. Plants typically die when exposed to high temperatures (44-50 °C). Heat stress due to increased temperature is an agricultural problem in many areas in the world. Transitory or constantly high temperatures cause an array of morpho-anatomical, physiological and biochemical changes in plants, which affect plant growth and development and may lead to a drastic reduction in economic yield. High temperatures can cause considerable pre- and post-harvest damages, including scorching of leaves and twigs, sunburns on leaves, branches and stems, leaf senescence and abscission, shoot and root growth inhibition, fruit discoloration and damage, and reduced yield. The plants stunted and if such a condition persists for a long period the plant is killed. High temperature causes coagulation of proteins, ammonia poisoning and disruption of protoplasmic structure and the protoplasm is killed. However, some plants tolerate high temperatures. Bacteria have been found growing in thermal springs and boiling springs in New Zealand at 101 °C.

Adaptations:

Dry seeds and spores, which have very low water content, are more resistant to heat injury than water soaked ones. High sugar content also increases the heat resistance in plants. Plants with thick bark can tolerate high temperature. Plants are bound to places. They, therefore, have to be considerably more adaptable to stressful environments and must acquire greater tolerance to multiple stresses than animals and humans. This is shown very clearly by the limitations in the distribution of particular types of vegetation, for example, the tree line on mountains. Extremely high light intensities, mechanical stress by wind, frequent periods of frost, and a winter period of many months have left their marks on these spruces and pines at about 3000 m altitude on Mt. Hood in Oregon (USA).



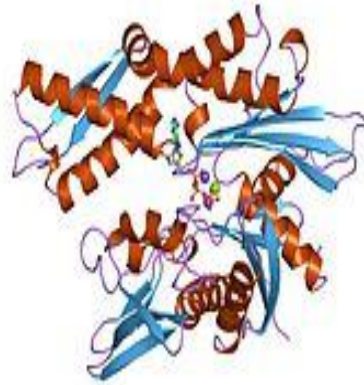
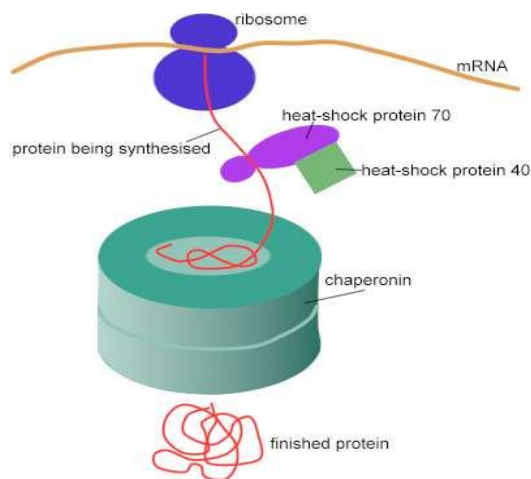
Heat Shock Protein:

Heat shock proteins are a class of functionally related proteins involved in the folding and unfolding of other proteins. Their expression is increased when cells are exposed to elevated temperatures or other stress. HSPs are found in virtually all living organisms, from bacteria to humans. Heat shock proteins function as intra-cellular **chaperones** for other proteins. Heat-shock proteins are named according to their molecular weight e.g. Hsp60, Hsp70 and Hsp90 (the most widely-studied HSPs) refer to families of heat shock proteins on the order of 60, 70, and 90 kilodaltons in size, respectively. Hsp70 was originally discovered by **FM Ritossa** in the 1960s. The Hsp70s are an important part of the cell's machinery for protein folding, and help to protect cells from stress. Hsp70 proteins can act to protect cells from thermal or oxidative stress. Hsp70 seems to be able to participate in disposal of damaged or defective

proteins. Hsp70 not only saves important components of the cell (the proteins) but also directly saves the cell as a whole.

Production of high levels of heat shock proteins can also be triggered by exposure to different kinds of environmental stress conditions, such as infection, inflammation, exercise, exposure of the cell to toxins (ethanol, toxic chemicals, particularly heavy metals such as arsenic, cadmium, copper, mercury, and ultraviolet light, among many others), starvation, hypoxia (oxygen deprivation), nitrogen deficiency (in plants), or water deprivation etc. As a consequence, the heat shock proteins are also referred to as stress proteins.

Housekeeping Heat-shock proteins also occur under non-stressful conditions, simply "monitoring" the cell's proteins. Some examples of their role as "monitors" are that they carry old proteins to the cell's "recycling bin" (proteasome) and they help newly synthesised proteins fold properly.



Effect on membrane: Molecules of biological membrane lose their chemical bond, integrity and functions due to heat stress. Decrease cell membrane thermo stability.

Salinity (More dissolved salts):

Salinity is the saltiness. Salt stress occurs mainly due to the presence of excess amount of sodium chloride, magnesium, calcium sulphate, bicarbonate, potassium etc in plant body. It causes dehydration of cell, changes in shape of cell and disturbance in metabolic processes. Thus cell growth and development of plants are retarded. Salinity is major threat for agriculture.

When there is a high concentration of Na^+ (Sodium) in soil, it is referred to as '**Sodicity**'. When Cl^- (Chloride) or other salts are involved, it is referred to as '**Salinity**'. There are two types of salinity:

1. Dry land salinity (occurring in non-irrigated).
2. Irrigated land salinity

Effect of salinity:

- 1) Invisible injuries: changes in bio membrane, enzyme activity etc.
- 2) Visible injuries: chlorosis, curling, necrosis, tissue damage and protein synthesis decline etc.
- 3) Plants and soil organisms killed.
- 4) It causes dehydration of cell, turgor losses change in shape of cell and disturbance in metabolic process.
- 5) Retarded growth, development, reproduction and productivity are severely affected.
- 6) Floristical changes on the level of population.
- 7) Ion toxicity: resulting from excessive uptake of less demand elements mainly chloride and sodium.



Chloride toxicity symptoms in avocado leaves; sodium toxicity in banana leaves, and Chloride 'tip burn' symptoms in citrus leaves.

Strategies to Resist Salinity:

1. Strategies include using molecular markers and genetic transformation as tools to develop salinity tolerant genotypes.
2. Use of potassium chloride (fertilizer), murate of potash.
3. Irrigation.

Chapter-9 Plant Defense Mechanism

How a pathogen fails to infect the host plant? In general plants defend themselves either

- i) By structural characteristics that act as physical barriers and inhibit the pathogen from gaining entrance and spreading through the plant. or
- ii) By means of biochemical reactions taking place in cell/tissues to produce chemical substances that inhibit pathogen.

Two things are relevant here. One, both structural and chemical defence may be used against pathogen at any stage of infection i.e. during prepenetration, or penetration or post penetration. Second, each of two lines of defence i.e. structural and chemical may be pre-existing (passive) existing before infection or active i.e. originating in response to infection.

I. Pre-existing structural and biochemical defense:

1. **Pre-existing defense structures:** The plant surface is the first line of defense. Such structures include:

- i) **Amount and quality of wax over the cuticle:** Waxes on leaf and fruit surfaces form a water repellent surface, thereby preventing the formation of a film of water on which pathogens might be deposited and germinate (fungi) or multiply (bacteria). A thick mat of hairs on a plant surface may also exert a similar water repellent effect and may reduce infection.
- ii) **Thickness of the cuticle:** A thick cuticle may increase resistance to infection in disease in which the pathogen enters its host only through direct penetration.
- iii) **Structure of the epidermal cell walls:** The thickness and toughness of the outer wall of epidermal cells are apparently important factor in the resistance of some plants to certain pathogens. Thick, tough walls of epidermal cells make direct penetration by fungal pathogens difficult or impossible.
- iv) **Size, location and shapes of stomata and lenticels:**
- v) The presence of **thick walled cell, trichomes** and other such structures on plant surface that may hinder the pathogen's advance.

2. **Pre-existing biochemical defence:** Several pre-existing lines of biochemical defense are as follows:

- i) **Inhibitors released by the plant in its environment:** Several inhibitory substances are secreted from the surface of roots as well as shoots of plants. Some of the compounds released by certain kinds of plants, however seem to have an inhibitory

action against certain pathogens. Fungitoxic exudates on the leaves of some plants e.g. tomato and sugar beet seem to be present in sufficient concentration to inhibit the germination of spores of fungi *Botrytis* and *Cercospora* respectively. Similarly, in the case of onion smudge, caused by the fungus *Colletotrichum circinans*, resistant varieties have red scales and contain, in addition to the red pigments, the phenolic compounds protocatechuic acid and catechol. In the presence of water drop or soil moisture containing conidia of the onion smudge fungus on the surface of red onion, these two fungitoxic substances diffuse into the liquid, inhibit the germination of the conidia, and cause them to burst, thus protecting the plant from infection. Both fungitoxic exudates and inhibition of infection are missing in white-scaled, susceptible onion varieties.

ii) Inhibitors present in plant cells before infection: Some plants are resistant to disease caused by certain pathogens because of one or more inhibitory antimicrobial compounds known as phytoanticipins, which are present in cell before infection. A variety of inhibitory substances may exist in the plant cells. These include chlorogenic acid (higher conc. in tubers of potato resistant to *Streptomyces scabies*, and in roots of potato resistant to *Verticillium*). Several phenolic compounds, tannins, and some fatty acid like compounds such as dienes which are present in high concentrations in cells of young fruits, leaves or seeds have been proposed as responsible for the resistance of young tissues to pathogenic microorganisms such as *Botrytis*. Strawberry leaves naturally contain (+) – catechin, which inhibits infection by *Alternaria alternata* by blocking the formation of haustoria. Several other types of preformed compounds such as the saponins, tomatin in tomato and avenacin in oats not only have antifungal membranolytic activity, they actually exclude fungal pathogens that lack enzymes (saponinases) that break down the saponin from infecting the host.

iii) Defense through lack of essential factors:

a) Lack of recognition between host and pathogen: Plants of a species or variety may not become infected by a pathogen if their surface cells lack specific recognition factors that can be recognized by the pathogen. If the pathogen does not recognize the plant as one of its host plants, it may not become attached to the plant or may not produce infection substances such as enzymes, appresoria, penetration pegs and haustoria necessary for the establishment of infection.

b) Lack of nutrients essential for pathogens: These may be several chemical factors necessary for the formation of an effective structure by the pathogen. In the absence of such factors, pathogens fail to develop. For instance, *Rhizoctonia* to infect a plant

needs a substance necessary for formation of a hyphal cushion. *Venturia inaequalis* needs a growth factor.

- c) **Absence of common antigens:** Plants do not produce antibodies against invading pathogens. However, a given variety may not have an antigen, present in particular race of pathogen. Thus variety becomes resistant to that race.

II. Induced Structural and Biochemical Defences Mechanism:

Recognition of the pathogen by the host plant:

Early recognition of the pathogen by the plant is very important if the plant is to mobilize the available biochemical and structural defenses to protect itself from the pathogen. The plant apparently begins to receive signal molecules i. e. molecules that indicate the presence of a pathogen, as soon as the pathogen establishes physical contact with the plant.

Pathogen elicitors: Various pathogens, especially fungi and bacteria, release a variety of substances in their immediate environment that act as nonspecific elicitors of pathogen recognition by the host. Such nonspecific elicitors include toxins, glucoproteins, carbohydrates, fatty acids, peptides and extracellular microbial enzyme such as protease and pectic enzymes.

1. Structures formed in response to infection: Some physical barriers may develop in response to infection and these affect their entry into the host. Some of these actively formed structures are as follows:

i) Histological barriers: these include the following:

a) Cork layers: Some bacteria and fungi and even some viruses and nematodes stimulate the host to form multilayered cork cells beyond the point of infection. These develop as a result of stimulation of host cells by substances secreted by the pathogens.

b) Abscission layers: these develop on young active leaves of stone fruits infected by any of several fungi, bacteria, or viruses. Such layers developing at the point of infection ultimately help discarding the infected area along with a few healthy cells from the host part.

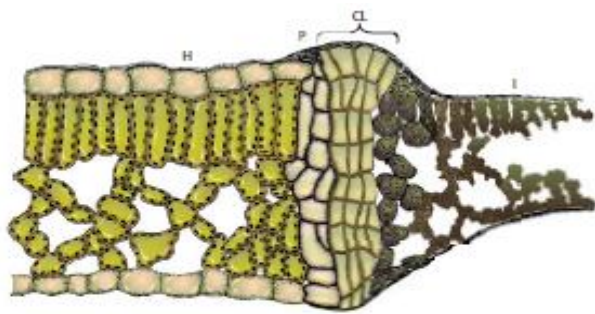


FIGURE 6-5 Formation of a cork layer (CL) between infected (I) and healthy (H) areas of leaf. P, phellogen. [After Cunningham (1928). *Phytopathology* 18, 717-751.]

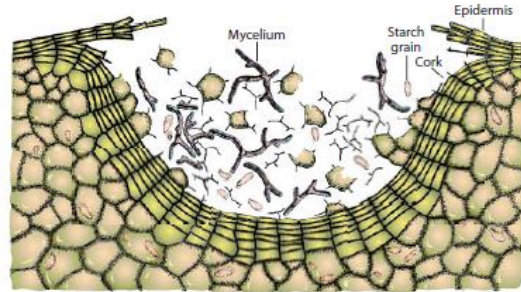


FIGURE 6-6 Formation of a cork layer on a potato tuber following infection with *Rhizoctonia*. [After Ramsey (1917). *J. Agric. Res.* 9, 421-426.]

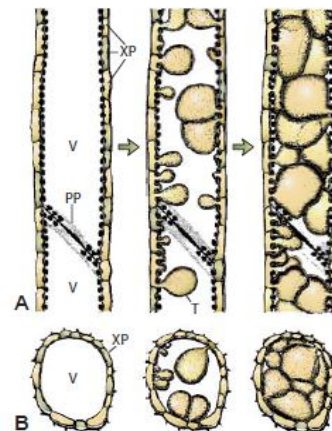
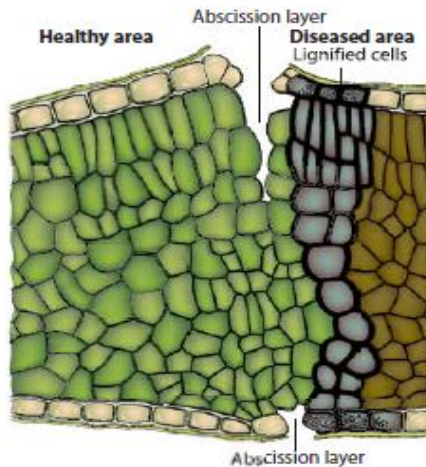


FIGURE 6-8 Development of tyloses in xylem vessels. Longitudinal (A) and cross section (B) views of healthy vessels (left) and of vessels with tyloses. Vessels at right are completely clogged with tyloses. PP, perforation plate; V, xylem vessel; XP, xylem parenchyma cell; T, tylosis.

c)Tyloses: The outgrowth of the protoplasts of adjacent live parenchyma cells protruding into x ylem vessels through pits, are known to develop under stress and in response to invasion by vascular pathogens.

d) Gum deposition: Plants produce variety of gummy substances around lesions in response to injury or infection. These are common in stone fruits. They are difficult to be invaded.

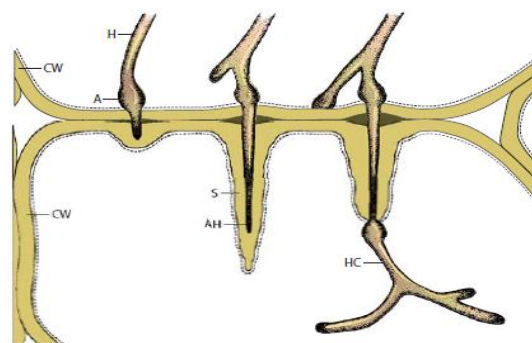
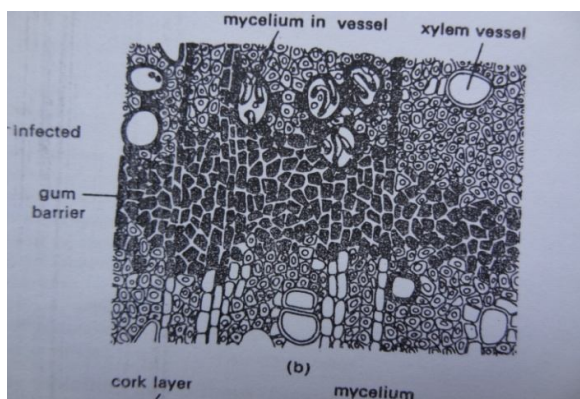


FIGURE 6-4 Formation of a sheath around a hypha (H) penetrating a cell wall (CW). A, appressorium; AH, advancing hypha still enclosed in sheath; HC, hypha in cytoplasm; S, sheath.

ii) Cellular structures: These involve changes in the cell wall morphology, or other structures derived from the cell wall of the cell being invaded. These include:

- a) Swelling of cell wall of epidermal and subepidermal cells
- b) Sheathing of hyphae penetrating a cell wall by enveloping them in a sheath formed by extension of cell wall inward in a way that surrounds and precedes the invading hypha.

iii) Cytoplasmic defence reaction: The plant cell cytoplasm surrounds the clump of hyphae and the plant cell nucleus is stretched to the point where it breaks in two. In some cells the cytoplasmic reaction is overcome and the protoplast disappears while fungal growth increases. In some of the invaded cells, the cytoplasm and nucleus enlarge. The cytoplasm becomes granular, dense and various particles or structures appear in it. Finally, the mycelium of the pathogen disintegrates and the invasion stops.

iv) Hypersensitivity: In some host-pathogen combinations, the nucleus moves towards the invading point and soon disintegrates. Resinlike granules develop in the cytoplasm, resulting in the death of the plant cell. This leads to degeneration of the pathogen structure and a fungal hypha.

2. Biochemical defence:

i) Active biochemical defence: These defence mechanisms develop in response to invading pathogen. A range of biochemical inhibitors and a range of biochemical processes are induced in the plant by the attacking pathogen. These are as follows:

- a) **Phenolic compounds:** These compounds are widespread in plants, healthy as well as diseased. However, their synthesis or accumulation is accelerated following infection. Such compounds may be called common phenolics. Certain other phenolics, not

present in healthy plants but are produced upon stimulation of plant by a pathogen or by a mechanical or chemical injury. Such compounds are known as phytoalexins.

- i) **Common phenolics:** These include chlorogenic acid, caffeic acid, scopoletin etc.
- ii) **Phytoalexins:** In general, these are not produced by healthy plants but are produced by plants following infection, injury, or at least stimulation by certain fungal, but not bacterial secretions. Muller and Borger (1940) first used the term phytoalexins for fungistatic compound produced by the plant in response to injury (mechanical or chemical or infection). Phytoalexins can be regarded as fungitoxic substances which arise from metabolic interaction of host and parasite and which are inhibitory to microorganisms attacking plants. Phytoalexins have been demonstrated in a number of plants as pea, sweet potato, barley, carrot.

Phytoalexin	Host	Pathogen
Pisatin-	<i>Pisum sativum</i> -	<i>Sclerotia fructicola</i>
Rishitin-	potato tuber-	<i>Phytophthora infestans</i>
Cicerin-	<i>Cicer arietinum</i> -	<i>Ascochyta rabiei</i>
Trifolirhizin-	<i>Trifolium pratense</i> -	<i>Helminthosporium turcicum</i>

Resistance: The ability of an organism to exclude or overcome completely or in some degree, the effect of a pathogen or other damaging factor. Different plants are resistant to certain disease for various reasons. Some plants are immune to particular pathogen even under most favourable conditions for disease development. Others exhibit some degree of resistance to a pathogen under most environmental conditions. Others are actually susceptible to the pathogen but under the conditions they are normally grown, may appear resistant.

Truly resistant varieties are actually those in which the pathogen and the host are incompatible with each other, or the host plant can defend itself against the pathogens by the various defense mechanisms activated in response to infection. Resistant may be-

- i) **Vertical resistant:** Resistance of a plant to a pathogen is provided by one or a few defence mechanisms controlled by one or a few genes, respectively and is either monogenic (one gene) oligogenic (a few genes), and the genes responsible for it are called major genes.
- ii) **Horizontal resistant:** A plant variety is provided by a combination of lesser defense mechanisms, each of which alone is rather ineffective against the

pathogen. Such mechanisms are controlled by a group or groups of complementary genes. It is almost always polygenic and the genes are called minor genes. Thus with horizontal resistance a given variety of plant is more or less uniformly resistant to all races of a pathogen.

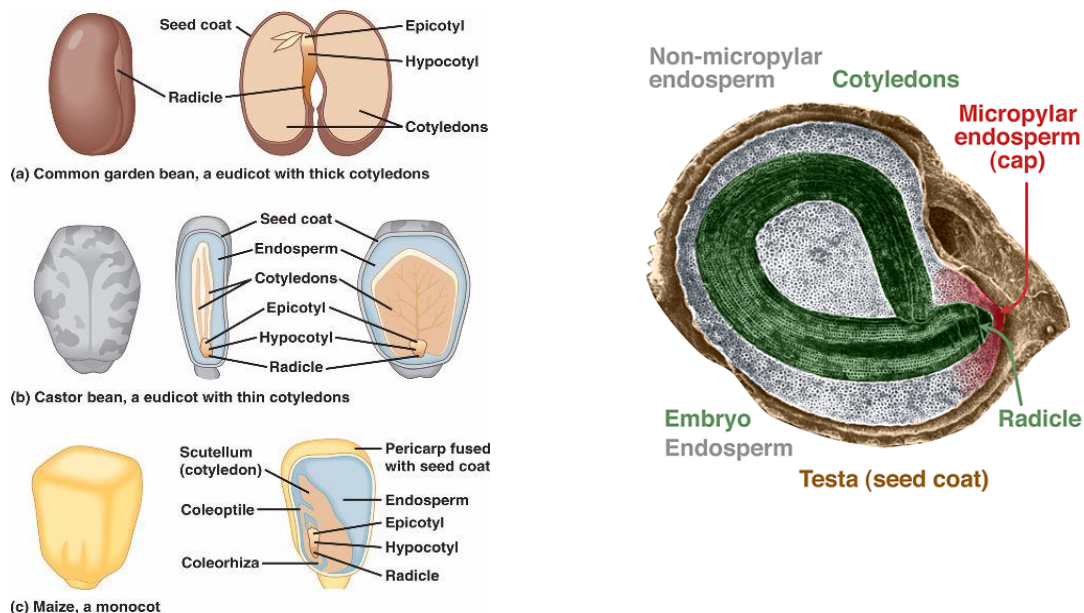
Susceptibility: The inability of a plant to resist the effect of a pathogen or other damaging factor.

Chapter-10 Dormancy and Seed Germination

Seed: Biologically, a seed can be defined as a ripened ovule consists of an embryo and a stored food supply both of which are enclosed in a seed coat or covering. A seed may be dicotyledonous or monocotyledonous. In dicotyledonous seed, the endosperm is absent while in monocotyledonous seed, the endosperm is present at maturity.

Germination:

The most common example of germination is the sprouting of a seedling from a seed of an angiosperm or gymnosperm. Germination is the growth of an embryonic plant contained within a seed; it results in the formation of the seedling. The seed of a vascular plant is a small package produced in a fruit or cone after the union of male and female sex cells. All fully developed seeds contain an embryo and, in most plant species some store of food reserves, wrapped in a seed coat. Some plants produce varying numbers of seeds that lack embryos; these are called empty seeds and never germinate.



Germination can be defined as a series of events, which take place when dry quiescent seed imbibes water resulting in an increase in metabolic activity and the initiation of the seedling from the embryo. In most cases, radicle emergence from the seed coat is the first visible sign of seed germination. The process of germination and seedling growth has been summarised in four events i.e.) i) imbibitions ii) hydration and enzyme activation iii) enzymatic degradation

of storage products and their subsequent transportation iv) radicle emergence and seedling growth.

1. **Imbibition of water (water uptake):** Imbibitions is the first phase of germination. After hydration, several enzymes reactivate due to water availability.
2. **Hydration and enzyme activation:** Hydration of seed causes formation or activation of various enzymes like lipases, proteinases, phosphatases, hydrolases etc. are either activated or *de novo* synthesized.
3. **Enzymatic degradation of storage products:** germination and seedling emergence include three types of changes inside the seed i.e.
 - i) Breakdown of reserve material in the seed
 - ii) Transport of breakdown products from one part of the seed to another
 - iii) Synthesis of new material from breakdown products.

Carbohydrate, fats and proteins are digested to produce ATP which is converted to ADP to release energy for biological activities.

Stages of Seed Germination

There are basically three steps of seed germination:

1. Water imbibitions results in rupture of seed coat, uniform imbibitions is important and approximately optimum temperatures are required
2. The imbibitions of the seed coat results in emergence of the radicle and the plumule, the cotyledon gets unfolded. It is important that the temperature and photo period are required in optimum amounts
3. This makes the final step in the germination of the seed where the cotyledons are expanded which are the true leaves.

Germination pattern:

The initial growth of seed follows two patterns i.e. epigenous or hypogenous.

In **epigeal germination**, the hypocotyl elongates and brings the cotyledons above the ground e.g. bean. In **hypogeal germination**, the epicotyl emerges and the cotyledons remain below the soil surface e.g. maize, cereals etc.



Germination of Seeds

Factors affecting germination:

Seed germination depends on both internal and external conditions. The most important external factors include **temperature, water, oxygen** and sometimes **light or darkness**. Various plants require different variables for successful seed germination. Often this depends on the individual seed variety and is closely linked to the ecological conditions of a plant's natural habitat. For some seeds, their future germination response is affected by environmental conditions during seed formation; most often these responses are types of seed dormancy.

1. Water: Water is required for germination. Mature seeds are often extremely dry and need to take in significant amounts of water, relative to the dry weight of the seed, before cellular metabolism and growth can resume. Most seeds need enough water to moisten the seeds but not enough to soak them. The uptake of water by seeds is called imbibition, which leads to the swelling and the breaking of the seed coat. When seeds are formed, most plants store a food reserve with the seed, such as starch, proteins, or oils. This food reserve provides nourishment to the growing embryo. When the seed imbibes water, hydrolytic enzymes are activated which break down these stored food resources into metabolically useful chemicals.

2. Oxygen: Oxygen is required by the germinating seed for metabolism. Oxygen is used in aerobic respiration, the main source of the seedling's energy until it grows leaves. Oxygen is an atmospheric gas that is found in soil pore spaces; if a seed is buried too deeply within the soil or the soil is waterlogged, the seed can be oxygen starved. Some seeds have impermeable seed coats that prevent oxygen from entering the seed, causing a type of physical dormancy which is broken when the seed coat is worn away enough to allow gas exchange and water uptake from the environment.

3. Temperature: Temperature affects cellular metabolic and growth rates. Seeds from different species and even seeds from the same plant germinate over a wide range of temperatures. Seeds often have a temperature range within which they will germinate, and they will not do so above or below this range. Many seeds germinate at temperatures slightly above (16-24 °C), while others germinate just above freezing and others germinate only in response to alternations in temperature between warm and cool. Some seeds germinate when the soil is cool -2 - 4 °C, and some when the soil is warm 24-32 °C.

4. Light or darkness: Light or darkness can be an environmental trigger for germination and is a type of physiological dormancy. Most seeds are not affected by light or darkness, but many seeds, including species found in forest settings, will not germinate until an opening in the canopy allows sufficient light for growth of the seedling.

Dormancy:

The failure of seeds to germinate even under favourable conditions is termed dormancy or Dormancy is a state in which seeds fail to germinate despite conditions of moisture, temperature and oxygen are favourable. The seed is called dormant and the phenomenon is termed dormancy.

Factors responsible for dormancy:

1. Hard seed coat:

Hard seed coat prevents entry of water and oxygen and thus prevents the germination of seed. Seed coat is very common in species belonging to the family Fabaceae. A structure called **strophliar plug** which occurs within the strophliar cleft of the seed coat does not allow the entry of water or oxygen.

i) Physical dormancy (impermeable seed coat): Seed coat dormancy is caused by hard seed coat which is impermeable to water and sometimes oxygen. Physical dormancy is the result of impermeable layer(s) that develops during maturation and drying of the seed or fruit. This impermeable layer prevents the seed from taking up water or gases. As a result, the seed is prevented from germinating until dormancy is broken.

Physical dormancy has been identified in the seeds of plants across 15 angiosperm families including: Anacardiaceae, Bixaceae, Cannaceae (monocot), Cistaceae, Cochlospermaceae, Convolvulaceae, Cucurbitaceae, Dipterocarpaceae, Geraniaceae, Leguminosae, Malvaceae, Nelumbonaceae, Rhamnaceae, Sarcolaenaceae, Sapindaceae

Physical dormancy has not been recorded in gymnosperms.

ii) Mechanical dormancy: In certain wild plants the hard seed coat acts as a physical barrier to the expansion of the embryo. In *Amaranthus* the seeds are readily permeable to water as well as oxygen but the resistant seed coat would not allow the embryo to germinate.

iii) Immature embryo: In certain species of Orchidaceae and Orobanchae and Ranunculus, the seeds are liberated at stage when the embryo is not fully developed. Dormancy of this type is natural consequence of the presence of an incompletely embryo in the seed and, therefore, the embryo should be allowed to complete development in an environment, which is favourable for germination.

2) Chemical dormancy:

Seeds of certain plants contain compounds which inhibit their germination. Such natural germination inhibitors have been found in the pulp of juice of fruits, seed coat, endosperm and embryo, etc. some of these inhibitors are ABA, ammonia, coumarin and parasorbic acid.

3) Physiological dormancy:

Physiological dormancy prevents embryo growth and seed germination until chemical changes occur. These chemicals include inhibitors that often retard embryo growth to the point where it is not strong enough to break through the seed coat or other tissues. Physiological dormancy is indicated when an increase in germination rate occurs after an application of gibberellic acid (GA_3) or after dry after-ripening or dry storage.

4) Photodormancy or light sensitivity affects germination of some seeds. These photoblastic seeds need a period of darkness or light to germinate. In species with thin seed coats, light may be able to penetrate into the dormant embryo. The presence of light or the absence of light may trigger the germination process.

5) Secondary dormancy:

Secondary dormancy occurs in some non-dormant and post dormant seeds that are exposed to conditions that are not favourable for germination, like high temperatures. It is caused by conditions that occur after the seed has been dispersed.

Methods of breaking the Seed dormancy:

The dormancy of seeds, though very useful for man, is not liked by the farmers who would like the seeds to germinate soon after they have been harvested. The methods employed vary from species to species depending upon the cause of the dormancy.

1. Scarification: One of the methods for breaking the seed coat is known as impactation.

The seeds are thoroughly shaken within a container. The strophilar plugs get

removed and germination takes place e.g. *Trigonella arabica*, *Crotolaria egyptica* etc. Scarification is a treatment by which the hard seed coat is ruptured or weakened. Several kinds of mechanical scarification are possible viz, threshing of the seeds by machine, rupturing the seeds with a hammer, cutting the seed coat by scapel etc. Chemical scarification by keeping the seeds in strong acids or certain organic solvents for some time helps in breaking the dormancy.

2. **Low temperature (chilling):** In many woody and in certain herbaceous species the dormancy can be broken by chilling treatments, just above freezing (0-5 °C).

It should be noted that while in some species it is only the intact seed which require a chilling treatment, in many other species the embryo shows dormancy, which has to be overcome by chilling.

3. **Alternating temperature:** Dormancy may be broken by exposing them alternately to low and high temperatures. Care is taken to see that the difference between the two extremes of temperature is not more than 10° or 20 °C.

4. **Light:** seeds whose germination is affected by light are termed **photoblastic**. The positively photoblastic seeds overcome their dormancy when they are exposed to light. The germination of a comparatively small number of negatively photoblastic seeds is inhibited by exposure of light.