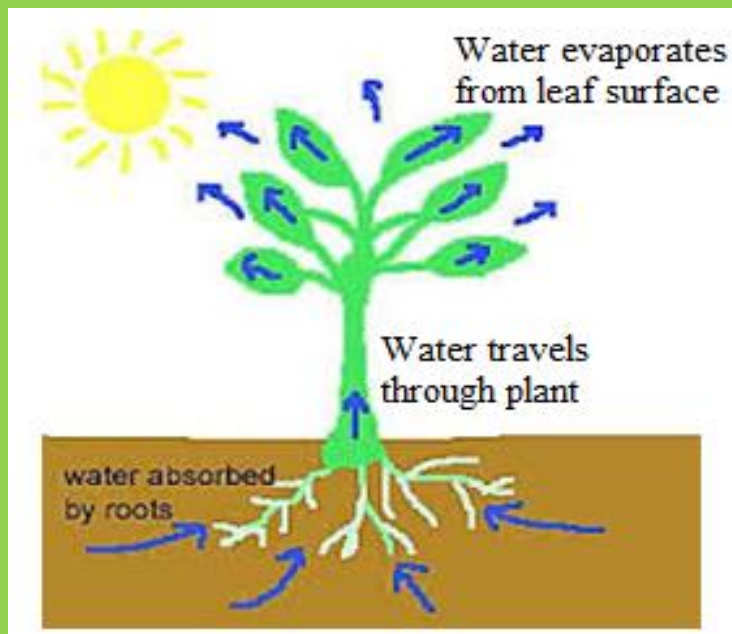




BOT(N)-302 & BOT(N)-302L

B.Sc. VI Semester

PLANT PHYSIOLOGY AND BIOCHEMISTRY



**DEPARTMENT OF BOTANY
SCHOOL OF SCIENCES
UTTARAKHAND OPEN UNIVERSITY, HALDWANI**

PLANT PHYSIOLOGY AND BIOCHEMISTRY



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BLOCK-1: PLANT - WATER RELATIONSHIP

UNIT-1: ABSORPTION OF WATER AND ASCENT OF SAP

Content

- 1.1 Objectives
- 1.2 Introduction
- 1.3 Importance of water to plant life
- 1.4 Physical properties of water
- 1.5 Diffusion and Osmosis
- 1.6 Absorption of water
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- 1.8 Summary
- 1.9 Glossary
- 1.10 Self Assessment Question
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- 1.13 Terminal Questions

1.1 OBJECTIVES

After reading this unit students will be able:

- To study the plant water relations and physical properties of water.
- To study physical processes of water, diffusion, osmosis, absorption of water and factors affecting water absorption process.
- To study the ascent of sap and its mechanism.

1.2 INTRODUCTION

Water is an important factor for plant growth as it helps to fulfill all the vital activities of plants. Water is essential for photosynthesis, respiration, absorption of minerals and nutrients, metabolism and even to maintain the soil temperature too. Beside this, water is also important in various other processes too, as it helps in the germination of seeds and in the process of transpiration etc. Water helps a plant by transporting nutrients through the roots. Nutrients are drawn from the soil and used by the plant. Without enough water in the cells, the plants droop so water helps a plant stand. Water carries the dissolved sugar and other nutrients through the roots. Plants absorb water through their entire surface- roots, stems and leaves. However, the majority of water is absorbed by root hairs.

To maintain the level of water inside the plant cells, it is necessary, to loss excess water from plant cells either in the form of evaporation or through transpiration. Evaporation of water from leaves is primarily controlled by stomata, sometimes lenticels and pores also helps in this process. This shows that, plants have a strong and significant relationship with water. Plant water relation means plants control the hydration of their cells including the collection of water from the soil, its transport within the plants and its loss by evaporation from the leaves. Transpiration also includes a process called guttation, which is the loss of water in liquid form from the uninjured leaf or stem of the plant principally through water stomata known as hydathodes. Studies have revealed that about 10 percent of the moisture found in the atmosphere is releases by plants through transpiration.

1.3 IMPORTANCE OF WATER TO PLANT LIFE

Water is most important and prime factor for life processes, as life itself has been originated in an aqueous environment. In course of evolution it became fully dependent on water in a number of ways. Thus we can say that **water is the liquid of life or elixir of life**. In general water is essential for life and is the main constituent of the protoplasm comprising 90 to 95% of its total weight. In absence of water, protoplasm becomes inactive and even killed. Water is a source of hydrogen atoms for the reduction of carbon dioxide in the reaction of photosynthesis and as mentioned earlier that water helps to fulfill different vital activities. Beside this water present in the vacuoles which helps in maintaining the turgidity of cells which is essential for proper

activities of life. Due to absorption of water the cell becomes turgid. The turgidity of cell helps in the elongation of cells resulting in growth. It is a well-known fact that the availability of water during winter and summer season is different and is the reason of formation of annual rings in higher plants. In summer the turgidity is less and hence formation of small cells, comparatively to winter when due to higher turgidity, formation of larger cells takes place. In nature we have different type plants e.g. aquatic, terrestrial, halophytes, xerophytes etc. and different plants absorb water in different ways. While orchids, absorb moisture directly from the atmosphere and not from soil. Land plants get their water supply from soil which serves as the source of water and minerals to them. The way in which water from soil enters into roots, particularly to root xylem is called “**Mechanism of water absorption**”

1.4 PHYSICAL PROPERTIES OF WATER

As per studies on global water covers about 73% of earth's surface and provides the most extensive medium for all aquatic life because of its unique properties from ecological point of view. Water occurs in all three physical forms in the earth at moderate temperature. It is present in either in the form of fresh water or in saline water form in sea and salt lakes. The fresh water of active ground water, glaciers and ice caps, rivers, lakes dams, streams, soil moisture etc. represents only 1.92% of the total water stock. But even from this small segment as much as 98.65% is shared between active ground water and ice on mountain tops and poles, lakes and rivers constitute only 0.98% and 0.004% fresh water stock respectively.



Fig. 1.1 Different states of matter

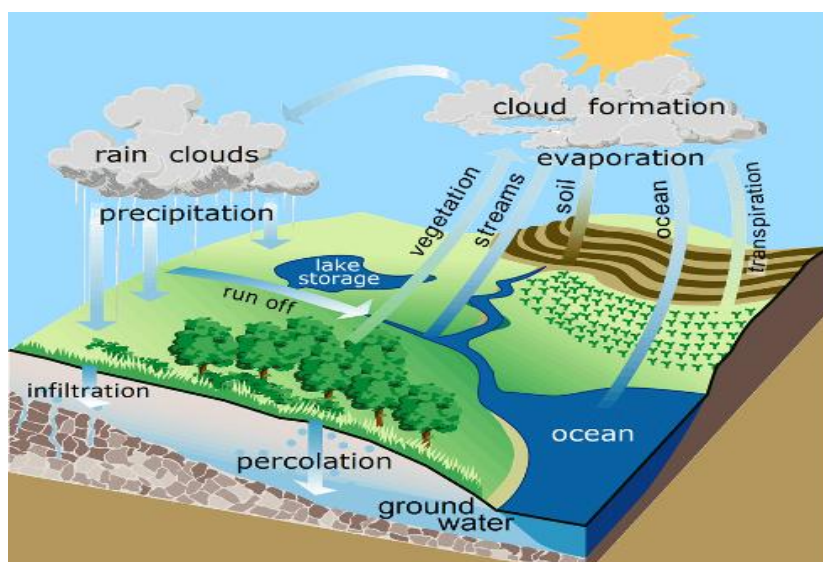
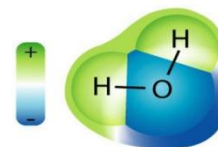


Fig.1.2 Water Cycle in the Atmosphere

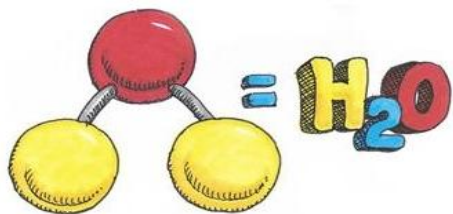
Water

Structure and Properties: Water comprises over about 90% of the chemical content of many organisms and so we can say justifiably that water is the fluid of life but before this it is necessary to understand the different physiological processes related to the diffusion and absorption of water, and fundamental chemical and physical properties of water and its interaction with other substances. Water participates in all metabolic reactions either directly or indirectly. Water is a remarkable compound with unique properties that results from its molecular configuration and hydrogen bonding.

Molecular Structure of Water: A single water molecule is composed of two hydrogen atoms bonded covalently to one side of an oxygen atom. Water absorbs large quantity of heat and tolerates other physical stresses without breakage of the bonds. Water is a polar inorganic compound at the room temperature at room temperature associates with each other because of the asymmetrical distribution hence due to these association i.e. adhesion or cohesion that are critical to the movement of water in soils and the translocation of water in plants.



The chemical composition of water in which the hydrogen atoms are attached to the oxygen atom causes one side of the molecule to have a negative charge and the area in the opposite direction to have a positive charge causes molecules of water to be attached to each other forming strong molecular bond. The strong hydrogen bond of water molecule results due to the



attraction of the positive hydrogen bond of water molecule for the negative oxygen atom of another water molecule. The resulting polarity of charges causes molecules of water to be attached to each other forming strong molecular bond. Water is a tasteless, odorless liquid at ambient temperature and pressure and appears colorless in small quantities, although it has its own intrinsic very light blue hue.

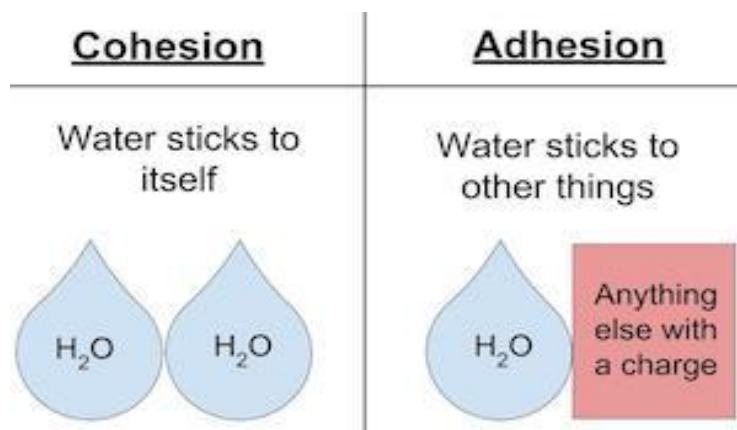
The most important property of water to the living cell is its solvent action. Water is referred as the 'universal solvent' because it forms a solution with a vast array of compounds. The solvent action of water is of tremendous importance for the living plants as all the essential elements necessary for normal plant growth and the compounds necessary for energy transfer and storage require water as a translocation and reaction medium. All these materials are present in water in the dissolved form this form, distributed throughout the plant. Thus all the physiological processes i.e. diffusion, osmosis, and imbibition are intimately associated with the essential function of translocation of water and solutes from site of origin of site of activity.

Adhesive and Cohesive Forces of Water

Water is attracted to many other substances because of its polar nature. This attraction between unlike or different molecules is called adhesion. In case of water, it involves hydrogen bonding between the water and other molecules. While the attraction of like molecules due to hydrogen bonding, is called cohesion. Cohesion force allows water to be pulled to the tops of trees through xylem elements in the form of a thin film of water. The cohesion between water molecules creates surface tension and then the molecules at the surface of a liquid are continually being pulled into the liquid by the cohesive (hydrogen bond) forces. This type of adhesion is called capillary action. Capillary action in a glass tube is caused by adhesive forces exerted by the internal surface of the glass exceeding the cohesive forces between the water molecules themselves. Due to surface tension a single drop of water works as spherical. The surface tension of water is high than that of most other liquids and the surface tension plays a great role in the physiology of plants.

There are various factors determined the rate of diffusion i.e. temperature, relative density, concentration gradient, concentration medium etc. the rate of diffusion increases as the temperature increases. Hydrogen diffuses 4 times faster as far as Oxygen and 5 times as fast as carbon dioxide, these rates are determined by the relative intensity of the gas. The gas diffuses through gases, liquids and solids, liquid diffuses through gases, liquids and solids and likewise solid diffuses through gases, liquids and solids. In some cases the rate of diffusion may be very fast or may be very low as per condition. There important example of diffusion as follows-

Blowing of wind, dispersal of scent, perfumes & agarbatti in a room, intake of CO_2 and liberation of O_2 in photosynthesis, and intake of O_2 and liberation of CO_2 during respiration, dissolution of sugar and salt in water, dissolution of KMnO_4 particles in water all are examples of diffusion. Some other interesting examples of diffusion are-

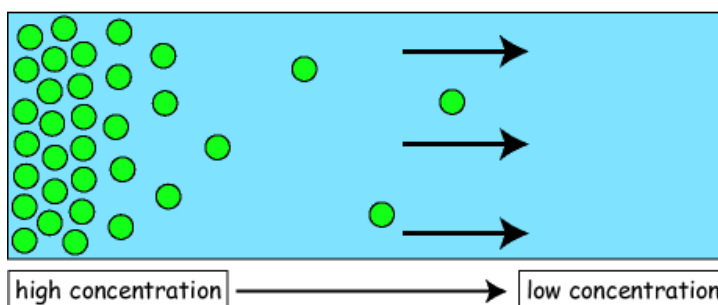


i) Gas into liquid- Foam, ii) Liquid into gas- Clouds, iii) Solid into solid- Smoke, iv) Solid into solid- Diffusion of copper into zinc and zinc into copper, although this process takes pretty long time, if the basis of two metals are kept upon one another.

Most animals and plants contain more than 60% water by volume. Water H_2O is a polar organic compound that is at room temperature a tasteless and unique liquid. In nature water can naturally occur in these as a liquid (water), solid (ice), and gaseous form (water vapour). Water is transported in plants through both cohesive and adhesive forces. These cohesive forces are related to water's property of adhesive forces on the attraction between water molecules and other molecule. Strong bonds determine almost every physical property of water and many of its chemical properties. This property of water of water allows for the transport of nutrients vital to life in animals and plants.

Diffusion: The diffusion means to spread; to flow out, to extend Diffusion can be simply defined as the movement of particles of matter due to their kinetic energy or the net movement from one point to another because of the random kinetic activities of molecules or ions is called diffusion. Diffusion refers to the process by which molecules intermingle as a result of their kinetic energy of random motion. However, the direction of movement of diffused particles is from the region of higher concentration to the region of lower concentration till both the concentrations equalize. The molecules in the region of higher concentration contain more kinetic energy and that is why they allow fast movement. The diffusion of particles still continues in both the directions though it is not detectable. Diffusion is random movement of molecules but has a net direction towards regions of lower concentration in order to reach equilibrium. Simple and passive diffusion occurs when small molecules pass through the lipid bilayer of a cell membrane.

Diffusion



● solute

Solute transport is from the left to the right;
movement of the solutes is due to the concentration
gradient (dC/dx).

In a solution the diffusion of particles of one substance is quite independent of the diffusion of particles of another substance. The diffusion of particles of both the substances is quite independent in the rate of flow of the particles as well as the direction. Each substance differs according to its own concentration and flow. There are various factors determined the rate of diffusion i.e. temperature, relative density, concentration gradient and concentration medium etc. Rate of diffusion of substances increases as the temperature increases.

The gases diffuse through gases. Liquids and solids; liquids diffuses through gases, liquids and solids and likewise solids diffuses through gases, liquids and solids. In some cases the rate of diffusion may be either very fast or very slow as per the condition. Hydrogen diffuses four times faster as far as oxygen and five times as fast as carbon dioxide, these rates are determined by the relative intensity of the gas.

Examples of diffusion are:

Gas into liquid- Foam,
Liquid into gas- Clouds,
Solid into gas-Smoke

Solid into solid- diffusion of copper into zinc and zinc into copper, although this process takes pretty long time, if the basis of two metals are kept one another.

Osmosis: A plant cell has a cell membrane and cell wall as its boundary. The cell wall is freely permeable to water hence it is not buried to movement of water. Osmosis is the net movement of solvent molecule through a semipermeable membrane into a region of higher solvent concentration to the region of lower solvent concentration in the direction that tends to equalize

the solute concentration on the two sides. If two solutions of different concentrations are separated by a semi-permeable membrane which is permeable to a small solvent molecules but not to the larger solute molecules than the solvent will tend to diffuse across the membrane from the less concentrated to more concentrated solution. Osmosis is essentially a special type of diffusion of liquids. In simple words, osmosis may be considered as diffusion when two solutions of different concentrations are separated by means of a semi-permeable.

The diffusion of water or in other words solvent from the solution of lower concentration to the solution of higher concentration until both the concentrations equalize. This process is defined simply as “the phenomenon, whereby, when a solution is separated from a weaker one by a semi-permeable into the stronger solution diffuses through the membrane into the stronger solution in an effort to equalize the strength of the two solutions”. Actually the diffusion of particles of solvent takes place both ways across the semi-permeable membrane but the diffusion of solvent is more from the solution of lesser concentration to that of the higher concentration.

The main difference between diffusion and osmosis is that in osmosis two substances are separated from each other by a semi-permeable membrane while in case of diffusion it is absent. The word semi-permeable membrane is used for a membrane that allows the passage for certain substances while checking the passage of others.

Osmosis Phenomenon: To explain osmosis if two different solutions of different concentration are separated by a semi-permeable and will not allow or permit soluble molecules to pass through it. As per the laws of diffusion the movement of solvent molecules will be from the region of higher concentration to the region of lower concentration or from dilute solution to concentrated solutions. The reason behind this is that the concentration of solvent molecules will lower in the concentrated solution and higher in the dilute solution. On the basis of concentration of solute molecules the solutions may be defined as hypertonic and hypotonic solutions.

Hypertonic Solution: A hypertonic solution is one in which the concentration of solutes is greater inside the cell than outside of it, while hypotonic solution: A hypotonic solution is one where the concentration of solute is greater outside the cell than inside it. When a cell is immersed into a hypertonic solution, the tendency is for water to flow out of the cell in order to balance the concentration of the solutes. Likewise, the symbol of the cell is conversely categorized as hypotonic, opposite of the outer solution hypotonic refers to a lower concentration.

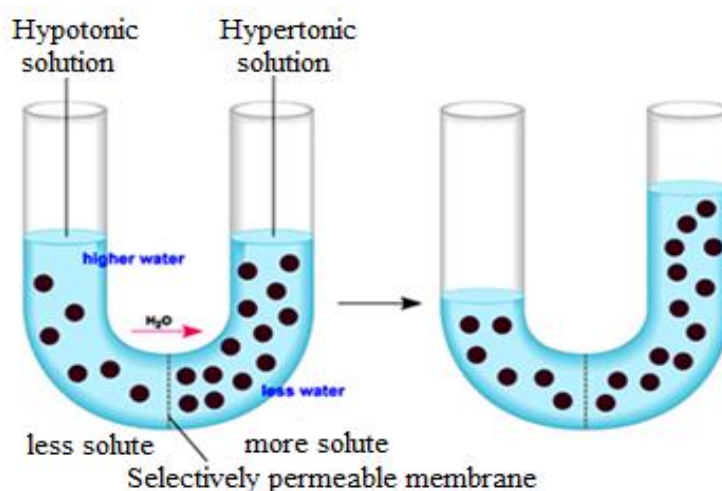


Fig.1.3 Demonstration of Osmosis Phenomenon

Exosmosis: Osmosis towards the inside of a cell or vessel or the flow of a substance from an area of lesser concentration to one of greater concentration, while the movement of water molecules from outside to inside of a cell through osmosis is known as **endosmosis**.

Or the process by which water molecules move out of the cell is called **exosmosis**. A solution is a mixture of two or more than two substance in which the concentration of the solute in solvent may be expressed as weight of solute per unit of solvent or in terms of molarity, normality or equivalent to this.

Osmotic Pressure or Osmotic Potential: If in a chamber a solute is added to water, so to keep the pressure constant an amount of water removed and concentration of water in the solution is decreased. If this solution is separated from pure water by a semi-permeable membrane a gradient of water potential is created and osmotic diffusion of water starts taking place from the chamber of higher water potential to that of lower water potential. If a mechanical pressure is applied to lower water solution and is gradually increased it will raise the water potential of the solution until the gradient no longer exists, this solution is separated from pure water by semi-permeable membrane and stop osmosis by applying the pressure. This pressure is termed the “**osmotic pressure**” and is usually expressed by the symbol π . Thus the osmotic pressure is defined as the excess hydrostatic pressure which much be applied to it in order to make its water potential equal to that of osmotic water.

The unit of osmotic pressure is measured in bars (a unit of the metric system, where 1 atm = 1.01 bars). The osmotic pressure of a solution is proportional to its molecular concentration. This osmotic pressure will be directly proportional to the molar concentration, if the solute is a non-electrolyte because the osmotic pressure depends upon the number of solute particles present in it. The electrolyte solution exerts a greater osmotic because greater part of the electrolyte is in an ionized conditions within a solution and addition of number of ions causes a higher osmotic

pressure. Temperature directly affects the osmotic pressure and increase in temperature result an increase in the osmotic pressure.

Water of Hydration: The water associated with the particles of hydrophilic solutes or colloids is known as water of hydration. An ideal molar solution at 0°C has an osmotic pressure of 22.4⁰ C atmosphere. Osmotic pressure increases at higher temperature. Modern workers have substituted the term osmotic pressure by osmotic potential is numerically equal to the osmotic pressure but is negative in sign, which indicates decrease in pressure that occurs due to addition of the solute. When we add more solute the osmotic potential increases more negatively, but dilution of solution with the solvent decreases the value of the osmotic potential. In a solvent the value of osmotic potential is zero.

Turgor Pressure and Wall Pressure: The pressure which develops in a cell from time to time due to the osmotic diffusion of water is called the turgor pressure. Unlike the osmotic pressure, the turgor pressure is also variable, which is always constant. During endosmosis in a cell, water enters inside the cell and due to this the turgor pressure increases gradually till it reaches its maximum when it is equal to osmotic pressure, provided the other liquid with which it is in a state of equilibrium is pure water or pure solvent. Turgor pressure is responsible for the growth of the young cells while in mature cells it develops a pressure of equal and opposite force known as 'wall pressure'.

Rate of Diffusion: Thus the rate of diffusion will be faster as the temperature increases. When a substance is diffusing between two compartments the greater the concentration differences between the two components, the substance will diffuse.

Factors affect the rate of Diffusion: Several factors affect the rate of diffusion of a solvent including the mass of solvent, the temperature of the environment, the solvent density, and the distance travelled etc.

Diffusion Pressure Deficit: The term diffusion pressure deficit or DPD was coined by B S Meyer, (1938), originally DPD was coined as suction pressure by Renner (1915). It is an older term that was used in place of water potential. The difference between diffusion pressure of pure water and solution is called diffusion pressure deficit (DPD). When a plant cell is hypotonic solution water enters into a cell by osmosis and as a result turgor pressure develops. The cell-membranes get stretched and osmotic pressure of cell decreases. Likewise gases and solutes, liquids also have a diffusion pressure. A pure solvent may have maximum diffusion pressure, when certain solute particles are added to the pure solvent, the diffusion pressure of the resulting solution is lowered. The amount, by which the diffusion pressure of a solution is lower than that of its solvent at the same temperature and atmospheric pressure is called the diffusion pressure deficit or DPD.

When a solvent is separated from a solution, the solvent molecules will diffuse towards the solution under a pressure which is being higher in concentration than in solution. This pressure is known as diffusion pressure deficit or we can say that this movement is due to certain deficit in the diffusion pressure of the solution as compared to solvent or may be due to diffusion pressure deficit (DPD) of the solution. In case of DPD the solution will always try to wipe off this deficit by pulling or sucking more of solvent molecules or DPD is also known as the index of sucking power or the suction pressure. Initially the DPD of the solution is equivalent to its osmotic pressure (OP). During osmosis in a cell, due to increasing pressure forces the cytoplasm out against cell wall. Since the cell wall is rather rigid and thus it will exert an equal and opposite pressure towards outside, and this is called wall pressure (WP). WP, results decrease in DPD. This WP may be shown as written as follows-

$$\text{DPD (SP)} = \text{OP} - \text{TP (WP)},$$

DPD= diffusion pressure deficit.

SP= suction pressure, OP= osmotic pressure

TP= turgor pressure, WP= wall pressure

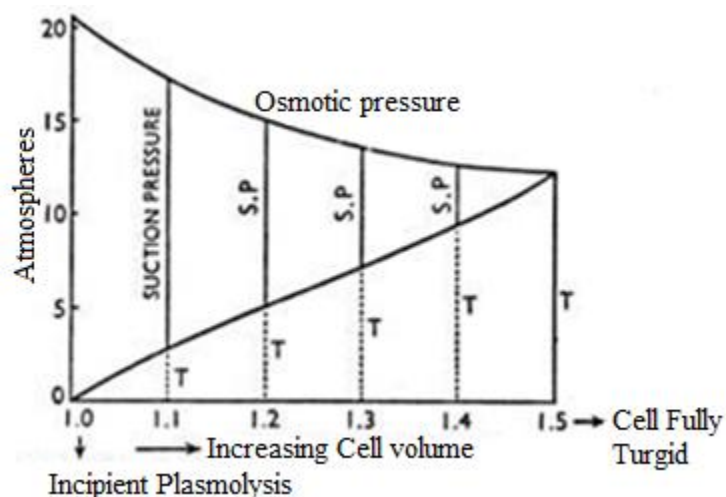


Fig.1.4 Diagram showing relationship of osmotic pressure (OP), turgor pressure (TP), and diffusion pressure deficit (DPD)

Plant Cell- Asan Osmotic System: The first cell of the plant which absorbs water and acts like an osmotic system or as an Osmometer is the root hair. The big vacuolated plant cell is an osmotic system which acts as follows:

When root hair comes in contact with a solution with higher concentration of water molecules than that of cell sap, then the process of osmosis begins. Firstly, the water or solution of external solutions (higher concentration) begins to enter inside the cell through osmosis (endosmosis). The movement of water takes place due to concentration differences between external and internal solutions in which plasma membrane acts as a semipermeable membrane.

The wall of root hair works as a specialized wall and facilitates water absorption. The high efficiency of root hair as an absorbing organ is due to the fact that the soil particles are attached to its pectic substances and bringing the soil water very close to wall of the root hair. Besides the pectic substances, the root hairs also secrete carbonic acid capable of dissolving everything required by plants.

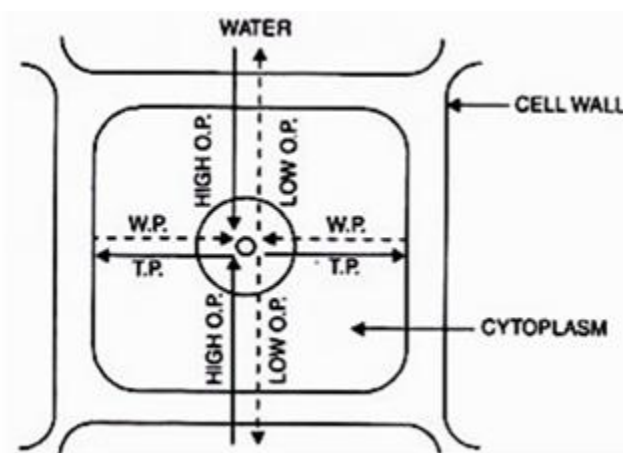


Fig.1.5 Different directions of the operating forces in a cell

Due to diffusion pressure deficit (DPD) water is absorbed by the root hair. The amount /quality of water absorbed by the root hair depends on deficit. If this deficit is greater, larger quantity of water will diffuse and greater amount of water will enter into the cell. The force per unit area of entrance of water is termed as suction pressure the potentiality of which depends upon DPD, the suction pressure that exists between the cell and environment. It can be said that-

$$\text{DPD} = \text{Osmotic pressure} - \text{turgor pressure.}$$

Entrance of water inside the cell affects it in two ways- i) it brings down the concentration of cell sap and ii) it stretches the elastic wall of the cell. This entered water results swelling of cell wall and causes a pressure known as turgor pressure. Due to this, cell increases in volume but due to elastic nature of the wall, offers resistance to this force. This resistance works in opposite direction to turgor pressure and is known as wall pressure. The wall pressure is exerted by wall in order to restore normalcy in size. The turgor pressure is fully turgid.

Water stops to enter or diffusion of water molecules in both the directions stops when the concentration of two solutions becomes equal, till the balance is fully stretched and thus entry of water is checked. At this stage cell is fully stretched and is said to be turgid. When osmotic pressure may exist the suction pressure will be zero in a turgid cell.

The entered water decreases simultaneously resulted in an increased turgor pressure and a decrease in osmotic pressure of cell sap. Due to this the concentration differences simultaneously and brings down the suction pressure. All forces are interrelated and work together.

In nature endosmosis is of common occurrence and is responsible for water absorption. Contrary to this natural process Exosmosis may also occur where the cell loses its water contact due to being placed in hypertonic (more concentrated) solution. Thus due to loss of water brings the turgor pressure of cell and protoplasm starts to withdraw from the cell wall. It tends to collect as irregular mass of Centre and resulted in the shrinkage of protoplast. This condition is known as **plasmolysis**. In case of extreme plasmolysis, the plant will die.

However, there are some exceptions also as mangrove plants grows in saline water, with very high salt concentration, which creates difficulties in absorption and the plants has to adopt accordingly. These mangrove plants are also known as Halophytes, has high osmotic pressure of the cell sap due to some reasons.

Soil Water: As mentioned earlier that generally plants absorb water from soil by their roots. Thus the water present in the soil either in the form of liquid or in vapour/moisture form, is known as “Soil Water” This soil water is the most important constituent of soil because it provides the medium for the absorption of minerals and organic matter by the roots and helps to activates various enzymes and metabolic processes. It also affects morphological, physiological and anatomical processes directly or indirectly. This water after absorption from roots later on reaches to the various other parts of the plant i.e. stem, leaves, flowers, fruits etc. So plants absorb water from their roots. The water is found in different forms in the soil. In soil the chief source of water is rain water. The total amount of water present in the soil is termed as “holard”, while the water among this, available to the plants is called “chesard” and the amount of water which cannot be absorbed by plants is termed as “echard”

After the rains, a part of water drains away and not available to plants it is known as “run-away water”. The water present in the soil may be classified as follows-

(i) Gravitational Water: The water which reaches deeply into the soil after rains due to gravitational force is called gravitational water. This water is not available to plants and percolates downwards through large pores between the soil particles under the gravity and hence is called gravitational water. It reaches the low water table in the earth.

(ii) Capillary Water: The water which remains present in the intercellular spaces of soil particles. This water is available to plants, which fills the spaces between then on- colloidal particles or it forms a thin film around the non- colloidal small particles of soil. This water is present in the capillaries of soil particles thus known as capillary water and one of the most important and significant form of water which is held by capillary forces and absorbed by the plants, or the water that is left in soil along with hygroscopic moisture and water vapours after the gravitational water has drained off is known as capillary water.

(iii) Hygroscopic Water: The water which is present around the soil particles in the form of thin vapour layer and held tightly by soil particles of colloidal complex due to adhesive force. This water is also non available water and cannot be drawn/absorbed by the plants.

(iv) Chemically Combined Water: The water which remains chemically bound to the soil particle is called chemically combined water. This water is also non- available to plants.

(v) Run- off Water: The water which run- off down through slopes after rains is called running water or run- off water. This is also non- available to plants.

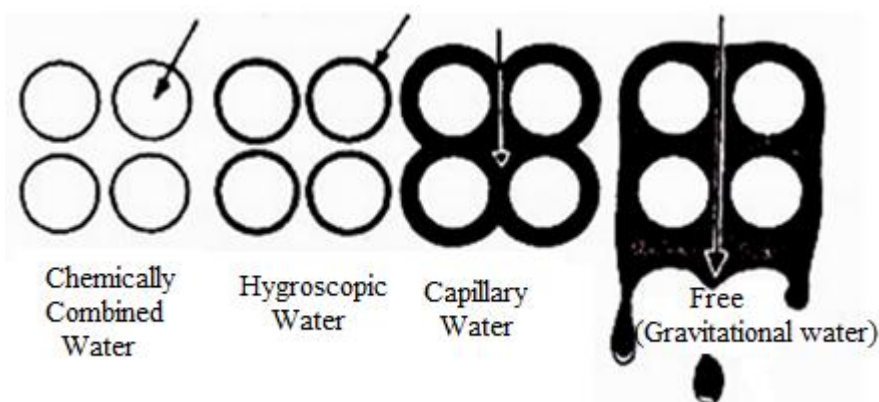


Fig. 1.6 Different types of water available in soil

A good amount of water is retained by the soil particles, is known as “Field Capacity”. Beside this, the movement of water is another important aspect regarding to soil moisture. Thus the movement of water into soil is called infiltration and the downward movement of water within the soil is called percolation, permeability or hydraulic conductivity. Pore space in soil (differs from soil to soil) is the conduit that allows water to infiltrate or percolate. Water differs in a fully saturated soil the percentage of moisture from soil to soil and depends upon the size of particles, e.g. 5% in sandy soil, 35% in loamy soil, 45% in clay soil and negligible in gravel and rocks.

Water Holding Capacity: In a fully saturated soil the percentage of moisture is held in the form of thin water film. It is known as water holding capacity. The maximum quantity of capillary water + Hygroscopic water + Chemically combined water in the soil represents the water holding capacity of that soil.

Wilting Co- Capacity: The percentage of water that remains in a soil when permanent wilting is attained is called wilting co- efficient or permanent wilting percentage.

On the basis of above studies it is clear that plants have a specific relationship with water. Hence there is a strong plant water relationship which means how plants control the hydration of their cells, including the collection of water from the soil, its transport within the plants and its loss by evaporation from the leaves. This water status of plants is usually expressed as water potential, which has unit of pressure is always negative and in simple form is algebraic sum of the hydrostatic pressure and the osmotic pressure of water. Flow of water through plant and soil over microscopic distance is driven by gradients in hydrostatic pressure over microscopic distances (e.g. across semi-permeable membrane). It is driven by gradients in water potential.

Field Capacity: The field capacity of any soil is the percentage of water present in the soil and remains after the gravitational water is drained away. Thus field capacity of any soil is the sum of the capillary water, hygroscopic water and chemically combined water or-

Field capacity= Capillary water+ hygroscopic water + chemically combined water.

Water Absorbing Parts of the Plants: Plant water relations concern how plants control the hydration of their cells, including the collections of water from the soil, its transport within the plant and its loss. Among them the first step is the absorption of water. Plants absorb water through their entire surface- roots, stems and leaves. However, the majority of water is absorbed by roots through root hairs. Root hairs are thin- walled, unicellular outgrowths of epidermis are in close contact with thin film of water surrounding the soil particles. The area of young roots where, most absorption takes place is the root hair zone. So a major portion of water required by plants is absorbed by the roots but exceptionally the absorption of water by leaves and stem has also been reported in a few plants. In some plants e.g. *Lycopersicum*, Beta, Sorghum and Phaseolus the water is absorbed by leaves. However, the epiphytes like orchids, which possess arid roots containing velamen tissue layer. The cells of this layer are hygroscopic in nature and absorb water or moisture present in the atmosphere. The following affects water absorption by leaves-

- a) Structure and permeability of cuticle and epidermis.
- b) The hairiness of leaf surface.
- c) Lack of water in the cells of cortex.
- d) The internal environmental deficiency of water in parenchyma cells close to epidermis.

Roots play the principle and significant role in absorption of water and due to this reason even the orchids develop modified roots for the absorption of water from atmosphere. In general, it is common knowledge that the root system of the plant is mainly responsible for the absorption of water (Meider, 1954) or moisture from humid air (Arvidson, 1950). Some of the plants have long and deep- seated root system while others have shallow roots and are spread out just below the surface of the soil. The deep feeder roots of the former type are in contact with large and permanent supply of underground water at different levels while the surface feeder roots of latter type are in contact with after a rainfall. Root system also affects the water absorption. Those plants which possess hairy and well developed root system show higher rates of water absorption comparatively to those which possess very small roots and less number of root hairs.

Roots: Roots absorb water mainly from its apical region on the basis of apical organization of roots- it shows three clear demarcations or zones-

- i) Zone of meristematic cells.
- ii) Zone of elongation and
- iii) Zone of absorption or differentiation.

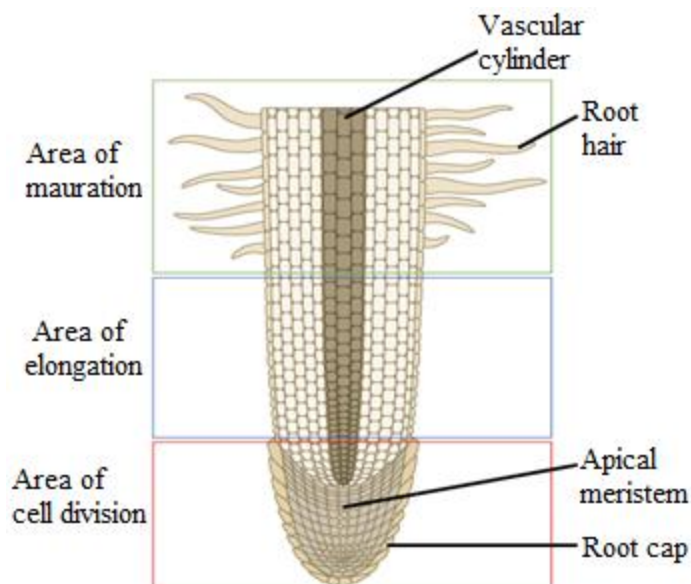


Fig.1.7 L.S. of apical region of root showing organization and differentiation of different parts

Although all the zones are important yet the third zone or zone of absorption is prime importance and it consists of three different types of tissue system i.e. outer most- dermal layer, the middle- cortical layer and the inner one is stellar region. Dermal tissue includes surface layer of cells. The epidermis in this region/ zone has enormous number of unicellular root hairs. The root hair projects almost at right angles to the long axis of proliferous cell. This generally less than one millimeter to about a centimeter in length and is about 10µm in diameter. The presence of large number of root hair on roots increases considerably the exposed surface for the absorption of water. Cortical tissue is simple and commonly consists of parenchyma cells while the stellar tissue system is complex and consists of pericycle, phloem and xylem etc.

Root Hairs: The absorption of water takes place in the terminal portions of roots but the maximum absorption takes place in the zone of root hairs, 1 to 10 cm behind the root tip. Kramer (1959) observed that the maximum intake of water occurs in the root hair zone. The cell wall of a root hair has two layers. The outer layer is composed of pectic compounds due to which soil particles are very closely adhered to the cell wall. The inner layer is made up of cellulose. Inside the cell wall, a thin layer of cytoplasm is present, which is continuous with cytoplasm of the piliferous layer of which the root hair is a part

Root hair is the special modified cell of epidermis for the absorption of water. Root hairs are thin- walled, unicellular outgrowths of epidermis. It is specialized root only in appearance but also in its internal structure. The wall of root hair consists of cellulose and pectic substances. Both these substances are highly hydrophilic in nature. These substances have a great capacity for water absorption. The root hair zone of root is the most effective region of water absorption. Each root hair zone has thousands of root hairs. Root hairs are specialized for water absorption. The cytoplasm encloses a central vacuole which contains cell sap. The cell wall has a great

property of imbibing water and is also permeable to it. The cytoplasm layer acts as a differentially permeable membrane.

The cell wall of root hairs acts as permeable layer next to cell wall is the plasma membrane enclosing cytoplasm, nucleus and a large vacuole, which is quite large and occupies most of the part of the cell and to give a peripheral arrangement to cytoplasm. This vacuole acts like controller during absorption of water. Endodermis layer also plays important role during water absorption by root hairs. It is a bounding layer of the central stele and in endodermis cells having a suberin and lignin thickening form “casparian’ strip’. While certain cells of endodermis lies opposite to protoxylem lacks such thickness or they are thin walled. Such cells are called ‘passage cells’, as they provide passage for the movement of water from cortex to stele.

Xylem: Xylem is the tissue most involved in water translocation. Xylem comprising several different types of cells living and non- living tissue. Xylem is the complex tissue and consists of tracheids, vessels, xylem fibres and xylem parenchyma of which the former two are thick walled due to lignin deposition. Of these the tracheary elements are the most prevalent and practically all water translocation takes place through these cells. The vessel elements and tracheids are the cells of xylem, most involved with water translocation. In many cases pits are present on the radial walls where lignin deposition is little and hence these are thickening areas helps to facilitate the movement of water. Beside the pits, the thickening of wall does not interfere in the movement of water because lignin is a hydrophilic substance and quite easily permits the easy movement of water through it. Another specified and modified cells for the movement of water are- vessels. Since their transverse walls are perforated, thus a continuous water column is maintained in the plant.

Thus water enters first to the cell wall of root hairs and the intercellular spaces of the root cells from the soil. The free space, where which water can easily reach or available is called “apparent-space”. This absorbed water then diffuses into the vacuole through the differently permeable membrane and the tonoplast. In plant cell the plasma membrane, the cytoplasm and tonoplast together act as a semi- permeable membrane.

Mechanism of Water Absorption: Entry of Water in Root Hair: Now the question arises that how water enters in the root hairs. Root hairs take active participation in water absorption as the root hair maintains contact with soil water and in nature it acts as a sole water absorbing organ. Due to diffusion pressure deficit gradient, water diffuses into the root hair. The cell sap contains a more concentrated solution than the water present outside the cell. Water enters inside the root hair, when the DPD of cell sap is greater than the osmotic pressure of solute. To know the exact mechanism of water absorption two main theories are proposed by the workers-

- (i) Active absorption
- (ii) Passive absorption

It was Renner (1912, 1915) who for the first time recognized the water uptake mechanism into active absorption and passive absorption. According to him the forces

responsible for the intake of water develop in the living cells of the roots and therefore, roots are actively concerned in the process. On the other hand during passive absorption the intake of water is controlled by the transpiration process of the plants and the roots simply acted as a passive absorbing surface.

Active Absorption: Water absorption takes place due to the activities of root, while shoot does not concern any affair. To explain the mechanism of active absorption various theories have been put forward by scientists/ workers from time to time.

There are two major theories to explain the active absorption of water-

- (a) Osmotic theory of active absorption and
- (b) Non- osmotic theory of active absorption

(a) Osmotic Theories: According to osmotic theories it is assumed that water moves by diffusion along a gradient of decreasing free energy or diffusion pressure (DP). If the xylem sap has a higher osmotic potential than that of the soil solution water can move from the soil into the xylem by osmosis.

For the first time Atkins (1916) and Priestley (1922) postulated the osmotic theory of absorption and according to them the water is absorbed due to osmotic differences between soil water and cell sap. The main cause of water absorption is the difference in osmotic pressure of soil water (remains below 1 atm.) and cell sap (generally 2 atm.) which may react up to 8 atm. Thus because of this difference, absorption of water takes place directly and requires no expenditure of metabolic energy. As per studies the value of osmotic pressure of the cell sap of the root hairs is generally 2 atm. and maybe as high as 2 to 8 atmospheric pressure in some cases. The osmotic pressure of the soil water is much lower comparatively to cell sap and generally less than 1 atm. The higher diffusion pressure deficit (DPD) of the cell sap of the root hair causes endosmosis of soil water across the semi- permeable plasma membrane. A number of investigators have supported the osmotic pressure theory, yet there are some objections also-

Objections: However, there are some problems too as according to this theory differential osmotic pressure is the sole movement of water from the cortex to xylem cells takes place. Xylem contains comparatively dilute sap and is metabolic inactive. This dilute sap of xylem has a low osmotic pressure (less than 2 atms.) as against several atm. of the cortical cells. The tracheids- membrane are not semi- permeable. Thus water absorption is not possible unless some other mechanism exists. Although in this process energy doesn't require but for the maintenance of solution these cells must require some energy yet this theory was not accepted.

(b) Non-Osmotic Theories: Thimann (1951), Boger & Prell (1953) and Kramer (1959) postulated that there is non- osmotic active water uptake mechanism in plants. Hence absorption of water takes place due to a concentration gradient. This active absorption of water will obviously require energy expenditure released from the metabolic activities (i.e. respiration etc.)

of the root cells or it could be explained simply that the absorption or uptake of water is an active process and occurs due to non- osmotic reasons even against diffusion pressure gradient. This process requires expenditure of energy obtained from physical activities i.e. photosynthesis etc. This theory is supported and accepted by most of the investigators on following points-

- i) In non- aerated soils such as flooded areas wilting of roots occurs.
- ii) Role of water absorption reduces when we use the respiratory inhibitors such as KCN while exudation from the stem started. Thus there is some correlation between these two processes.
- iii) The water absorption is also affected by hormones such as auxin concentration increases water uptake and exudation, water uptake is stimulated by auxin etc.
- iv) There is a distinctive diurnal variation in water uptake and root pressure supports living activities.
- v) Water absorption process thus it is a vital occurs only in living cells activity.

Passive Absorption: This theory believed that the forces responsible for absorption of water into the roots are governed by other cells. The governing force originates or develops in the cells of transpiring shoots rather than in the roots. The governing forces developed due to the process of transpiration. Due to the occurrence of transpiration, the DPD of leaf cells increases which results in the movement of water from the xylem cells to adjacent mesophyll cells. Hence presence of a continuous column of water from leaves to roots through xylem channel and simultaneously the deficit is transmitted to the xylem of roots through endodermis to cortex. A gradient of DPD develops across the cortex to root hair along which radial movement of water gradient takes place and it puts these cells under tension. This creates the tension of several atmospheres in the xylem comparatively to other root cells with comparatively low pressure and this puts the root in tension. The question arises that how the movement of water takes place from cortical cells to xylem tracheids, which is supported by mass flow of water against diffusion.

In support of this theory, several evidences have been put forward by different workers and reveals that the rate of water absorption is approximately equal to that of transpiration. Thus it is revealed that the water is absorbed through the roots and pulled up to the transpiring (leaf) surface and not pumped into the plants by roots. It has been further supported by following points-

- (i) Water uptake is a regulated process and the rate of absorption is under the control of transpiration, means transpiration regulated and controls the rate of water absorption in plants.
- (ii) The rate of absorption is almost equal to the rate of transpiration it means much as water transpired it creates a force/ gradients which helps in absorption.
- (iii) It is a purely physical process which plays important role i.e. physical environment (sunlight, temperature, wind etc.)
- (iv) Movement of water across cells can be much faster than the simple osmotic pressure.
- (v) The cut ends of stem are also capable of absorbing water in rapidly transpiring plants.

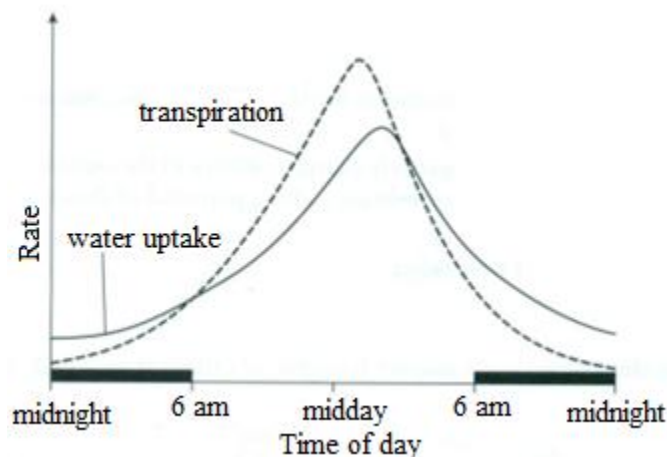


Fig.1.8 Graph showing transpiration-absorption ratio

Significant Points of Active and Passive Absorption: In water uptake process active and passive absorption are two different types of mechanism of which one is related to operate in plants. Further the famous root pressure theory, explained in next chapter ‘Ascent of Sap’ serves as the best evidence to support active absorption of water in plants. But on the basis of recent studies active absorption could not favor much owing the following reasons:

- (i) In very tall trees like gymnosperms, root pressure is not observed.
- (ii) Root pressure is also not observed in fast transpiring plants.
- (iii) There is a clear difference in the amount of water exuded by stems/ cut ends of stumps due to root pressure and that of lost by transpiration.
- (iv) The submerged hydrophytic plants, although roots are present, the water is absorbed from general surface.

Thus on the basis of these evidences it is observed that passive absorption accounts for most of the water absorption and if active absorption exists so either it cooperates with passive absorption or has no importance.

Pathway of Water across the Root Cells: The root hair absorbs water from soil and becomes fully turgid. Its diffusion pressure deficit falls. The absorbed water in the root hair moves to the cortex cells. As the adjoining cell of the cortex which has a much higher diffusion pressure deficit absorb water from the root hairs. These cortical cells are interconnected through the plasmodesmata. Then water moves from cell to cell from cortex to endodermis. The endodermis possess thick casparian strips, which cannot allow water but the thin walled cells or passage cells of endodermis helps to diffuse water. Young endodermal cells possess suberized thickenings on lateral walls, the casparian strips and old cells are completely suberized, hence water can pass only through passage cells present in between them. These facts favors the theory of water potential gradient. Earlier it was believed that water moves from the region of root hair to pericycle through osmotic system of high DPD present in cortical cells as compared to root hair cells but recent approach being different and does not believe in it.

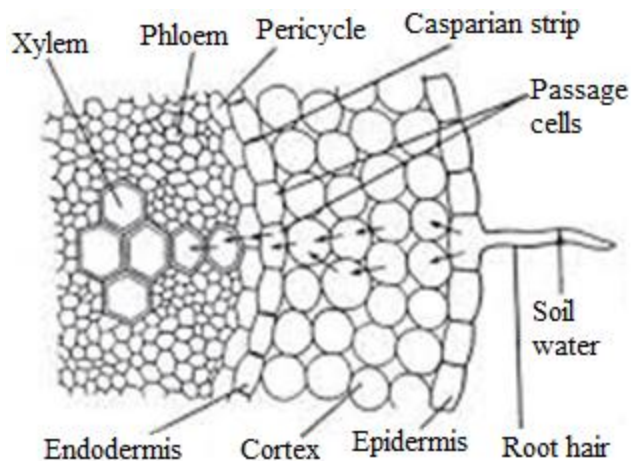


Fig. 1.9 T.S. of root (a portion) showing the direction of movement of water(radial movement) after absorbing from soil

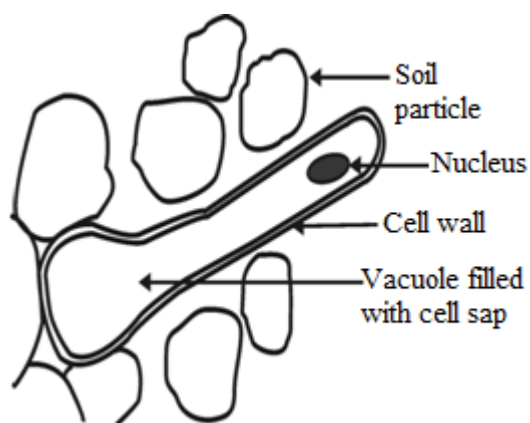


Fig.1.10 An enlarged root hair showing details of structure

Factors affecting water Absorption rate: Plants absorb water from soil through their root hairs. This phenomenon is affected by external as well as internal factors.

Plant metabolism, transpiration and root system are the internal factors while all other factors related to soil and its environment are categorized under internal factors.

(A) Internal Factors:

(a) Metabolism: Absorption of water and metabolism are closely related. However, it is not clear that during water absorption, plants use energy or not, yet the factors inhibiting rate of respiration i.e. poor aeration, application of KCN and anesthetics reduces the rate of absorption. Thus it reveals that the metabolic activities are expected to participate indirectly by forming a constantly elongated root system and always providing new constantly with soil water.

(b) Transpiration: Based on studies it is proved that the rate of water absorption is nearly directly proportional to that of rate of transpiration. The rate of absorption increases as the rate of transpiration increases because of cohesion theory of ascent of sap because transpiration

produces a tension or pull which is transmitted to roots through hydrostatic system of plants and creating a favorable or suitable conditions for the absorbing of water.

(c) Root System: Presence of a well- developed root system and a large number of root hairs are responsible for water absorption. The efficiency of water absorption directly depends upon the absorbing system or root system. While development of root hairs depend upon existing environment i.e. in moist conditions root hairs are well developed and in large quantity. The coniferous plants or gymnosperms although bears few or no root hairs but they absorb large amount of water with the help of micorrhizal hyphae. This shows that root hairs play significant role in water absorption.

(B) External Factors: The external factors includes those factors related to external environment of the plant and includes mostly related to soil these are- available soil water, soil temperature, soil aeration, concentration of soil.

(a) Available Soil Water: As studied earlier that water in the soil is available in different forms i.e. gravitational capillary, hygroscopic and chemically bound water. Among these only capillary water is available for the absorption in plants. The rate of absorption of water is generally uniform in the range of water between the field capacity and permanent wilting percentage. If the amount of water is increased beyond the field capacity then it creates a bad effect on soil aeration and also reduces the rate of water absorption as the aeration of soil badly affected. Similarly in extremely dry soil wilting is observed because dry soil water reduces the rate of absorption.

(b) Soil Aeration: In a well aerated soil water absorption takes at faster rate and it is greatly retarded in poorly aerated soil or oxygen deficit soil. Probably the reason behind this may be respiration as normally roots failed to respire anaerobically. The deficiency of O_2 inhibits the growth and metabolism of plants. The reduction in rate of water absorption is due to lack of O_2 and accumulation of CO_2 increases the viscosity of protoplast, decrease in the permeability of the cell membrane, reduction in the size and growth of roots, reduction in respiratory rate etc. These may be the reasons for plant death in flooded areas. Such soil is usually physiologically dry and is known as physiological dryness and not fit for water absorption.

(c) Soil Temperature: The variation of temperature affects the rate of absorption. Generally, maximum rate of water absorption recorded between 20° - 30° C. Thus the temperature between 20° - 30° C is the most suitable temperature for absorption. Beside this the low temperature reduces and moderate high temperature increases the rate of absorption. A very high temperature kills the protoplasm of cell and results the death of plant. The low temperature, rate of water absorption is affected directly or indirectly as follows-

1. Decrease in the concentration of protoplasm.
2. Reduced permeability of plasma membrane.
3. Reduction in the growth and length of soil water into roots.
4. Reduction in the metabolic activities of root cells.
5. Increased viscosity of water.

(d) Concentration of Soil Solution: A Large number of elements remains dissolved in soil water, known as soil solution. Due to the presence or absence of these elements the concentration of soil water changes. In highly concentrated soil solution it increases the osmotic pressure and when it reaches higher than the cell sap, water absorption does not take place. Because the osmotic pressure of any solution is directly proportional to its concentration. If soil water contains more salts (elements) and its concentration and the osmotic pressure will also be more. Due to this reason, the plants growing in alkaline and marsh soil absorb a very little or no water. This is also an example of physiological dryness.

Internal Environmental Factors: Internal environmental factors or commonly known as plant factors also affects the rate of absorption including following factors-

(i) Transpiration: The rate of absorption of water is nearly directly proportional to that of transpiration. An increased rate of transpiration increases the rate of absorption directly because of cohesion theory of ascent of sap i.e. due to transpiration a pull or tension transmitted to roots through hydrostatic system of plants and hence creating a suitable condition for entrance of water.

(ii) Absorbing Root Systems: The efficiency and amount of water absorption directly depends upon the root system or absorbing system. The presence of number of root hairs responsible for water absorption though depends on environment e.g. the xerophytic plant has well developed and extensive root system. Similarly development of root hairs depends on environment, as in maize plant produces large and well developed root hairs in soil but completely failed to produce the root hairs when grown in culture solution. Most of the gymnosperms or coniferous plants bear either few or no root hairs but absorb water with the help of mycorrhizal hyphae. Thus root systems play a major role in absorption of water.

(iii) Metabolism: The absorption of water and metabolism are closely related. The metabolic activities are expected to participate indirectly by forming a constantly elongated root system. The factors- poor aeration, application of anesthetics and KCN reduce the absorption rate, these factors inhibiting the rate of respiration, however, doubts exist in use of energy during absorption. The metabolic activities may be expected to participate indirectly by forming a constantly elongated root system and always providing new contacts with soil water.

(iv) Ascent of Sap: As described previously that the absorption of water is takes place by root hairs of the plant from where it reaches to xylem via cortical cells and passage cells. It reaches top to the plant through xylem and then it transpired by leaves and also used for other metabolic activities. From time to time by a number of experiments it has been demonstrated that xylem is the main water conducting tissue. Thus the upward movement of water from stem base to tree top is called *ascent of sap*.

The ascent of sap in xylem tissue of plant is the upward movement of water and mineral from the root to the crown is the complex tissue consisting of living and non- living cells. The conducting cells in xylem are non- living and include in various groups of plants, vessels

membranes and tracheids It is fascinating to understand how water moves in plants to such great height much as 100 feet or more. This process of upward movement of water and minerals are also known as translocation of water as well as Ascent of sap. Sometimes it covers a height of more than 90 metres against gravitational pull as in case of the biggest and tallest trees of the Australian Eucalyptus and Californian Sequoia. By adding the distance between root hairs and stem base, the actual distance travelled by water from root hair to the crown of the tree becomes much more than 90 metres. In fact, to lift the water column over 10 m is not possible because to pull the water up to this height needs more than one atmosphere, than to rise the water up to such height needs some special mechanism.

Mechanism of Ascent of Sap: To explain this mechanism a number of theories have been put forward from time to time by various scientists. Some of them considered the living cells as being actively involved in pumping water while others have explained the mechanism is purely a physical activity. The various theories can be broadly classified under three headings- (i) vital theories (ii) root pressure theories and (iii) physical force theories.

(i) Vital Force Theories: All those theories which considered the living cells to be responsible for the upward movement of water and minerals or ascent of sap are categorized under vital theories. Westerrmeir, Godlewski and Jones in 1880-84 stated that the living cells of a stem play a significant role in ascent of sap, according to them the living tissues involved in the ascent of sap.

Westerrmeir in 1883 stated that upward movement of water in the stem was affected in wood parenchyma and the tracheids and vessels also acted as water reservoir and not only as conducting tissue.

Godlewski (1884) put forward ‘Clambering’ (or relay pumping) theory to explain mechanism of ascent of sap. He proposed that living tissues in the xylem play an important role to bring about a pumping action of water in an upward direction and the xylem element i.e. tracheids and vessels acts as water reservoir.

Jones (1887) Supported Godlewski and showed that if the lower portion of a branch was killed the above leaves were affected within a few days.

Previously these theories have little experimental background but latter considerable experimental evidences were provided by Ursprung (1905-07), Edwart (1905-08) etc. in support of vital theories but these evidences were enables to convince many workers in the field.

Sir J.C. Bose (1923), the Indian scientist proposed “Pulsation Theory of Ascent of Sap” and observed pulsatory activities performed by the innermost cortical cells lying just outside the endodermis. According to him absorption of water from the water from the outside and again pump it into the vessels is furnished due to pulsatory activities of cells. The external factors such as temperature, poison, anesthetics etc. were also found pulsatory activities. Sir Bose applied his observations in explaining the phenomenon of ascent of sap.

Objections to vital Theories: To verify the vital theories Strasburger (1809) experimentally demonstrated that the ascent of sap can take place even in segments of xylem in which living cells have been already killed by heat or poison. According to him, oak tree aged 75 years of about 122 m in height was cut across at the base close to the ground and was placed in picric acid solution, which is normally poisonous for living cells. He observed that surprisingly the solution ascending slowly to the top of the tree killing all living cells in between solution. However, after three days, addition of Fuchsin with picric acid, shows the movement of this solution up to top of the tree, through all living cells were already killed.

Thus such experimental evidences rejected the vital theories on ascent of sap.

Root Pressure Theory: It is noted that if a plant stem is cut a few inches above from its base with a sharp knife, the xylem sap is seen flowing out through the cut end. This phenomenon is called “exudation or bleeding” This process of upward flow of water by Priestley. He proposed that this flow is due to a hydrostatic pressure developed in root system. He said that root pressure is assort of hydrostatic pressure which develops in the roots due to accumulation of absorbed water. The term root pressure was postulated by Stephan Hales (1727) and observed that water rise in a 8 mm diameter tube to a height of 63 metres, (in several days) connected to a cut stump of vine system.

According to her the origin of root pressure depends largely due to osmotic phenomenon because when the roots are watered with isotonic (equal0 or hypotonic (higher) concentration of solution. The observed average range of root pressure is usually between +1 or +2 bars.

The externally applied factors such as poison and oxygen supply affected the root pressure. These factors are also known to affect the release of energy during respiration of living cells either than affecting semi -permeability of protoplasm. This proves the involvement of living cells in the process and root pressure is the outcome of active participation of living cells of the cells. It is believed that in the process, the casparian’ strips of endodermal cells of the root help to present downward leakage of water and surrounding cells force water to enter in xylem vessels. Stocking (1956) believes the root presence to be an active process and defined it as “a pressure developing in the tracheary elements of the xylem as a result of metabolic activities of roots.

It is believed by some scientists that root pressure is responsible for during water to any height in the plant

Objection to root pressure theory: Today root pressure is considered to be of little importance in the ascent of sap. Some scientists- Kramer, Unger, Renner, Dixon &Jolly and Steward after analyzing the facts of root pressure theory have objected very strongly the involvement of root pressure in the ascent of sap. The main objections are as follows-

1. Root pressure has not been observed in all plants. In the plants of gymnosperms either no or little root pressure is found, which as a matter of fact have the tallest trees in the world.
2. Water continues to rise upward even in the absence of roots.

3. In plants, root pressure is always below 2 atmospheres and therefore, due to root pressure water can rise up to 21 m if all favorable conditions are available.
4. Root pressure is commonly observed during favorable periods of growth when the transpiration rate is low, whereas at the time of need when the rate of transpiration is high, root pressure is not observed.
5. The amount of water exuded by the root pressure is very low and constitutes about 5% of the total water lost during excessive transpiration.
6. The rate rise of water by root pressure is too slow to explain the actual rate of ascent of water in a plant.

Physical Force Theory: According to this theory it is believed that living activities or living cells are not involved in ascent of sap. It is purely a physical activity. To explain the mechanism of ascent of sap several theories have been proposed. Some important ones are as follows-

Capillary Theory: This theory was proposed by Boehm (1805), according to him “water rises in the narrow tubes due to surface tension while others suggested that water moves through the lumen of the tracheids and vessels as a result of capillary action was not perfect because in the smallest tracheid with 0.02 mm diameter rise of water can be affected only up to 150 m cm, while in larger vessels with 0.5 mm diameter, rise of water was recorded up to 6cm. Comparatively to this capillaries of 1µm diameter, water can be rise up to 29.95 metres which fall within the range of imbibitional absorption, but the resistance to flow becomes very high. The capillary flow theory faces the following objections-

- (i) The capillary works easily in narrow vessel plants but tall plants have rarely such vessels.
- (ii) The vessels with vessels of 0.03 mm. diameter water can rise only to a little more than one meter. This diameter is considered as an average and standard among the plants.
- (iii) Among gymnosperms, the tallest trees, in which vessels are absent and only tracheids are present, capillary cannot operate or capillary theory cannot be applicable due to the presence of end walls in tracheids.
- (iv) The soil water have not direct connection to vessels hence this theory cannot be applicable in vessel bearing plants.
- (v) To maintain the capillary a free surface must be present in the xylem vessel.

Imbibitional Theory: Unger (1868) was the pioneer worker of imbibitional theory of ascent of sap while later Sachs (1878-1879) advanced this theory and assumed that water moves upward in the stem through the wall of the xylem elements due to the process of imbibition. Hence the water movement through imbibition by colloids is extremely slow yet to carry water to any distance the imbibitional pressure (100-1000 atm is found quite adequate. After some time as it became evident, that water moves through the lumen of the xylem and not carry by walls. The imbibition theory was discarded. Moreover, the imbibitional movement of water has been found not only slow but negligible also.

Atmospheric Pressure Theory: According to this theory atmospheric pressure is responsible for ascent of sap. With the fall of atmospheric pressure at the transpiring surface due to the loss of water during transpiration, water rises upward to fill up the gap.

This theory was also rejected with the following objections-

- (i) For the proper functioning of atmospheric pressure it is essential to have free surface at the lower end of the plant which cannot be found in plants.
- (ii) For the application of this pressure vacuum at the transpiring surface is necessary. Thus it is not applicable because there is no vacuum is observed in the plants. However, even if complete vacuum is created at the transpiring surface, the maximum rise of water can be 10 m while certain plants are far more taller.

Transpiration Pull or Cohesion- tension Theory: This theory was proposed by Dixon and Jolly (1894) and has been supported by other workers too e.g. Curtis & Clark (1951), Milburn and Johnson (1960), Levitt (1960) etc. This theory is now popularly known with various names- such as Dixon and Jolly's theory of cohesion, Cohesion hypothesis, Cohesion-tension theory, Theory of cohesive force and transpiration pull theory. It is the most accepted theory.

What is Cohesion? Attraction between similar molecules is known as cohesion. The water molecules have strong mutual attraction known as cohesion force due to this strong force they cannot be separated easily from one another. The measured magnitude of cohesion force of water is up to 350 atm. which is sufficient for the ascent of sap in the tallest trees.

Cohesion- tension theory: Due to transpiration pull the water forms a continuous column from base of the plant to its top and remains under cohesion-tension. Thus the water is pulled up to the top of the tree according to the need of the plant.

Nature of Cohesion-tension theory: This most accepted and important theory has the following significant features.

- i) Water forms a continuous column from base of the plant to its top.
- ii) First of all due to transpiration water is lost from mesophyll cells and develops a pulling force. It puts these cells under tension.
- iii) This tension may cause a break in the water column but due to cohesive property of water molecules or due to tensile strength of the column the continuity of column is not broken.
- iv) This tension of transpiration pull is transmitted to the base or root region to regulate absorption.

Mechanism of ascent of sap: Due to transpiration, loss of water from the surface of the mesophyll cells of the leaf reduces the amount of water and resulted in the increase in osmotic pressure of these cells. Thus in mesophyll cell a reduced water potential is developed, i.e. DPD increases. The water is pulled from the adjacent cells and ultimately from the conducting tissue and as a result a pull is developed in the mesophyll cells and the xylem cells of the leaf. No water

present in the xylem cells is placed under tension which is ultimately transmitted to the root through the stem tracheids.

This downward transmission of tension develops due to cohesion properties of a continuous water column in the vessels and tracheids from leaves to roots through the stem.

Ultimately the water column moves upward by mass flow due to transpiration pull and simultaneously the process of ascent of sap is accomplished.

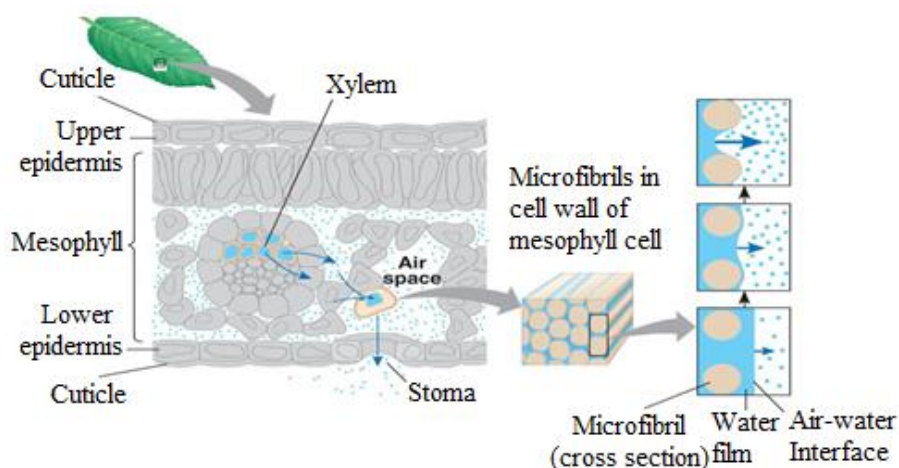


Fig.1.11 V.S. of leaf showing stomatal transpiration generating transpiration pull

Evidences in support of the theory: Several experimental evidences have been in support of this theory. Some of them are as follows-

- i) The osmotic pressure of mesophyll cells has been recorded very high up to 20 atm, which is quite sufficient for the upward movement of water. As per records it has been calculated that 1 atm. Osmotic pressure can result in water rise up to 10 metres.
- ii) It does not require any metabolic energy since it is purely a physical process, and even if it requires, it is negligible, because for 100 metre rise of 1 ml. water only 0.5 cal. Energy would be needed.
- iii) It is recorded that the tensile strength of xylem sap is between 25- 300 atm. which is sufficient enough to maintain a continuous water column without any break.
- iv) For further support of this theory it is compared with the porous pot experiment in which at the top of a continuous water column a porous pot is placed and its water is subjected to evaporate, the water column is put under tension. It is recorded that this column is not broken due to cohesive property of water molecules while evaporation continues. Thus it proves that a similar kind of mechanism may operate in plants too.

Further with the help of certain calculation based experiments strengthened this theory. Zimmerman (1965) explained that a pressure of 0.15 atm. per meter is required for the fastest movement of water in plants means that the rise of water in the tallest tree a water potential difference of 45 atm, will be sufficient. However, the cohesive property or tensile strength of

water is quite high (1000atm.) and about 1300 atm is necessary to break a stretched water column.

Kramer and Kozlowski (1960) while using dendrographic measurements of the tree trunk diameter variation observed that the diameter of the xylem elements decreased during excessive transpiration period due to strain exercised on the water column by tension. This also supports the theory.

Scholander and coworkers (1965) adopted the pressure bomb technique by using a cut shoot and enclosed it in the vessel placing the cut surface in the bomb. An artificial tension was created in the vessel in order to pull the liquid back and measured the counter pressure. This was found to be as high as 80 atm. These results are also supports the cohesion- tension theory.

Objection to Cohesion- tension theory: There is one objection to this theory which is relevant but refutable. As there is variation of temperature during day and night and in vessels of larger diameter, there are fair chances of gas bubbles entering the water from the soil which may break the continuity of water column. Although it is true that such bubbles are not rare among the plants yet this objection has been successfully explained.

Explanation: During excessive tension the vessels are gas filled. This phenomenon is known as “cavitation” and has been demonstrated by Milburn and Johnson (1966). This problem is overcome by the presence of many parallel columns of vessels side by side and the gas filled or injurious effect temporary cavitation are eliminated or resolved. The gases are dissolved in the solution when the tension is relieved by rain or simply at night and the column becomes continuous.

1.8 SUMMARY

Absorption of water is highly essential for plants for various metabolic activities. Land plants get their water supply from soil which serves as the source of water and minerals to them. The way, in which water from soil enters roots, particularly to the root xylem is called mechanism of water absorption. The ascent of sap in the xylem tissue of plants is the upward movement of water and minerals from the root to the crown. The conducting cells in xylem and tracheary non- living and include in various groups of plants, vessels members and tracheids. Thus the upward conduction of water from the plant body is called ascent of sap.

1.9 GLOSSARY

Absorption of water- The way in which water from soil enters roots, particularly to the root xylem is called mechanism of water absorption.

Ascent of sap- The upward conduction of water from the roots to different parts of the plant body is called Ascent of sap.

Capillary Water- Water held by capillary forces.

Combined water- Water chemically bound to soil surface.

Diffusion- The process whereby particles of liquid, gases or solids are intermingled as the result of their spontaneous movement caused by them.

Endosmosis- In plants diffusion of solvent inside the cell is called endosmosis.

Exosmosis- In plant diffusion of solvent outside the cell is called endosmosis.

Field capacity- Field capacity is the amount of soil moisture or water content held in the soil after excess water has drained away and the rate of downward movement has decreased.

Guttation- Exudation of water usually through structures called hydathodes present at the tip of veins of leaves.

Gravitational water- Free water moving goes down due to gravity.

Hydathodes- Structures present at the ends of vein lets of leaves, specially meant for exudation of water through a pore.

Hygroscopic water- Water held lightly by soil particles.

Hypertonic- Having an osmotic pressure higher than that of an isotonic solution.

Osmosis- A process by which molecules of a solvent tend to pass through a semipermeable membrane from a less concentrated solution into more concentrated one.

Water vapour- Un- combined water as moisture in soil.

Wilting coefficient- The level of soil moisture at which water becomes unavailable to plants and caused permanent wilting ensures.

1.10 SELF- ASSESSMENT QUESTION

1.10.1 Short Answer type Questions:

1. The maximum absorption of water by roots occurs in the zone of-

- | | |
|-----------------------|-----------------|
| (i) Cell division | (ii) Root cap |
| (iii) Cell elongation | (iv) Root-hairs |

2. The movement of water is along-

- | | |
|------------------------|------------------------|
| (i) Diffusion gradient | (ii) DPD gradient |
| (iii) Turgor gradient | (iv) Osmotic- gradient |

3. Water supply in the plant is due to-

- | | |
|-----------------------|-----------------|
| (i) Guttation | (ii) Osmosis |
| (iii) Cohesive forces | (iv) Imbibition |

4. The principle by which blotting paper absorbs water is

- | | |
|----------------------|--------------------------|
| (i) Capillary action | (ii) Absorptive capacity |
| (iii) Root- pressure | (iv) Transpiration pull |

5. Which one explains ascent of sap-

- (i) Cohesion-tension theory of Dixon and Jolly (ii) Photosynthesis
(iii) Starch- sugar inter conversion (iv) None of the above
6. The process in which loss of water occurs in the form of water vapour is-
(i) Guttation (ii) Respiration
(iii) Transpiration (iv) Exosmosis
7. Ascent of sap takes place through-
(i) Phloem (ii) Cambium
(iii) Xylem (iv) Epidermis
8. Who was the first to evolve vital force theory?
(i) Godlewski (1884) (ii) Janes (1887)
(iii) Priestley (1886) (iv) Westermeir (1883)
9. Who demonstrated that the ascent of sap occurs to the pulsatory activity of innermost cortical cells?
(i) Strasburger (1891) (ii) J.C. Bose (1923)
(iii) Janes (1887) (iv) Molisch (1828-29)
10. Most accepted theory of ascent of sap was given by-
(i) Bose (ii) Dixon and Jolly
(iii) Sachs (iv) Strasburger
11. Imbibition theory was given by-
(i) Schas (ii) Scolander
(iii) Boehm (iv) Curtis

1.10.2 Fill in the blanks:

1. In absence of water ----- becomes inactive and even killed.
2. Generally plants absorb water from soil by their-----
3. The total amount of water present in the soil is termed as-----
4. The water among this, available to the plants is called-----
5. The water drains away and not available to plants is known as-----
6. The gravitational water is -----available to plants
7. The only available water to plants is-----water
8. Capillary water+ hygroscopic water + chemically combined water = -----
9. The upward movement of water is known as-----
10. Attraction between similar molecules is known as -----

1.10.1 Answers Key: 1- (iv), 2- (ii), 3- (iii), 4- (i), 5- (i), 6-(iii), 7- (iii), 8- (iv), 9-(ii), 10- (ii), 11- (i).

1.10.2 Answers Key:

1-Protoplasm, 2-Roots, 3-Holard, 4- Chesard, 5-Run-off water, 6- Not, 7-Capillary water, 8-Field capacity, 9-Ascent of sap, 10-Cohesion

1.11 REFERENCES

- S.N. Pandey and B.K. Sinha (2015).A Text Book of Plant Physiology. Vikas Publishing House Pvt. Ltd.
- Susheela M Das (2010-11). Latest Portfolio of Theory and Practice in Plant Physiology. Dominant Publishers and Distributors New Delhi- 110002.
- V. Burma (2001). A Text Book of Plant Physiology for under graduate and Post graduate students. Edited and production associated by Prem kumar Mehta. Emkay Publishing House, Swami Dayanand Marg, Delhi- 110051.

1.12 SUGGESTED READINGS

- S.N. Pandey and B.K. Sinha(2015).A Text Book of Plant Physiology.Vikas Publishing House Pvt. Ltd.
- Susheela M Das (2010-11). Latest Portfolio of Theory and Practice in Plant Physiology.Dominant Publishers and Distributors New Delhi- 110002.
- V. Burma (2001). A Text Book of Plant Physiology for under graduate and Post graduate students. Edited and production associated by Prem kumar Mehta. Emkay Publishing House, Swami Dayanand Marg, Delhi- 110051.

1.13 TERMINAL QUESTION

1.13.1 Short Answer type Questions:

1. What is the hygroscopic water?
2. Define the gravitational water?
3. What do you meant by ascent of sap?

1.13.2 Long Answer type Questions:

1. Explain the phenomenon of osmosis?
2. Define diffusion with suitable examples?
3. Plant cell works as semi-permeable membrane, explain it?
4. How does water moves through the soil, explain it?
5. Explain the mechanism of absorption of water by land plants?
6. Differentiate between active and passive absorption?
7. Explain the cohesion-tension theory?

UNIT-2 WATER LOSS IN PLANTS: EVAPORATION AND TRANSPIRATION

Content

- 2.1 Objectives
- 2.2 Introduction
- 2.3 Loss of water from plants
- 2.4 Transport of water
- 2.5 Evaporation
- 2.6 Transpiration
- 2.7 Physiology of stomata
- 2.8 Summary
- 2.9 Glossary
- 2.10 Self Assessment Question
- 2.11 References
- 2.12 Suggested Readings
- 2.13 Terminal Questions

2.1 OBJECTIVES

After reading this unit students will be able:

- To study the phenomenon of transpiration.
- To study the mechanism of opening and closing of stomata.
- Different factors affecting transpiration.

2.2 INTRODUCTION

Plants absorb a considerable amount of water through their roots and carried to the top of the plant. The absorbed water is utilized by the plant. The remaining water is lost from a plant, primarily in the form of water vapours and rarely in the form of liquid from the aerial parts of plant. The loss of water from the living tissue of aerial parts of the plant in the form of water vapours is termed as "*transpiration*" and in the form of liquid is known as "*guttation*".

2.3 LOSS OF WATER FROM PLANTS

Transpiration is the process of water movement through a plant and it evaporate from aerial parts such as leaves, stem and flowers. Water is necessary from plants but only a small amount of water taken up by the roots, is used for growth and metabolism. Transpiration is both important and costly process for plants and it requires that a delicate balance between the amount of water absorption and its loss.

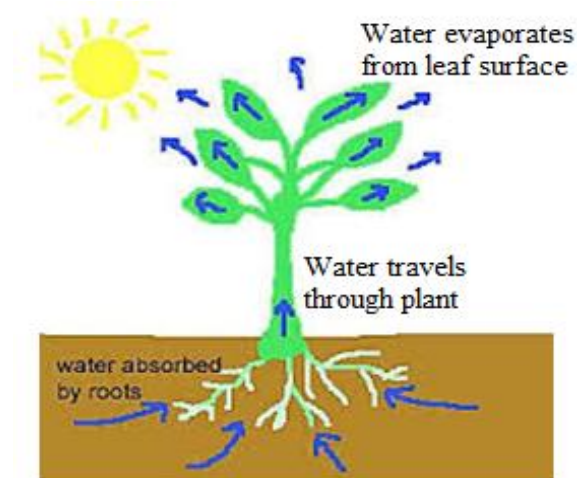


Fig.2.1. The process of transpiration and evaporation

2.4 TRANSPORT OF WATER

Transpiration involves the evaporation of water. The cells of the leaves are exposed it to direct sunlight. Energy from the sunlight can be transferred from the plant cell to the water caring it to evaporate. The process of transpiration is a very important procedure for plants. It creates a negative pressure gradient that helps to draw water and minerals up through the plant from its roots. It helps to keep the plant cool on the hot weather- a method of evaporative cooling.

Water moves from the soil into plant roots, up through the sapwood into the leaves. The water warmed by the sun turns into vapour (evaporate) and passes out through thousands of tiny pores (stomata) mostly on the underside of the leaf surface.

Table- 1 Differences between transpiration and evaporation

S.No.	Transpiration	Evaporation
1.	Transpiration is found in the plants and is modified physical phenomenon.	It is a pure physical process and taking place on any free surface.
2.	This phenomenon is regulated by the activity of guard cells	In evaporation no such mechanism is found
3.	In this process, only living cells exposed to the atmosphere are involved.	It can occur from living and non-living species.
4.	In transpiration different types pressures. Such as vapour pressure, osmotic pressure, diffusion pressure etc.	In this process no such forces are involved.
5.	It helps to protect the surface of leaf and young stem from sun burning and keeps Surfaces wet.	It causes dryness of the free surface.

Table- 2 Differences between transpiration and guttation

S.No.	Transpiration	Guttation
1.	It occurs during day time.	It occurs usually in the night.
2.	The transpired water is given out in the form of water vapour.	The water is given out in the form of liquid
3.	The transpired water is pure.	Guttate water contains dissolved salts and sugar.
4.	Water transpired through stomata lenticels and cuticles.	It occurs through hydathodes, the special Structures found only on leaf tips or margins.
5.	Transpiration is a controlled process.	It is an uncontrolled process.
6.	It helps to lowers down the temperature.	It lacks such a relationship.

2.5 EVAPORATION

Evaporation is a physical process that converts water from a liquid to a vapour, primarily under the influence of solar energy. Evaporation occurs on all free water surfaces, including soil, water bodies, and moist surfaces, but in plants, it plays a unique and regulated role as part of the transpiration process. Plants absorb a significant amount of water from the soil by the roots. This absorbed water is transferred up through the xylem to the plant's leaves and other aerial parts. However, only a small percentage of this water is used for metabolic processes like photosynthesis and cell development. The remaining, much greater amount is lost to the atmosphere in the form of water vapour, a process called evaporation.

Physical Basis of Evaporation in Transpiration

Evaporation in transpiration is mainly a physical process driven by solar energy. When the leaf surface gets light, some of the energy is transformed into heat energy. This heat boosts the temperature of the water within the leaf cells, increasing the vapour pressure.

As a result, water molecules escape into the surrounding atmosphere. The rate of evaporation is determined by the difference in water vapour pressure inside the leaf and that of the surrounding atmosphere. When the outside atmosphere is dry (low humidity), evaporation occurs quickly. When humidity is high, the rate of evaporation decreases.

The process of evaporation in transpiration also causes cooling of the plant surface, which helps in the regulation of the plant's internal temperature, a phenomenon known as evaporative cooling. This is essential for plant survival, particularly in hot and dry climates.

Significance of Evaporation in Transpiration

1. **Maintains Water Flow:** Continuous evaporation keeps water flowing upward from roots to leaves.
2. **Mineral Transport:** The flow of water that results from evaporation aids in the transport of dissolved minerals nutrients.
3. **Cooling Effect:** Evaporation decreases the temperature of plant tissues, preventing overheating in direct sunlight.
4. **Maintains Turgidity:** Evaporation helps cells maintain their turgor pressure by regulating water loss.
5. **Gas Exchange Support:** The opening of stomata for evaporation promotes gas exchange, including CO₂ for photosynthesis and oxygen for respiration.

2.6 TRANSPIRATION: MECHANISM OF TRANSPIRATION

The process of transpiration fulfills into two stages-

1. Water evaporates from the cell walls into the intercellular spaces and
2. Diffusion of these water vapors of the intercellular spaces into the outside atmosphere.

The stomatal movement is directly related to increase or decrease in the osmotic potential of the guard cells and surrounding environment. It occurs in two steps-

- i) In the first stage - from the surface of turgid cells, water gets evaporated and collects in the nearby intercellular spaces results the increasing water vapour pressure and lowering its diffusion pressure deficit (DPD).
- ii) In the second stage the collected water diffuses through stomata, lenticels or cuticle in the outer atmosphere because of low water vapour pressure and high DPD value outside.

The difference in all three type transpiration is that in stomatal transpiration, pores are involved and controlled by guard cells while in lenticular transpiration, pores involved with uncontrolled opening and closing and in cuticular transpiration pores are not involved at all.

Types of Transpiration: As mentioned earlier that transpiration may occur either through cuticle, lenticels or stomata thus accordingly it is called cuticular, lenticular or stomatal transpiration.

i) Lenticular Transpiration: Loss of water in the form of water vapour taking place through the lenticles present in woody stem and fruits.

In the bark there are areas of small pores which are filled with loosely arranged cells known as complementary cells. About 0.1 percent water vapour lost through lenticels. It is quite negligible in comparison to the total loss by the whole plant.



Fig. 2.2 Lenticular transpiration

ii) Cuticular Transpiration: The loss of water in the form of water vapour through the cuticle is known as cuticular transpiration. The wax like covering on epidermis of leaves and herbaceous stems is known as cuticle. Its thickness varies from plant to plant. It is also a meant to check transpiration. Due to some crakes in it the cuticle is rarely completely impermeable. Upto 20 percent of the total transpiration may take place through it. With the increase in the thickness of cuticle the loss of water is also reduced.

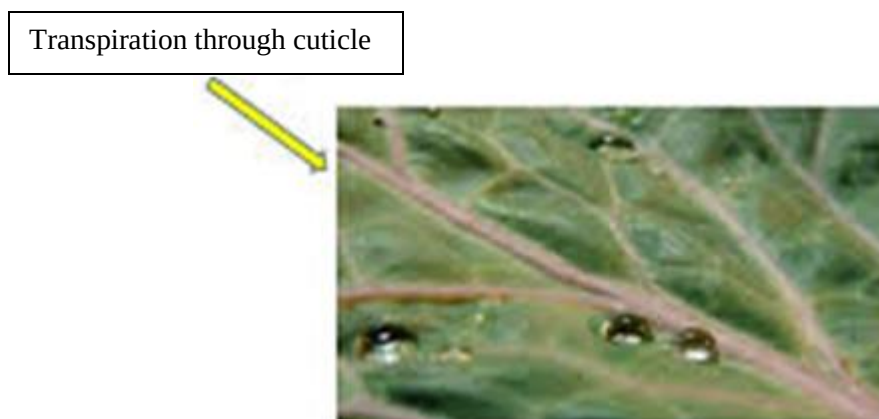


Fig. 2.3 Cuticular transpiration

iii) Stomatal Transpiration: The epidermal surface of a leaf bears a great number of pores called stomata. Stomata are minute pores in the epidermis, their opening and closing being controlled by guard cells. Maximum diffusion of water vapors takes place through stomata. Stomata are mostly situated on the leaves but in herbaceous stem they may be found in the epidermis. Maximum transpiration, about 80- 90 percent water vapors is lost through stomata.

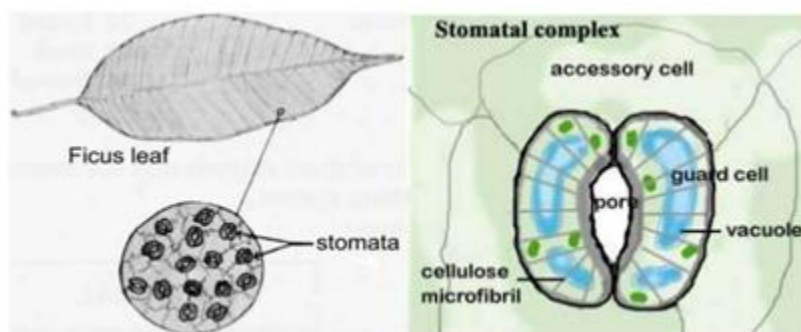


Fig. 2.4 Stomatal transpiration

2.7 PHYSIOLOGY OF STOMATA

Stomata

The Structural Details- Stomata are minute, tiny pores of elliptical shape structures. In dicots the guard cells are kidney or bean shaped and in monocots, or in the members of Graminae (Grasses family), dumble shaped. The stomata are microscopic and bordered by two specialized epidermal cells called *guard cells*, which control the opening and closing of the stomata. The surrounding wall of the guard cells inside the *epidermal cells* is thick and inelastic due to presence of secondary layer of cellulose. While the outside walls of the guard cells is thin, elastic and permeable. Each guard cell has a cytoplasmic lining and central vacuole containing nucleus and number of chloroplast, often poorly developed and incapable of photosynthesis. The guard

cells are surrounded by specialized epidermal cells called *subsidiary cells*, supports the movement of guard and the number of cells vary from plant to plant. e.g. in *Phaseolus vulgaris* it ranges from $7 \times 3\mu$, in *Avena sativa*- $38 \times 8\mu$, and in *Zea mays*- $4 \times 26\mu$.

Stomata

Stomata (sing. Stoma) = pores in a leaf, mostly on the undersurface

- (a) Each pore is surrounded by a pair of guard cells
- (b) Guard cells can change shape to open or close the stoma

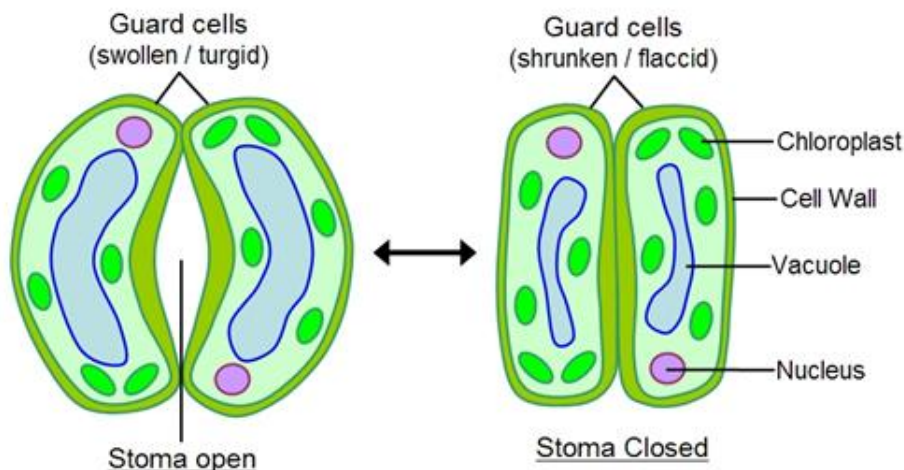


Fig. 2.5 Structural differences of stomata during opening and closing stages

The number of stomata in a definite area varies from plant to plant and Xerophytes possess larger number of stomata than mesophytes. About 1 - 2 percent of the total area of leaf area is occupied by stomata.

On the basis of distribution of stomata on leaf surface the plants may be categorized in five categories as follows-

- 1. Potato Type:** When stomata are distributed more on the lower surface and less on its upper surface e.g. potato, bean, pea, tomato, cabbage etc.
- 2. Apple or Mulberry Type:** The stomata are distributed on the under surface of the leaf only, e.g. peach, mulberry, walnut etc.
- 3. Oat Type:** Stomata occur equally on the both surfaces of leaf, e.g. Oat, maize grasses etc.
- 4. Wild Lily Type:** Stomata are found only on the upper surface of the leaf, e.g. water lily, Nymphaea and many aquatic plants.
- 5. Potamogeton Type:** Stomata are either altogether absent or functionless, e.g. potamogeton and submerged aquatics.

Daily Movement of Stomata: to study the daily movement of stomata Von Mohl (1856) prepared a stomatal clock and observed that stomata open in daylight and close at night. On the basis of this Loft field had classified three main groups of stomata in accordance with their daily movement.

i) Alfalfa Type: The stomata remain open throughout the day and closed all night, e.g. Pea, bean, mustard etc.

ii) Potato Type: The stomata close for a few hours in the evening, e.g. Allium, cabbage etc. and

iii) Barley Type: The stomata open only for a few hours in a day, Barley, and other cereals.

Considering variable behavior of stomatal movements five categories have been recognized as -

1. Autonomous movements
2. Hydroactive movements
3. Passive and active movements
4. Skotoactive movements

Mechanism of Stomatal Opening and Closing: Microscopic direct observation and measurement studies show that the movement and the opening and closing of stomata are brought about by changes in the volume and shape of the guard cells. It is revealed that the expansion and contraction of the guard cells must be due to turgidity and flaccidity respectively i.e. when guard cells are pores are open but when flaccid the pores are closed. The size of the pore depends on upon the degree of turgidity of guard cells. When the guard cells absorb water from the surrounding cells and become turgid. When turgidity increases, the outer thin walls of guard cells stretch outward causing outward stretching of their inner wall. The inner inelastic wall becomes concave and as a result the space surrounding the pore become wide and the pore opens. Thus in the opening and closing of stomata, turgidity of guard cells plays significant role. So what is the mechanism working behind the change in turgidity in guard cells has a question of great controversies. To explain it many theories has been proposed.

1. Theory of Photosynthesis in Guard Cells: According to Von Mohl (1956), stomata open in day and close at night. Based on this hypothesis he proposed that in the presence of night, photosynthesis occurs in the guard cells and produces carbohydrates due which osmotic pressure of guard cells increases. The opening sequences explains the mechanism of stomatal opening-In presence of light----- Photosynthesis

(in guard cells)-----Sugar formation takes place-

-----Osmotic pressure of cell sap increases-----Resulting Endosmosis (water enters from neighbouring epidermal cells) ----- Turgidity of guard cells increases-----Stomata increases

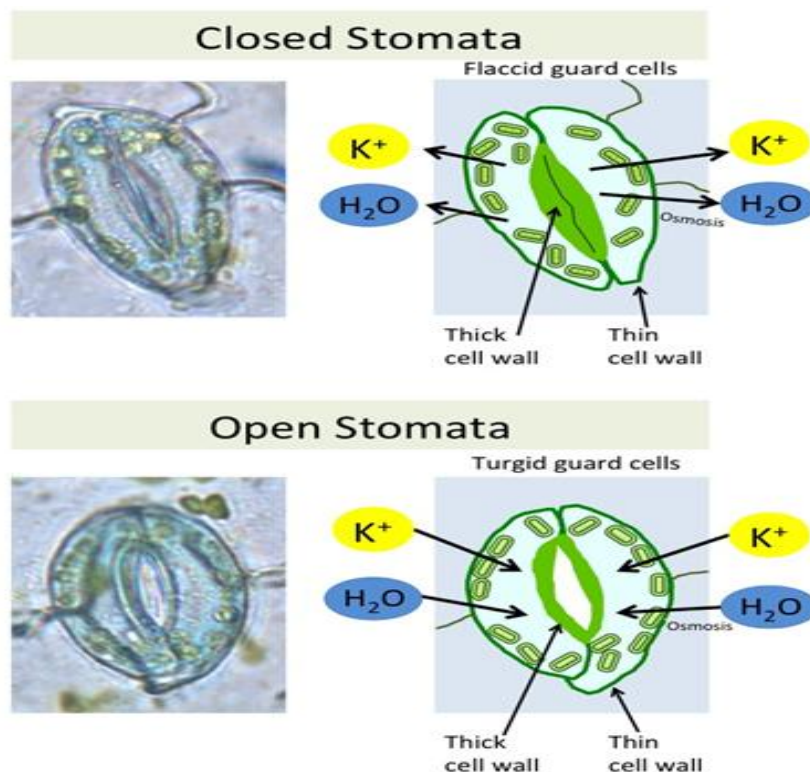
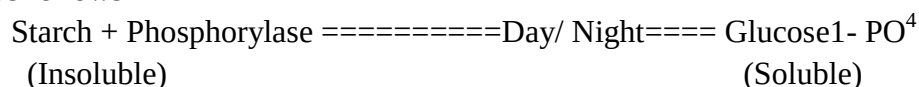


Fig. 2.6 Movement of H_2O and K^+ ions in guard cells

This theory was rejected due to the following causes-

- i) The photosynthesis by guard cells is too slow.
- ii) The chloroplast of the guard cells are either totally incapable of carrying sufficient photosynthesis.
- iii) The leaves of some plants which are chlorophyll also possess starch grains in the guard cells of stomata.
- iv) To bring about endosmosis and turgidity for sufficient accumulation of sugar is too slow.

2. Theory of Starch -----Sugar Inter-conversion: According to Lloyd (1905), Loftfield (1921) and Sayre (1926), the amount of starch in the guard cells increases during night and decreases during day time. Hence the insoluble starch present in the guard cells is hydrolysed into soluble glucose -1- P in presence of phosphorylase enzyme during day time and soluble glucose -1-P is converted into soluble starch during night. Thus both these reactions are reversible as follows-



Sayre (1926) observed that for the opening and closing of stomata depends on different P_H medium e.g. stomata remain open in neutral or alkaline P_H (in the atmosphere of ammonia vapour) even in the dark and close in the acetic P_H (in the atmosphere of acetic vapour) even in

night. According to him starch converted into sugar and vice versa. How this change in P_H occurs in guard cells was explained by Scarth (1932) and proposed the theory of Starch $\rightleftharpoons$ Sugar inter-conversion.

The steps involved in opening and closing of stomata may be summarized as below.

Yin and Tung (1940), Stewart (1964) proposed a modified scheme for stomatal opening with the discovery of the presence of phosphorylase enzyme in guard cells.

The mechanism proposed for stomatal opening is below-----

Light-- In mesophyll cell of the leaf rate of photosynthesis----- from intercellular spaces CO_2 removed----- P_H of guard cells increases----- enzymatic conversion of starch into glucose----- increase in osmotic pressure of cell sap----- results into endosmosis----- guard cells become turgid----- stomata open, Contrary to this during closure of stomata a reverse scheme would follow.

Inter-conversion of starch and sugar based theories have been criticized on the basis of the following points-

- Some guard cells lack starch although they work just as well as those of other plants.
- The chloroplasts of stomatal guard cells of certain plants are completely unable to photosynthesis carbohydrates.
- The guard cells already possess much amount of stored sugar.
- The starch $\rightleftharpoons$ sugar or glucose inter - conversion is too slow to account for opening and closing of stomata.

The guard cells of stomata of young leaves sometimes also possess starch grains before the opening and formation of buds.

However, some plants when kept in dark, their leaves still possess starch. Stomata can open independent of carbon dioxide concentration such as by light and temperature.

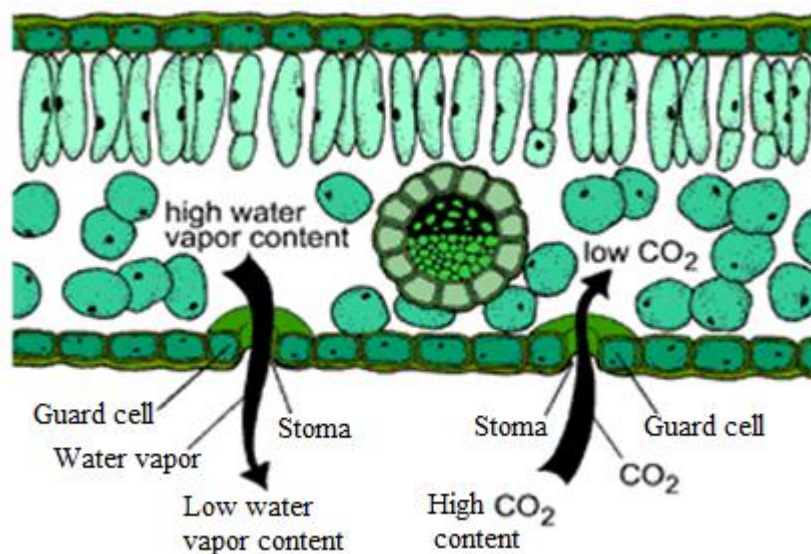
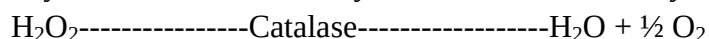


Fig.2.7 Stomata open to allow carbon- dioxide CO_2 to enter a leaf and water vapor

Theory of Glycolate Metabolism- Zenith (1963) was proposed this theory considering the fact that change in CO_2 concentration in the environment of stomata is insufficient to bring its opening and closing. According to this theory production of glycolic acid in the guard cells plays an important role in the opening of stomata. Glycolate is produced under low concentration of CO_2 . Glycolate also synthesizes carbohydrates hence rising the osmotic potential of guard cells with the formation of glycolate which requires ATP for its synthesis.



(non-cyclic photosynthesis)



(Hydrogen peroxide)

According to Zelitch (1963) ATP produced by this mechanism participate in active pumping of water into the guard cells. The whole process refers to transformation ATP through glycolate, glycolate shuttle which helps in reoxidation of NADPH.

The theory however, has been rejected due to several reasons-

Explanations-

- i) Opening and closing of stomata in darkness in field.
- ii) It also fails to explain the opening of stomata in light even in complete absence of CO_2 i.e. glycolate.
- iii) For stomatal opening blue light is more effective than red light could not be explained.
- iv) In some plants stomata have been seen to be starch content.
- v) It also fails to explain the opening of stomata during night in succulent plants.

The opening and closing of stomata summarized as below-

Opening of stomata-

- i) Starch + phosphate $\xrightarrow{\text{(phosphorylase)}}$ Glucose - 1- P
- ii) Glucose - 1-P $\xrightarrow{\text{(Phosphoglucomutase)}}$ Glucose + i P

Closing of Stomata-

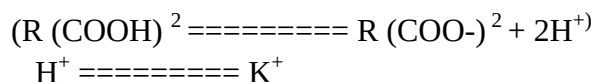
- i) Glucose + i P + ATP $\xrightarrow{\text{(hexokinase)}}$ Glucose-1-P
- ii) Glucose - 1-P $\xrightarrow{\text{(phosphorylase)}}$ Starch + Phosphate.

Many physiologists rejected the theories of starch sugar inter-conversion and starch glucose inter-conversion due to following reasons.

- i) The guard cells of monocotyledons plants functions like other dicotyledones plants although they contain starch.
- ii) The stomata become closed in noon without any change in the quantity of starch.
- iii) The rate of inter-conversion of starch to sugar and glucose is insufficient for opening and closing of stomata.
- iv) The concentration of CO_2 is insufficient to for activation of guard cells and change in the P_H of cell sap.

v) It is observed that sometimes starch is converted into malic acid in place of sugars.

Theory of Proton Transport-This theory was proposed by Levitt (1974) base on proton - transport concept. It explains the mechanism of opening and closing of stomata. According to this theory, potassium ions (K^+) have been found to play a critical role. The opening and closing of the stomata are the result of an active transport of potassium ions into the guard cells and out of them. At first malic acid is formed from starch in the guard cells which dissociates into cations and anions-



The organic acid provide H^+ in exchange for Potassium (K^+) and anions to balance the charges of K^+ . Malic acid is synthesized in illuminated guard cells which accomparises the influx of potassium ions. The exact biochemical steps involved are not fully known.

One of them possible step many be that during day time starch is metabolized to malic acid and then light triggers the excretion of malic acid from chloroplast into the cytoplasmic guard cells.

For stomatal opening and closure Noggle and Fritz (1976) have summarized the events--

i) During day time, light- induced stomatal opening as follows:

Light -----Malic acid production ----- Dissociation into hydrogen and malic ions ----- Influx of K^+ and efflux of H^+ -----Transport of Potassium malate into the vacuoles -----Osmotic entrance of water into guard cells ----- increase of turgor pressure -----stomata open.

Closing to this an abscissic acid (ABA), an inhibitor involves in the closing of stomata, which functions in presence of CO_2 . ABA inhibits K^+ uptake by changing the diffusion and permeability of guard cells. The K^+ moves out to the subsidiary cells. ABA results in lowering of P_H of guard cells and induces the process of acidification. At low P_H starch is synthesized and thus osmotic pressure of guard cells lowers and water moves out of guard cells to subsidiary cells. Due to this the guard cells became flaccid and stomatal pore is then closed.

Studies on isolated protoplast of guard cells have been carried out recently and it has been observed that the swelling response on the protoplast to K^+ is a specific property of guard cells. The protoplast swells when treated with K^+ ions, both in light and dark, but in light the reaction was more than in dark. It also observed that K^+ ions, PEP carboxylase, cytosolic and mitochondrial malate and ATP play critical role in the stomatal opening.

In this process for the import of K^+ ions energy from ATP is required in specific channels into the guard cells and malate is used as a substance for the ATP for the ATP supply. When malate level in the mitochondria falls, the ATP supply is depleted and then protoplast of guard cell does not swell. Contrary to this with decreasing level of malate in mitochondria, the ATP supply is also decreases and then protoplast of guard cells does not swell. On the other side, the light, when K^+ ions are imported into the guard cells, PEP carboxylase is activated which activated directly or indirectly increases malate in the cytosol and it is transported to the mitochondria. The supply of ATP is kept maintained. The ATP is transported into cytosol where

it may be hydrolyzed by plasma membrane bound ATPase to ADP or AMP. The released supply is restricted K^+ ions import is blocked. In light about 30% supply of ATP in guard cells is supported by the process called phosphorylation.

Plant Anti -respirants: As a fact the total water absorbed by the plant, almost 98% of the total water is lost in transpiration and only an insignificant amount is utilized by the plant for its own purpose. Due to this, plants have to face several problems, this enormous loss of water can be reduced, it will be an asset to nature and to the agriculturists. Recently scientists made efforts to find antirespirant substances reducing the transpiration rate without adversely affecting exchange of gases during photosynthesis and plant growth.

Any material applied to plants for the purpose of retarding transpiration is known as antiaspirant.

Examples of antiaspirants are colorless plastics, silicon oils, low viscosity waxes, abscissic acid, CO_2 etc. Among them colorless plastics, silicon oils and low viscosity waxes, sprayed on the leaves and these substances form a thin film permeable to CO_2 and oxygen but not to water. This approach gets only limited success. Similarly the fungicide phenyl mercuric acetate when applied in low concentration (10^{-4}), it exercised very little toxic effect upon leaves and resulted in partial closure of stomatal pores for over two weeks, it works as antirespirant.

Carbon-dioxide: Carbon-dioxide is an effective and antiaspirant. It is reported that a little rise in CO_2 concentration from the natural 0.03 to 0.05% in atmosphere includes partial closure of stomata. But its higher concentration is harmful which results in complete closure of stomata and adversely affecting photosynthesis and respiration, while use of CO_2 inhibited phosphorylation. Its usage cannot be economical and is practically feasible in experimental glass houses.

Factors Affecting the Role of Transpiration: The rate of transpiration is affected by a number of factors. These factors can be categorized under two categories.

i) External or Environmental Factors: That includes the temperature, light, water supply, wind velocity, atmospheric pressure, sprays and dusts and other vital activities.

ii) Internal or Structural Factors: It includes frequency of stomata and its structure, structural (specialties) peculiarities of leaf and water content of mesophyll cells.

(i) External Factors: All those environmental factors which affect the steepness of the DPD gradient affect the rate of transpiration. Some of the factors are as follows:

(a) Temperature: Temperature is the prime factor which directly affects the rate of transpiration. As increase in temperature increases the rate of transpiration by increasing the rate of evaporation of water from cell surface and decreases the humidity of the external atmosphere. It works according to Vant Hoff's rule that 10° rise in temperature the rate of transpiration doubled.

(b) Light: Comparatively to temperature, light has no direct effect on the rate of transpiration but indirectly it affects the rate in two ways

- i) By controlling the stomatal opening and
- ii) By affecting the temperature.

Increase in intensity of light the rate of transpiration increases because the stomata get opened and the temperature rises. Light also has directly effects on transpiration rate as it increases markedly in light and decreases in dark. There is a close relationship between the opening of stomata and presence of light.

c) Humidity of Air: The relative humidity of the atmosphere affects the rate of transpiration to a great extent by influencing the DPD gradient between the intercellular spaces and outer atmosphere. It shows inverse effect on rate of transpiration i.e. rate of transpiration increases with the increase in the humidity of the external atmosphere upon water vapour saturation of the air, because the capacity of atmosphere to take up more moisture which depends upon the difference between the amount of water present in the air and the amount needed to saturate it completely.

d) Wind Velocity: Velocity of wind has direct effect on rate of transpiration and the increase in the wind also increases the rate of transpiration by removing water vapour of the atmosphere and hence lowers the relative humidity. The wind of much higher velocity, however, decrease the rate of transpiration probably as the guard cells become flaccid and stomata are closed while the transpiration is faster in mild wind.

e) Atmospheric Pressure: The reduction of atmospheric pressure reduces the density of the external atmosphere and hence permitting more rapid diffusion of water.

f) Water Supply: Rate of transpiration decreases with the deficiency of water in soil indirectly by decreasing the rate of water absorption.

g) Vital Activities: Some vital activities of plants may also affect the rate of transpiration e.g. Spray and Dusts: Sprays and dusts affect the rate of transpiration by affecting the permeability of the cuticle and temperature of leaves. For example Bordeaux mixture lower the leaf temperature but increases the permeability of the cuticle and hence increases cuticular transpiration particularly at night.

(ii) Internal Factors:

a) Stomatal Frequency: Stomatal frequency means the number of stomata per unit area of the leaf surface. It varies from plant to plant and also depends upon the effect of environment. Salisbury used a term known as stomatal index to represent as-

$$I = \frac{S}{E + S} \times 100$$

Where I stand for stomatal index

S for number of stomata per unit area and

E for the number of stomatal cells in the same unit area.

Rate of transpiration increased with the increase in stomatal frequency which is also depends upon the degree of opening of stomata.

b) Structural peculiarities of Leaf: Besides normal leaves certain plants adapted to reduce the rate of transpiration i.e. by reducing the size of leaves and hence reduce the rate of transpiration.

The rate of transpiration is also checked by the deposition of wax and cutin like substances on the surface of leaves as in *Calotropis*.

Some plants have needle like spine like leaves or the xerophytic plants to reduce the total evaporating surface *Pinus*, *Opuntia* etc.)

In some other plants stomata are sunken or in cavities surrounded by cavities also helps to reduce rate of transpiration (*Cycas*, *Nerium* etc.).

High osmotic pressure of leaf cells and presence of hydrophilic compounds such as gums, mucilage etc. helps in retarding the rate of transpiration.

Leaf area, intercellular spaces of leaf and extent of root system are several other factors which affect the rate of transpiration.

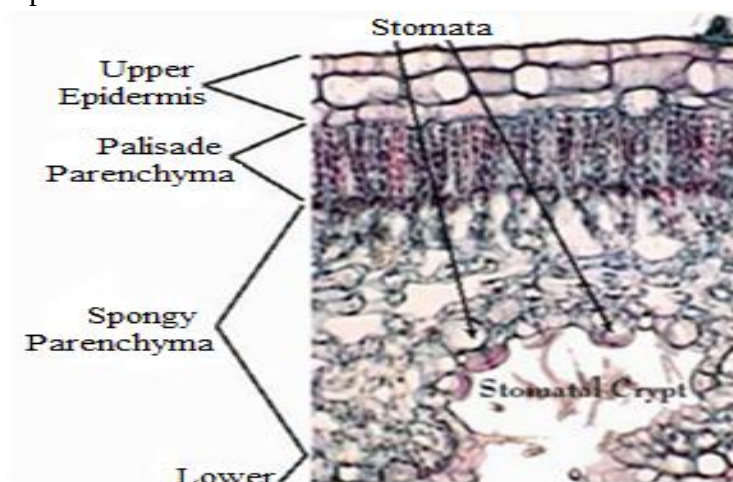


Fig. 2.8 C.S. of Nerium leaf showing specific stomatal crypt

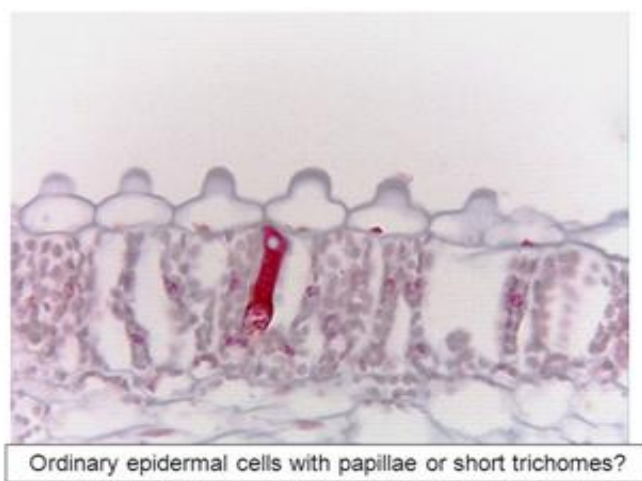


Fig.2.9.Colocasia leaf with Papillae

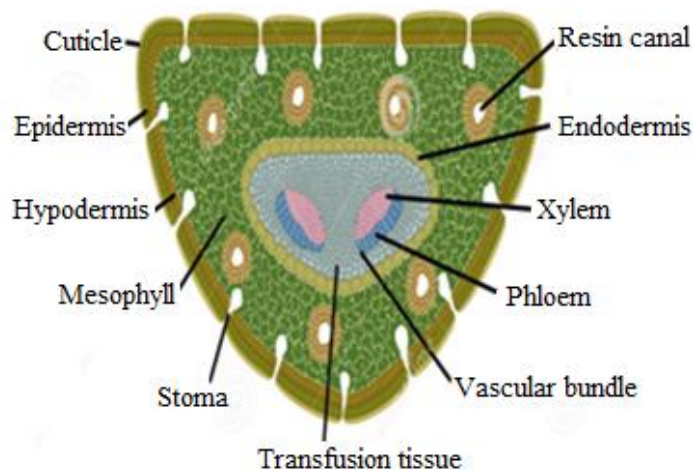


Fig. 2.10. Pine leaf showing cuticle layer in upper side

Guttation: Exudation of the water from plants in the form of liquid is known as Guttation. However, the amount of water lost by this process is negligible as compared to that by transpiration. The word guttation was proposed by Burgerstein for the first time. It is not universally reported in all plants and only a few genera of plants mostly herbaceous among which about 333 genera belonging to 115 families are reported to guttate.

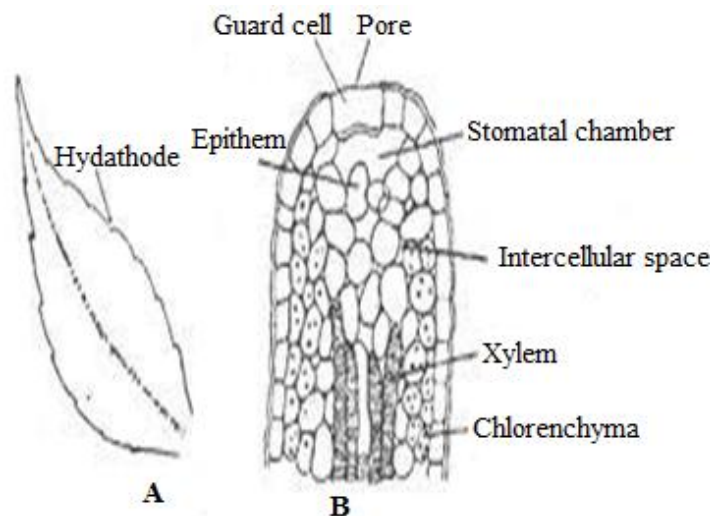


Fig. 2.11 Leaf margins with hydathode (A), Internal structure of hydathode (B)

The plants showing guttation have specialized structures called hydathodes situated on the tips and margins of leaves. Due to this, the water droplets are seen deposited on margins of leaves of some grasses and dicotyledones. Structurally each Hydathode consists of a pore in epidermis followed by large intercellular spaces, loosely arranged parenchyma and blindly ending xylem elements. The loosely arranged mass internal structure of parenchyma is known as *epithum*.

Colocasia antiquorum is known to guttate heavily and the amount ranges from a few drops to 100 ml or even more water per day. *Tropoelum*, tomato, grasses, barley etc. are other common examples showing guttation.

The main cause of guttation is root pressure cold and dry aerated soil reduces the root pressure resulted to bring down the guttation rate. The mineral deficiency also reduces guttation rate.

Guttate water has been containing various kinds of sugars, enzymes, amino acids, vitamins, organic acids, and other mineral salts.

2.8 SUMMARY

Evaporation serves as the physical basis of transpiration. It is the driving force that allows water to travel through the plant's body and eventually exit into the atmosphere. It is biologically regulated in plants by guard cells and stomata.

Transpiration is the process by which moisture is carried through plants from roots to small pores on the underside of leaves, where it changes to vapour and is released to the atmosphere. Transpiration is essentially evaporation of water from plant leaves. Stomata are pores in the leaf that allow gas exchange where water vapors leave the plant and carbon di oxide enters. Special cells called guard cells control each pore's opening and closing. When stomata are open, transpiration rates increases, when they are closed transpiration rates decreases.

Transpiration also includes a process called guttation, which is the loss of water in liquid form from the injured leaf or stem of the plant, principally through water stomata known as hydathodes. Studies have revealed that about 10 percent of the moisture found in the atmosphere is released by plants through transpiration. However, so many internal and external factors affect the rate of transpiration. Scientists proved that transpiration is both an important and costly process for plants, and it requires that a delicate balance for the survival of plant.

2.9 GLOSSARY

ADP: Adenosine di- phosphate.

ATP: Adenosine tri- phosphate.

ATPase: Also called myosine, an enzyme catalyzing TTP, CTP, GTP, UTP and ATP to yield orthophosphate and the corresponding diphosphate.

Amyl: means starch.

Carbohydrate: Compound containing carbon, hydrogen and oxygen with general formula $C_n(H_2O)_n$ e.g. sugar, starch, cellulose.

Chlorophyll: Green pigment found in autotrophic plants. It's a magnesium containing porphyrin compound and by this plants become capable of manufacturing own food.

Chloroplast: Chlorophyll containing plastid bodies and the site of photosynthesis.

Guttation: Loss of water in form of water drops through hydathodes.

Hydathode: Water excreting gland occurring on the edges or tips of leaves.

Transpiration: Loss of water in the form of vapour by the aerial parts of terrestrial plants.

Vacuole: A fluid filled (cell sap) space bounded by a membrane (tonoplast) found in the cell.

2.10 SELF ASSESSMENT QUESTION

2.10.1 Objective type questions:

1. The process in which loss of water occurs in the form of water vapors is-

- | | |
|---------------------|------------------|
| (i) Guttation | (ii) Respiration |
| (iii) Transpiration | (iv) Exosmosis |

2. Hydathode occurs in-

- | | |
|------------|-------------------|
| (i) Roots | (ii) Leaves |
| (iii) Stem | (iv) All of above |

3. The stomatal type of cereals which open only for a few hours during the day is-

- | | |
|-------------------|-------------------|
| (i) Barley type | (ii) Bean type |
| (iii) potato type | (iv) alfalfa type |

4. Transpiration is high under-

- | | |
|---------------------------------|----------------------|
| (i) Low atmospheric temperature | (ii) Dry environment |
| (iii) High temperature | (iv) All the above |

5. Sunken stomata-

- | | |
|------------------------------|---------------------------|
| (i) Hinder transpiration | (ii) Decrease environment |
| (iii) Increase transpiration | (iv) Stop transpiration |

6. Stomatal frequency indicates-

- | | |
|-------------------------------------|-------------------------------|
| (i) Number of stomata per unit area | (ii) Rate of gaseous exchange |
| (iii) Rate of water loss | (iv) All the above |

7. Excess of CO_2 (0.05%) in the atmosphere will-

- | | |
|---------------------------------|------------------------------------|
| (i) Has no effect | (ii) Will reduce the transpiration |
| (iii) Enhance the transpiration | (iv) Reduce transpiration |

8. When Oxygen is deficient in the atmosphere-

- | |
|--|
| (i) Stomata start closing |
| (ii) Stomata open fully |
| (iii) Stomata close up provided temperature is high |
| (iv) Stomata open provided water in the soils is maximum |

9. The number stomata per unit area of leaf are generally more on lower surface in case of --

- (i) Herbaceous stem
- (ii) Dorsiventral leaf
- (iii) Isobilateral stem
- (iv) In woody stem

10. A leaf with hair on its surface-

- (i) Reduces guttation
- (ii) Reduced transpiration
- (iii) Increases transpiration
- (iv) Reduces exchange of gaseous

11. Guttation takes place through-

- (i) Lenticels
- (ii) Stomata
- (iii) Hydathode
- (iv) Wounds

12. Who proposed the term guttation?

- (i) Bergerstein
- (ii) Levitt
- (iii) Shantz
- (iv) Darwin

13. A passive hydathodes comprises a group of loosely arranged colorless and parenchymatous cells known as-

- (i) Aerenchyma
- (ii) Spongy parenchyma
- (iii) Epithem
- (iv) Prosenchyma

14. Anti respirants-

- (i) Reduces the rate of transpiration without affecting carbon dioxide assimilation
- (ii) Reduces the rate of transpiration affecting protein synthesis of plant
- (iii) Reduces the rate of transpiration affecting growth of plant
- (iv) Reduce the rate of transpiration affecting carbon assimilation.

15. One of the following is an antitranspirant but its effect persists only for a few hours-

- (i) Malic acid
- (ii) Abscissic acid
- (iii) Phenyl mercuric acetate
- (iv) Silicon emulsion

16. In which type the stomata are present exclusively on the upper surface of the leaves-

- (i) Barley leaf
- (ii) Potamogeton type
- (iii) Potato type
- (iv) water lily type

17. Number of stomata present per cm^2 of a common leaf is about-

- (i) 1 million
- (ii) More than 100,000
- (iii) Less than 100
- (iv) 1000

18. Transpiration differs from evaporation in-

- (i) Transpiration is a physical process while evaporation is a physiological process
- (ii) Rate of water loss
- (iii) Transpiration is a physiological process while evaporation is a physical process
- (iv) Frequency of water loss

2.10.2 Fill in the Blanks -

1. Transpiration is reduced due to deposition of-----
2. Frequency and position of stomata can be determined by-----
3. In thin leaved mesophytes, stomata open during the day and closed during the night they belong to-----
4. Phenyl mercuric acetate (PMA) result in-----
5. A leafy twig of mesophyte plant dipped in water would demonstrate-----

2.10.1 Answers:

1. (iii) 2. (ii), 3. (i), 4. (iv), 5. (ii), 6. (i), 7. (ii), 8. (i), 9 (ii), 10 (ii), 11 (iii), 12 (i), 13 (iii), 14 (i), 15 (ii), 16 (iv), 17 (iv), 18 (ii),

2.10.2 Answer: 1. Cutin, 2. Porometer, 3. Alfalfa, 4. Reduces transpiration, 5. Transpiration

2.11 REFERENCES

- S. N. Pandey and B. K. Sinha (2015) A Text Book of Plant Physiology. Vikas Publishing House, Pvt. Ltd.
- Susheela M. Das (2010-11) Latest Portfolio of Theory and Practice in Plant Physiology. Dominant Publishers and Distributors New Delhi-110002.
- Burma (2001) A Text Book of Plant Physiology for under graduate and Post graduate students. Edited and Production associated by Prem Kumar Mehta. Emkay Publishing House. Swami Dayanand Marg. Delhi-110051.
- Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). *Plant Physiology and Development* (6th ed.). Sunderland, MA: Sinauer Associates.
- Salisbury, F. B., & Ross, C. W. (1992). *Plant Physiology* (4th ed.). Belmont, CA: Wadsworth Publishing.
- Nobel, P. S. (2009). *Physicochemical and Environmental Plant Physiology* (4th ed.). Academic Press.
- Kramer, P. J., & Boyer, J. S. (1995). *Water Relations of Plants and Soils*. Academic Press.
- Larcher, W. (2003). *Physiological Plant Ecology: Ecophysiology and Stress Physiology of Functional Groups* (4th ed.). Springer.
- Levitt, J. (1980). *Responses of Plants to Environmental Stresses* (Vol. 1). Academic Press.
- Campbell, N. A., Reece, J. B., & Urry, L. A. (2017). *Biology* (11th ed.). Pearson Education.

2.12 SUGGESTED READINGS

- S. N. Pandey and B. K. Sinha (2015) A Text Book of Plant Physiology. Vikas Publishing House, Pvt. Ltd.
- Susheela M. Das (2010-11) Latest Portfolio of Theory and Practice in Plant Physiology. Dominant Publishers and Distributors New Delhi-110002.
- Burma (2001) A Text Book of Plant Physiology for under graduate and Post graduate students. Edited and Production associated by Prem Kumar Mehta. Emkay Publishing House. Swami Dayanand Marg. Delhi-110051.

2.13 TERMINAL QUESTIONS

1. Write an essay on transpiration and its advantages to the plant?
2. Write short notes on-
 - i) Guttation
 - ii) Significance of transpiration
 - iii) Proton transport Concept
 - iv) Antirespirant
- 3 Explain with suitable diagrams the opening and closing mechanism of stomata?
4. Explain different type of transpirations occurs in plants?
5. Write short notes on-
 - i) Theory of photosynthesis in guard cells
 - ii) Theory of starch= sugar inter- conversion
 - iii) Theory of starch= glucose inter- conversion
 - iv) Theory of glycolate metabolism.
6. Transpiration is a “necessary evil”, do you agree with this?
7. Discuss the mechanism of stomatal movement and conditions influencing them?
8. What is transpiration? Explain the mechanism of stomatal movements?
9. How do environmental conditions bring out opening and closing of stomata?
10. What is transpiration? What is its significance for plants? Mention the various factors that affect the rate of transpiration?
11. Differentiate between transpiration and guttation?
12. Write notes on guttation?
13. What is transpiration? Mention various factors that control the process?

UNIT-3 MINERAL NUTRITION AND ABSORPTION OF MINERAL SALTS

Content

- 3.1 Objectives
- 3.2 Introduction
- 3.3 Essential Mineral Elements
- 3.4 Macro-elements
- 3.5 Micro-elements
- 3.6 Absorption of Mineral Salt
- 3.7 Summary
- 3.8 Glossary
- 3.9 Self Assessment Question
- 3.10 References
- 3.11 Suggested Readings
- 3.12 Terminal Questions

3.1 OBJECTIVES

After reading this unit student will be able understand-

- about the Mineral nutrition
- about essential macro-elements, their role, deficiency symptoms, toxicity symptoms
- about the essential micro-elements, role, deficiency symptoms, toxicity symptoms
- about Absorption of mineral salt
- process of Mineral uptake

3.2 INTRODUCTION

To complete the life cycle normally a living organism requires the supply of a large number of substances from outside. The supply is called nutrition.

The autotrophic plants manufacture organic food by the process of photosynthesis. These plants also require some inorganic salts like Potassium, Calcium, Iron, Sulphur etc for their growth. These inorganic substances occur in soil in form of solution. This inorganic nutrition of plants is commonly known as Mineral nutrition.

A complete ash analysis of a plant may show nearly half the elements of the periodic table, but all of them are not required by the plant for nutrition. After they have been absorbed elements are never present inside the plant in the free state. They are generally in ionic form or as constituents of organic compounds.

When plant material is burnt in air, the organic matter is destroyed and a residue of inorganic salts, the ash remains.

3.3 ESSENTIAL MINERAL ELEMENTS

In fact, all elements found in a plant are not essential for its growth and life cycle. An essential element is known without which the plant cannot complete its life cycle. It is clear physiological role to play. For finding out whether an elements is essential or not for a plant, the plant is raised in complete absence of that particular elements under controlled culture conditions. If the plant grows normally, the elements are non-essential and if it does not grow normally, it means that the element is truly essential.

The important criteria for essential elements are as follows-

- 1- These elements are absolutely necessary for supporting normal growth and reproduction of plant.
- 2- These elements are always specific and cannot be replaced by only other elements.
- 3- These elements is directly involved in the metabolism of the plant.

It has since long been known that carbon, hydrogen and oxygen are essential elements for the plant. In the middle of last century water culture and sand culture experiments has established

that the elements nitrogen phosphorus, potassium, magnesium, calcium and iron were indispensable for the plants. In the absence of any one of these elements the growth of shoots or roots are stunted.

The essential elements are classified into two broad categories called (i) Macronutrients and (ii) Micronutrients.

The macronutrients are carbon, hydrogen, oxygen nitrogen, phosphorus, sulphur, potassium, calcium, magnesium are generally present in plant tissues in concentrations of 1 to 10mg per gram of dry matter.

The micronutrients or trace elements are manganese, copper, molybdenum, zinc, boron and chlorine, recently some other such elements have also been discovered, e.g. cobalt, vanadium and nickel.

The microelements are required in very low quantity. i.e., about 0.1 mg per gram of dry matter.

Role of Essential Elements

The most important role of the elements is to participate in various metabolic activities such as regulation of permeability of cell membranes; some elements are required for maintenance of osmotic pressure of cell sap. While others take part in an electron transport system.

3.4 MACRONUTRIENTS

3.4.1- Carbon, Hydrogen and Oxygen

These are not minerals in origin but are discussed here because they enter into the composition of practically all organic compounds present in the plant and accounts for a major part of the dry weight. The significance of water (H₂O) can be felt when it is said that water is the liquid of life. The source for carbon and oxygen is atmosphere and for hydrogen it is water.

3.4.2-Nitrogen

Functions of Nitrogen: The sources of nitrogen are soil and atmosphere. About 78% of nitrogen is found in atmospheric air but this is of no use to plants in its free state. This enters in the plants through stomata along with other gases and comes out in the same state unused. The plants can take nitrogen from the soil in the form of nitrates, nitrites and ammonium salts. The chief sources of nitrate are sodium nitrate, potassium nitrate, ammonium nitrate and calcium nitrate. Besides, there are certain highly specialized organisms called nitrogen fixers, such as bacteria and cyanobacteria. They fix atmospheric nitrogen into the soil in the form of nitrites (NO₂) and Nitrates (NO₃).

Nitrogen deficiency symptoms: Nitrogen deficiency causes yellowing of older leaves (Chlorosis). The plant growth is stunted as protein content, cell division and cell enlargement are decreased. It also causes dormancy of lateral buds, late flowering, purple coloration and wrinkling of cereal grains.

3.4.3-Sulphur

Functions of Sulphur: Sulphur is the constituent of amino acids, vitamin B, coenzyme A and volatile oils. It is absorbed from the soil as sulphate iron. Through the different amino acids it participates in protein synthesis. Sulphur affects an increase in nodule formation in root of leguminous plants.

Sulphur deficiency symptoms: Sulphur deficiency causes yellowing (i.e. chlorosis) of leaves younger leaves are affected first tips and margins of leaves roll inward a hard woody stem due to development of sclerenchyma. Sulphur starvation results in shortage of protein.

3.4.4. Phosphorus

Functions of Phosphorus: Phosphorus is absorbed by the plant from the soil in the form of phosphate ions. It is one of the most important elements for the plants.

Phosphorus is vital structural component of the nucleic acids nucleoprotein, phytin, phospholipids, sugar phosphates, ATP, NADP and numerous phosphorylated compounds. It is an essential element participating in the skeleton of Plasma membrane.

Phosphorus deficiency symptoms: Phosphorus deficiency causes decrease in the rate of protein synthesis. It causes premature leaf fall and purple anthocyanin pigmentation. The leaves become dark blue green in colour and brown necrotic areas are developed on leaves and petioles. The growth of root and shoot is extremely restricted. Flowering is delayed.

3.4.5. Calcium

Functions of Calcium: This element is always found in green plants. The middle lamella of the cell wall consists of calcium pectate. Only because of these elements the permeability of the protoplasm is maintained calcium affects the hydration of colloids. Calcium is believed to be important in regulating metabolic activities as it activates certain enzymes.

Calcium deficiency symptoms: Calcium deficiency causes disintegration of growing meristematic regions of root, stem and leaves. Chlorosis generally occurs along the margins of younger leaves. It also causes malformation of the younger leaves.

3.4.6. Potassium

Functions of Potassium: Potassium is the only monovalent cation essential for plant growth. This element is usually found in the growing regions of the plant. It is one of the constituents of protoplasm. Potassium is essential for the formation of sugar and starch and also for their translocation throughout the plant. It is also needed in cell division, reduction of nitrate, development of chlorophyll, stomata movements etc.

Potassium deficiency symptoms: Potassium deficiency inhibits synthesis of proteins, which results in the accumulation of organic nitrogenous compounds in the plant cells Carbohydrate metabolism is checked. The rate of respiration increases. Mottled chlorosis of leaves occurs Necrotic areas are developed at the tips and margins of leaves.

3.4.7. Magnesium

Functions of Magnesium: It is a constituent of chlorophyll and therefore essential for the formation of this pigment. Magnesium activates enzymes in respiration and photosynthesis. It plays an important role in synthesis of ATP from ADP and inorganic phosphates.

Magnesium deficiency symptoms: Magnesium deficiency causes interveinal chlorosis of the leaves. stem becomes yellowish green, often hard and woody. Deficiency symptoms develop on the older leaves and proceed systematically towards the younger leaves.

3.4.8. Iron

Functions of Iron: Iron is normally absorbed in the ferrous, form though it can be absorbed in the ferric form as well. It plays an important role in the formation of chlorophyll, constitution of the chlorophyll. It plays the role of catalyst. Iron is found in ferredoxin, FRS, flavoprotein and the iron porphyrin protein, which include cytochromes peroxidases and catalases. It therefore plays an important role in respiratory mechanism.

Iron deficiency symptoms: Iron deficiency causes rapid chlorosis of the leaves which is generally interveinal chlorosis may produce a mottled pattern of the leaf may show complete bleaching, or often become necrotic.

3.5 MICRONUTRIENTS

3.5.1 Manganese

Functions of Manganese: Manganese activates many enzymes which are involved in photosynthesis, respiration and nitrogen metabolism. It also plays some role in the synthesis of chlorophyll and in the transfer of electron from H₂O to photo-oxidized chlorophyll in photosynthesis (Homann, 1967)

Manganese deficiency symptoms: Manganese deficiency causes chlorosis which is distinct from that of iron deficiency. The leaf takes mottled appearance. The chloroplast loss chlorophyll and starch grains and become yellow green in colour. Dead tissue spots are found scattered over the leaf.

3.5.2 Copper

Functions of copper deficiency: The element is required in very small quantity. It is very toxic when present in larger quantity. It acts as catalyst in oxidation reduction reactions, since it is a constituent of certain oxidising and reducing agents. Copper helps in formation of starch. It is required for the overall metabolism in plants.

Copper Deficiency symptoms: Copper deficiency causes necrosis of the tips of young leaves. Both vegetative and reproductive growths are reduced. In crops, the younger leaves wither and

show marginal chlorosis of the tips. Grain formation is more severely restricted than vegetative growth.

3.5.3. Zinc

Functions of Zinc: Zinc helps in the formation of chloroplasts. It functions as activator of certain enzymes. e.g. carbonic anhydrase, alcohol dehydrogenase, hexose kinase etc. Zinc is required in synthesis of auxins.

Zinc deficiency symptoms: Zinc deficiency causes reduced stem growth due to decreased synthesis of auxin. It causes chlorosis of older leaves which starts from tips and margin. The absence of zinc also suppresses seed formation and causes malformation in fruiting trees.

3.5.4. Boron

Functions of Boron: Boron differs from the other micronutrients in that there is no evidence to suggest its connection with the enzyme systems and it also differs in that it is absorbed as an anion, i.e. borate and tetraborate, rather than a cation, like the other metallic nutrients.

It is necessary for translocation of sugars and involved in the reproduction and germination of pollens. It is concerned with water reactions in cells and regulates the intake of water into the cell. It also affects flowering and fruiting, cell division, metabolism, active salt absorption, photosynthesis etc.

Boron deficiency symptoms: Boron deficiency causes death of the shoot tip. Flower formation is suppressed, root growth is stunted and shoot apices die. Fruit becomes of small size and root nodules in leguminous plants are not formed and leaves become coppery in texture.

3.5.5. Molybdenum

Functions of Molybdenum: The main role of molybdenum in plants has been found in the nitrogen metabolism. It acts as an activator for the enzyme nitrate reductase. It also helps in formation of proteins. This is absorbed by plant from soil in the form of molybdenum ion (MoO_4^{2-}).

Molybdenum deficiency symptoms

Molybdenum deficiency causes chlorotic interveinal mottling of the older leaves. This may cause nitrogen deficiency, as it is component of enzymes involved in nitrogen metabolism. It also inhibits the flower formation.

3.5.6 Chlorine

Functions of chlorine

Chlorine helps in determining solute concentration and anion cation balance in cells. It is required for cell division in roots and leaves. Chloride ions are essential in the transfer of electrons from H_2O to photo oxidised chlorophyll in photosynthesis.

Chlorine deficiency symptoms: The deficiency of chlorine in plants causes wilting of leaves.

3.6 ABSORPTION OF MINERAL SALT

Besides water, the plant absorbs from the environment consideration quantities of mineral salts, gases and various other salts. All these are absorbed in the form of aqueous solutions. The mineral salts are absorbed from the external solution by the roots.

In plants **mineral absorption**, also called **mineral uptake**. In plants, the entrance portal for mineral uptake is usually through the roots. (Roots, 2005) Some mineral ions diffuse in-between the cells. In contrast to water, some minerals are actively taken up by plant.

Most of the elements required by the plants are absorbed by them from the soil. The clay particles of the soil are present in the form of colloids. The micelles of colloidal clay are usually negatively charged. These charges are balanced by the binding of positively charged ions (cations) which are taken up from the soil solution. In acidic soil H^+ and in alkaline soil Ca^{2+} are the principal cations associated with the clay particles. In the acidic soil the particles may also take up and binds potassium, ammonium and other cations. This reversible binding of cations, a property possessed by clay particles is known as cation exchange. The soil also contains the anions like Cl^- , SO_4^{2-} , HCO_3^- , $H_2PO_4^-$, NO_3^- , and OH^- . Most anions except the phosphate ions leach out of soil rapidly.

Passive Absorption: In most cases, the movement of mineral ions into the root occurs by diffusion. Molecules or ions diffused from a region of their higher concentration to a region of their lower concentration. As these substances diffuse they exert a pressure. The movement of mineral ions into root cells as a result of diffusion is called passive absorption.

1. Mass flow theory (Bulk Flow): According to this theory ions are taken up by the roots along with mass flow of water under the influence of transpiration. Russel and Barber (1960) also supported this theory but raised a question whether the effect of transpiration is direct or indirect. Lopushimsky (1964) worked in this problem and studied the uptake of radioactive P^{32} and Ca^{45} , they found that an increase in the hydrostatic pressure (comparable to transpiration pull) increases ion uptake. So transpiration effect on salt absorption is direct. However, both mass flow theory and direct influence of transpiration have been challenged in view of recent research. Both of these fail to explain salt accumulation against osmotic gradient.

2. Ion exchange: In ionic exchange mechanism anions or cations from within the cells are exchanged for anions or cations of equivalent charge of the external solution in which the tissue is immersed.

The phenomenon has been experimentally confirmed in excised barley roots in which radioactive K^+ ions exchange place with the non-radioactive K^+ ions. A similar exchange mechanism operates between soil solution and clay micelles. The ions get accumulated against a concentration gradient without the participation of metabolic energy because cations and anions

of the external medium get exchanged with H^+ and OH^- ions, which always remain absorbed on the surface of the membrane. H^+ and OH^- are readily available from water.

The process of ionic exchange has been explained by two theories 1 the contact exchange theory and 2-carbonic acid exchange theory. According to the contact exchange theory an ion may be absorbed by the plant root without being first dissolved in the soil solution. An ion absorbed electrostatically to a solid particle such as a plant root or clay micelle, is not held too tightly, but oscillates within a certain small volume of space. An exchange of ions takes place when the oscillation volume of one ion overlaps to oscillation volume of another ion. The soil solution, however, plays an important part in the carbonic acid theory in the it provides the medium for the exchange of ions between the roots and the clay micelles. Carbon dioxide released in respiration combines with water to form carbonic acid in the soil solution. Carbonic acid dissociates into (H^+) and (HCO^-) ions. A cation absorbed to the clay surface may be exchanged with H^+ of the soil solution. This cation then may diffuse to the root surface in exchange for H^+ . The cation then may diffuse to the root surface in exchange for H^+ . The cation may also be absorbed as ion pairs with bicarbonate. Thus ion exchange mechanism would allow for greater absorption of ions from the external medium than could normally be accepted for by the free diffusion.

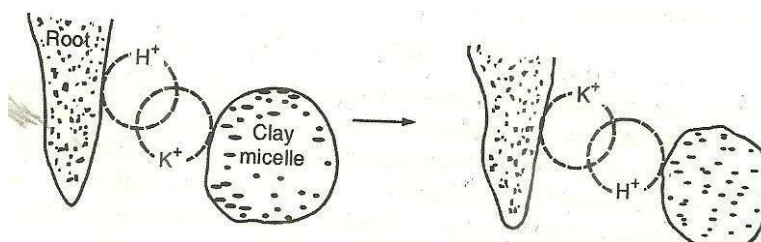


Fig.3.1 Figure explaining cation exchange theory (Contact exchange theory)

3. Donnan equilibrium: This theory explains the passive accumulation of ions that are non-diffusible, which may be present on one side of the membrane (Donnan, 1927). Unlike diffusible ions, the membrane is not permeable to non-diffusible ions. Such ions are called fixed ions. They may be anions or cations. In which there are no fixed ions, there are equal number of anions and cations on both sides of the membrane at equilibrium. But in Donnan equilibrium, in order to balance the charge of the fixed ions (anions) more ions of the order charge (cations) would be required.

For example there is a membrane that separates a cell from the external medium and allows exchange of some ions and not others. To the inner side of this membrane there are anions, which are fixed and non diffusible and therefore the membrane becomes impermeable to these anions. In such a situation for equilibrium to be reached additional cations are needed to balance the negative charges of the anions that are structurally formed to the inner side of the above membrane.

According to the theory, Donnan equilibrium is attained if the product of anions and cations in the internal solution becomes equal to the product of anions and cations in the external solution, depicted by the equation as follows:

$$\frac{\text{Cations inside, } \text{Ci}^+}{\text{Cations outside } \text{Co}^+} \qquad \frac{\text{Anions outside, } \text{Ao}^-}{\text{Anions inside, } \text{Ai}^-}$$

For example, a membrane which is permeable to Mg^+ and Cl^- ions and to X^- ions present inside the cell. Here, membrane has 6X^- fixed ions on the inner side. Cl^- ions make across the membrane by diffusion along the concentration gradient. The concentration of anions on the inner side is now more than that of cations. In order to balance electrochemical equilibrium within the cell sap, Mg^{++} ions move across the membrane against the concentration gradient. Similarly, if these are fixed cations the anions shall move against the concentration gradient to bring about equilibrium.

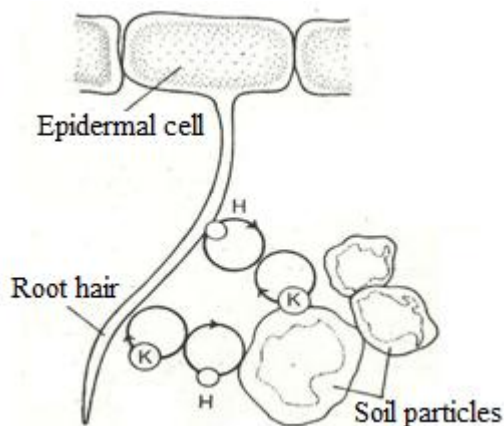


Fig.3.2 Contact exchange theory

Objection to Passive Absorption Concept

Certain strong objections have been raised against passive absorption concept of salt uptake. A few of them are:

- (i) In actual process, the rate of absorption of minerals is too rapid to be explained by passive absorption.
- (ii) No theory of passive absorption adequately explains absorption and accumulation of salts or ions against the osmotic gradients (or against the laws of diffusion). However, cases of extra accumulation of K^+ ions within the cells (like 1000 times as against the surrounding medium) are now frequently known in *Nitella translucens*, *Chara australis* and *Hydrodictyon africanum* (Hober, 1945 and Raven, 1967).
- (iii) It has been experimentally demonstrated that there is a close relationship between salt uptake and metabolic activities. This may be supported by the following examples:
 - a) A quantitative relationship has been found between anion absorption and respiration.
 - b) A close relationship between salt accumulation and respiration is found in all cases. Hopkins (1956) observed that salt accumulation is slowed, and even prevented completely, with the decrease in the oxygen content of the nutrient medium.

- c) The active phase of salt absorption is inhibited by the absence of oxygen, i.e. oxygen is required during salt uptake.
- d) There is a close relationship between metabolic activities and ability to absorb and accumulate solutes.
- e) The metabolic inhibitors influence the salt absorption. Lundegardh (1955) reported that salt uptake is inhibited by oxidase inhibitors azides, carbon monoxides and cyanides (all metabolic inhibitors)
- f) Salt uptake has been found to stimulate and increase the rate of respiration. This increased respiration has been termed as *salt induced respiration*.
- g) Factors like pH, light, oxygen tension and growth affect the salt absorption suggesting that there is some essential role of metabolic activities in salt uptake.

Active Absorption: According to active absorption concept of salt uptake, it is believed that this process is supported by metabolic energy, thus the absorption of ions, involving use of metabolic energy is called active absorption. There have been modification from time to time to discuss the nature of participation of metabolic energy and that is why several theories have been proposed.

Active absorption of solutes or theory of salt accumulation

According to this theory Hoagland (1923) suggested that absorption of solutes takes place against higher concentration of salts. The cells near the tips of roots also have the capacity of accumulating ions (Hoagland and Broyer 1936). If the initial salt content of the root cells is low and if other conditions are favorable, the concentration of ions in the absorbing cells may greatly be increased than that present in soil solution. This involves an expenditure of energy. This energy is supplied by the respiratory activity of the absorbing cells. The rate of accumulation is often influenced therefore by the previous metabolic status of the absorbing cells.

The phenomenon of salt accumulation seems confined largely to cells which have the capacity for cells division and growth, Meristematic cells and cells in the early stages of enlargement are particularly active in absorbing ions. As cells lose their capacity for growth they also lose their capacity of mineral salt accumulation. As already referred earlier that accumulation of salt requires expenditure of energy which is supplied by respiratory activity of cells.

Hoagland and Broyer (1936) found that if excised roots (young roots) are immersed in dilute solutions of mineral salts through which N_2 is bubbled little or no accumulation of salts occurs in the root cells. If on the other hand O_2 is bubbled through the solution, a rapid accumulation of salts within root cells takes place. Lack of O_2 checks aerobic respiration and prevents absorption of ions. The accumulation of ions of root cells and their retention within these cells in a free condition requires an expenditure of energy which is supplied by the process of respiration.

Salt accumulation is also affected by the rate of photosynthesis because on this process depends the supply of carbohydrates which are the respiratory substrates for efficient respiration. So any factor which reduces photosynthesis, also reduces salt accumulation.

The fact that salt accumulation by root cells is dependent on respiration, suggests that temperature may have also marked effect on salt accumulation process.

1. Carrier concept: Ions, which are accumulated in cells, may move into the inner space against concentration and for this movement additional energy is required. This additional energy is derived directly or indirectly through metabolism. This theory of active absorption has been supported by various evidences which show that active ion uptake is carried out by carrier mechanism for both influx and efflux of ions.

Unlike ion channels, the carrier proteins do not have pores. The membrane does not allow the ions to pass through as it is. The activated ions combine with carrier proteins and form ion-carrier complex, which is capable of moving across the membrane. The complex moves across the membrane and reaches the inner surface. Here, the complex breaks and release ions into the cytoplasm of the cell. Carriers are specific, and combine with particular types of ion.

2. Ion Movement into the Root: Mineral nutrients absorbed by the root are carried to the xylem. This absorption takes place by two pathways. They are (i) Apoplast pathway and (ii) Symplast pathway.

(i) Apoplastic pathway- This pathway essentially involves diffusion and mass flow of water from cell to cell through spaces between cell wall polysaccharides.

The ions that enter the cell wall of epidermis move across cell wall of cortex, cytoplasm of endodermis cell wall of pericycle and finally accumulate in xylem vessels.

(ii) Symplast pathway- In this pathway, ions that enter the cytoplasm of epidermis move across the cytoplasm, cortex, endodermis and pericycle through plasmodesmata, and finally reach to xylem vessels.

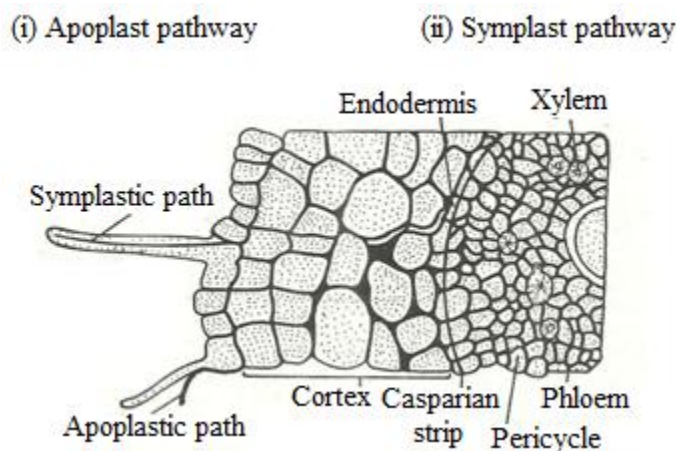


Fig.3.3 Apoplastic and symplastic pathways of ion absorption

Table-1 Differences between Passive Absorption and Active Absorption

S.No.	Passive Absorption	Active Absorption
1	This processes physical driving force, which is non-metabolic	Here the driving force is energy derived from metabolic processes.
2	This type of absorption of ions and molecules is spontaneous and proceeds towards equilibrium	This type of absorption is not spontaneous and does not proceed towards equilibrium
3	Such absorption of a substance occurs across a protoplasmic membrane from its higher to lower chemical potential.	The active absorption of a substance occurs across a protoplasmic membrane from its lower to higher chemical potential i.e. against concentration gradient.
4	In passive absorption the energy yielding metabolic processes are not involved.	When energy yielding metabolic processes are weakened, active transport system is also checked.
5	The passive transport takes place through the protoplasmic layer in between the cell wall and the vacuole.	The active transport takes place across the protoplasmic membrane (i.e. plasma membrane, tonoplast, etc.)

3.7 SUMMARY

In this unit we discussed about the mineral nutrition. The plants require some inorganic salts like Potassium, Calcium, Iron, Sulphur etc for their growth. These inorganic substances occur in soil in form of solution. This inorganic nutrition of plants is commonly known as Mineral nutrition.

An essential elements are without which the plant cannot complete its life cycle. In fact, all elements found in a plant are not essential for its growth and life cycle. If the plant grows normally, the elements are non-essential and if it does not grow normally, it means that the element is truly essential.

The essential elements are classified into two broad categories called (i) Macronutrients and (ii) Micronutrients.

The macronutrients are carbon, hydrogen, oxygen nitrogen, phosphorus, sulphur, potassium, calcium, magnesium. The micronutrients or trace elements are iron, manganese copper, molybdenum, zinc, boron and chlorine, Recently some other such elements have also been discovered, e.g. cobalt, vanadium and nickel.

Besides water, the plant absorbs from the environment consideration quantities of mineral salts, gases and various other salts. All these are absorbed in the form of aqueous solutions. The mineral salts are absorbed from the external solution by the roots. In plants **mineral absorption**, also called **mineral uptake**. In plants, the entrance portal for mineral uptake is usually through

the roots. Some mineral ions diffuse in-between the cells. In contrast to water, some minerals are actively taken up by plant.

3.8 GLOSSARY

Adenosine-A nucleoside containing a heterocyclic nitrogen base (adenine) and a pentose sugar.

Adenosine monophosphate (AMP) Adenine nucleoside with two phosphate molecules of which terminal one is energy rich.

Adenosine diphosphate (ADP) Adenine nucleoside with two phosphate molecules of which terminal one is energy rich.

Adenosine triphosphate (ATP) Adenine nucleoside with three phosphate molecules, the last two molecules are energy rich.

Anthocyanins- Blue and red glycoside pigments (flavonoid) found dissolved in cell sap.

Assimilation-Uptake and formation of simple foodstuff.

Auxin-A group of hormones of plants which is synthesized by growing tips of stems and roots and regulate many aspects of plant growth, e.g. IAA.

Biotin-Vitamin H.

Carbohydrate- Compound containing carbon, hydrogen and oxygen with general formula $C_n(H_2O)_n$ e.g. sugars, starch cellulose.

Carotenoids-Yellow, orange or red pigments, soluble in oil solvents. These pigments are absent such as carrot roots, floral petal etc.

Cell wall-In plant cells the outer layer surrounding the plasma membrane. It consists of cellulose, hemicelluloses and pectic substances.

Chlorophyll-Green pigment found in autotrophic plant.

Chloroplast-Chlorophyll containing plastid bodies and the site of photosynthesis.

Chlorosis-Disease of green plants in which green colour is lost and characteristic yellow colour appears.

Cytoplasm-All the protoplasm of a cell excluding nucleus.

Diffusion pressure deficit-Net capacity of plant cell for absorbing water.

FAD-Flavin adenine dinucleotide.

FMN-Flavin mononucleotide.

Starch-The principle reserve food materials of the green plants, frequently found in colourless plastid.

Tonoplast-Also called vacuolar membrane.

3.9 SELF ASSESSMENT QUESTION

3.9.1 Multiple Choice Questions:

1. In soil, water available to the root is:

- (a) Capillary water (b) Hygroscopic water
(c) Gravitational water (d) Combined water

2. Water enters into root hairs from soil on account of:

- (a) Turgor pressure (b) Osmotic pressure
(c) Suction pressure (d) Root pressure

3. In passive absorption water is absorbed due to:

- (a) Osmosis (b) transpiration pull
(c) High PDP of root hairs (d) All of the above

4. Which one of the following is not required by plants for their normal healthy growth?

- (a) Magnesium (b) Lead
(c) Calcium (d) Iron

5. Presence of phosphorus in a plant:

- (a) Brings about healthy root growth (b) Promotes fruit ripening
(c) Retards protein (d) None of the above

6. Die back disease of citrus is caused by:

- (a) Virus (b) fungus
(c) Bacterium (d) Cu-deficiency

7. On the basis of symptoms of chlorosis in leaves a student inferred that this was due to deficiency of nitrogen. This inference could be correct only if yellowing appeared first in:

- (a) Young leaves (b) Old leaves
(c) Young leaves followed by old leaves (d) Old leaves followed by young leave

8. Plastocyanin is protein containing:

- (a) Mo (b) Zn
(c) Mg (d) Cu

9. Absence of Mg^{++} ions from plant tissue results in:

- (a) Plasmolysis (b) Hydrolysis
(c) Chlorosis (d) Necrosis

10. Sick leaf disease occurs due to deficiency of:

- (a) P (b) K
(c) Cu (d) Proline

11. Sulphur is the constituent of

- (a) Tryptophan
- (b) Valine
- (c) Methionine
- (d) Proline

12. Khaira disease of rice is the consequence of:

- (a) Boron deficiency
- (b) Zinc deficiency
- (c) Magnesium deficiency
- (d) All of the above

13. The importance of micro-elements was recognized rather belatedly because

- (a) They were toxic to plants
- (b) They were lost by plants through roots
- (c) Plants physiologists did not find them in plants
- (d) They were being absorbed as contaminants

14. Which elements is common to nucleic acids phospholipids ATP ADP NADPH

- (a) Boron
- (b) Zinc
- (c) Phosphorus
- (d) Sodium

15. Which of the following is required for synthesis of ascorbic acid

- (a) Zinc
- (b) Iron
- (c) Copper
- (d) Cadmium

16. Which of the following are required for chlorophyll synthesis

- (a) Cu and Ca
- (b) Fe and Mg
- (c) Mg and Cu
- (d) B and Fe

17. An element concerned with the photolysis of water during light reaction of photosynthesis is

- (a) Mg
- (b) Mn
- (c) Zn
- (d) Fe

18. Active transport of elements across the cell membrane required

- (a) ATP
- (b) Phlorogucinol
- (c) Acetyl choline
- (d) Cyclic AMP

19. Deficiency symptoms of nitrogen first appears in

- (a) Young leaves
- (b) Old leaves
- (c) Both a and b
- (d) Developing fruit

20. In plants Ca is present in the form of

- (a) Calcium acetate
- (b) Calcium pectate

- (c) Calcium carbonate (d) Calcium oxalate
21. Molybdenum is components of
(a) Phosphate reductase system (b) Nitrate reductase system
(c) Phosphorylase (d) Photolysing water system
22. Plants obtain nitrogen from the soil in the form of
(a) Nitrates (b) Free Nitrogen
(c) Nitrogen oxide (d) None of these
23. Elements which maintains the solubility of calcium in the cell is
(a) Manganese (b) Copper
(c) Iron (d) Boron
24. Which elements has be taken exclusively from external atmosphere by the terrestrial plants
(a) Nitrogen (b) Phosphorus
(c) Carbon (d) Potassium
25. Which of the following in not a part of enzyme but it activates the enzyme
(a) Mg (b) K
(c) Zn (d) Mn
26. Absence of magnesium ions from the soil usually results in
(a) Plasmolysis (b) Chlorosis
(c) Necrosis (d) Etiolation
27. In Plants chlorosis is caused due to deficiency of
(a) Boron (b) Iron
(c) Copper (d) Calcium
28. Four elements found in abundance in plants and making upto 90% of all elements are
(a) H, O, C, N (b) C, H, O, S
(c) C, H, O, P (d) C, N, O, P
29. Which of the following elements in the constituent of chlorophyll?
(a) Fe (b) Mn
(c) Mg (d) Cu
30. The following are common in translocation of carbohydrate in the plants?
(a) Fe (b) Mo

(c) B

(d) Zn

31. The following are common symptoms developed due to deficiency of Ca, Mg, K and Mo

(a) Formation of anthocyanins

(b) Appearance of necrotic spot

(c) Chlorosis

(d) Vein banding

32. Which of the following are called storage elements?

(a) C, N, S, P

(b) Ca, C, N, K

(c) N, P, K, B

(d) Ca, B, N, O

33. What features of an element is not required to prove essential

(a) Plants cannot complete vegetative and reproductive cycle in absence of it

(b) It cannot be substituted by another element

(c) It directly participates in metabolism

(d) It is found in all tissue of plants

34. Advantages of hydroponic cultures are that:

(a) Plants can be grown with controlled nutrients

(b) Natural calamities such as floods and drought can be avoided

(c) Pest problems can be kept controlled

(d) All of these

35. Deficiency symptoms of Mg, Zn, K and N occur first in old leaves which indicates that:

(a) These elements are non mobile

(b) These are readily mobile

(c) These are less mobile

(d) These require ATP for their mobility

36. Symptoms like--- terminal buds die, young leaves become hooked, die back at margins or tips appears due to deficiency of:

(a) Ca

(b) K

(c) B

(d) Mo

37. Necrosis occurs due to deficiency of:

(a) K and Mg

(b) Zn and Ca

(c) Mo

(d) All of these

38. Symptoms of phosphorus deficiency are

(a) Symptoms appear in young leaves

(b) Symptoms are localized

(c) Lower leaves are usually red or yellow

(d) Terminal buds die and chlorosis

39. Whiptail of *Brassica* is caused due deficiency of

- (a) Zinc (b) Molybdenum
(c) Copper (d) Manganese

40. Top sickness of tobacco is caused due to deficiency of

- (a) Molybdenum (b) Boron
(c) Manganese (d) Copper

3.9.1. Answer Key:-

1-(a), 2-(c), 3-(b), 4-(b), 5-(c), 6-(d), 7-(a)8-(d), 9-(c), 10-(a), 11-(d), 12-(b), 13-(d), 14-(c), 15-(c), 16-(b), 17-(b), 18-(c), 19-(b), 20-(b), 21-(b), 22-(a), 23-(d), 24-(c), 25-(b), 26-(b), 27-(b), 28-(a), 29-(c), 30-(c), 31-(b), 32-(a), 33-(c), 34-(d), 35-(b), 36-(a), 37-(d), 38-(c), 39-(b), 40-(b)

3.10 REFERENCES

- S.N. Pandey and B.K. Sinha, Plant Physiology
- B.P. Pandey, Botany for Degree Students
- V.Verma, Plant Physiology
- S.K. Gupta, Plant Physiology

3.11 SUGGESTED READING

- Plant Physiology by S.N. Pandey and B.K. Sinha
- Botany for Degree Students by Dr. B.P. Pandey
- Plant Physiology by V.Verma
- Plant Physiology by S.K. Gupta

3.12 TERMINAL QUESTION

3.12.1 Long Answer Type Questions:

1. Describe two important functions each of K, Fe and Zn in green plants and also write deficiency symptoms of any two of them.
2. Write an essay on mineral nutrition in plant.
3. List the macronutrients and mention their major function.
4. Differentiate between micronutrient and macronutrients. How they are helpful in plant growth?
5. Describe the role and deficiency symptoms of the following elements in plants
(a) Nitrogen
(b) Molybdenum

(c) Zinc

(d) Phosphorus

(e) Copper

6. What are macronutrients? Discuss the role of potassium phosphorus, sulphur and iron in plant metabolism.

7. Make a list of macronutrients and mention their major functions.

UNIT-4 ORGANIC SUBSTANCES: THEIR TRANSPORT AND TRANSLOCATION

Content

- 4.1 Objectives
- 4.2 Introduction
- 4.3 Transport of organic substances
- 4.4 Mechanism of phloem transport
- 4.5 Concept of source and sink
- 4.6 Factors affecting translocation
- 4.7 Summary
- 4.8 Glossary
- 4.9 Self Assessment Question
- 4.10 References
- 4.11 Suggested Readings
- 4.12 Terminal Questions

4.1 OBJECTIVES

After reading this unit students will be able-

- To study structural organization of phloem tissue
- To understand significance and mechanism of phloem translocation
- To learn about different theories of translocation
- Different environmental factors affecting the process of translocation.

4.2 INTRODUCTION

Green plants are photosynthetic organisms which prepare their own food by the process of photosynthesis. This process of photosynthesis occurs in leaves (mesophyll cells), the photosynthetic products after synthesis are confined to mesophyll cells. But photosynthetic product (sucrose) is required by all the parts of plant for their growth and development. Hence translocation of solutes is a process by which solutes (mainly photosynthetic product) is transported from leaves (where they are synthesized) to other parts of plant (stem, roots, fruits, etc). The process of translocation of solutes occurs through phloem tissue. Phloem tissue is made up of different types of cells, each of which performs a specific function in the process of translocation. Translocation of solutes is a pressure driven transport process, solutes are transported from the region where their concentration is high to the regions which require nutrients. Several physiological and environmental factors such as temperature, oxygen, age of plant, seasonal variation, developmental stage of plant, water etc affect the rate of translocation.

Structure of phloem tissue

In most plant species, phloem is made up of phloem fibres, phloem parenchyma, sieve cells (sieve elements) and their accompanying companion cells

Sieve cells

Sieve elements are responsible for conduction of sugar and other organic materials in all parts of the plant. Sieve elements have no nucleus and only a sparse collection of other organelles. Sieve tube elements join to form continuous tube. **Pores** in sieve plate between sieve tube elements are open channels for transport. Each sieve tube element is associated with one or more **companion cells**.

Companion cells

Companion cells are living cells, nucleated with protoplasm and are associated with sieve cells. These cells are attached to sieve cells through protoplasmic strands and help in phloem loading. Companion cells contain large number of mitochondria for cellular respiration to provide the energy which acts as driving force for translocation of solute. There are three types of companion cells ordinary companion cells, transfer cells and intermediary cells.

- (a) **Ordinary Companion cells:** These type of companion cells contain chloroplasts with developed thylakoids, smooth inner cell wall, and few plasmodesmata and are connected to it's own sieve plate only.
- (b) **Transfer cells:** These also contain well developed thylakoids. They possess fingerlike cell wall ingrowths which increase surface area of plasma membrane for better solute transfer. Ordinary as well as transfer companion cells are specialized for taking up solutes from apoplast or cell wall space
- (c) **Intermediary cells:** These types of companion cells take up solutes through cytoplasmic connections. They have large number plasmodesmata connects to their surrounding cells. Compared to ordinary and transfer cells they have poorly developed thylakoids.

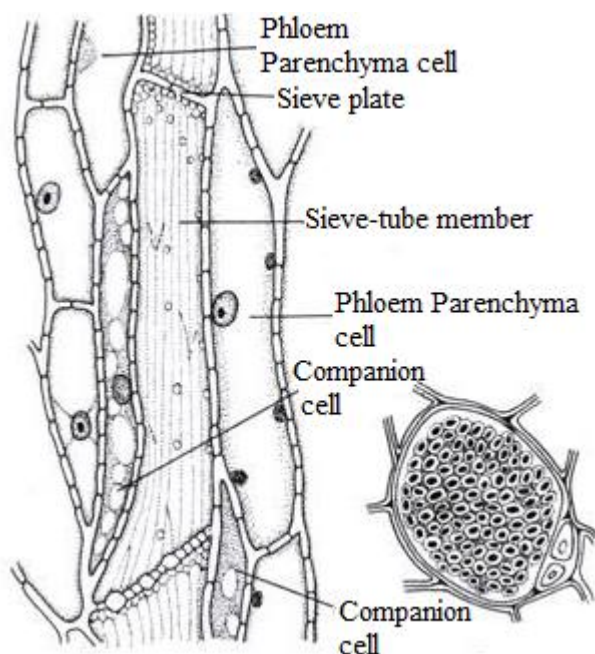


Fig.4.1 Organization of phloem tissue

Phloem parenchyma cells are living, with thin cells and store food materials. These help a lateral translocation of water and other solutes.

Phloem fibres are thick walled cells and provide mechanical support to phloem cells. Transfer cells are helpful in lateral transport of food materials and water.

Protective mechanism of phloem

Several factors are known to cause damage to phloem tissue. Major factors include insect feeding on sugars, damage caused by wind, effect of high and low temperature, pollution, etc. Damage caused by any of the factor results in loss of sugars, hence to prevent this plant possesses different protective mechanism which includes deposition of P-protein and callose protein. P-protein is found to occur in several forms such as tubular, fibrillar, crystalline and also depends plant species and age. P protein function to seal off damage caused to sieve elements as

they plugg up pores present in sieve plate. P- protein is a short term solution to damage caused to sieve cells. In comparison to P- protein callose is a long term solution to seal off damage caused to phloem tissue. Callose is a β -(1,3)-glucan, which is synthesized in functioning sieve elements by their respective plasma membranes.

Evidences which show translocation occurs through phloem

Experimental evidences in support of the fact that translocation of sugars takes place in phloem are quite substantial and satisfactory. A few of them are given here:

(i) Ringing experiment: Curtis (1925) conducted ringing experiments. In one set he removed a ring including phloem and in another set a ring including xylem but excluding phloem. A third set was kept intact; shoot parts above the ringed parts in the ringed sets were defoliated. To keep the cut portion moist it was enclosed in a glass cylinder filled with water. Phloem removed plants showed much less growth and elongation in the upper defoliated part while the stem in which only xylem was removed showed good growth and more elongation. It shows that phloem is the tissue through which most of the food is translocated in upward direction.

(ii) Exudation incision in bark: When an incision is made in the bark of a deciduous tree, there is an exudation of liquid containing sugars in high concentration. A careful examination reveals that the exudates come from sieve tubes elements.

(iii) Evidences from tracer techniques: The use of tracer techniques has extended further support to the fact that phloem is the tissue through which organic compounds are translocated downwards. Burr and others (1945) allowed a bean leaf to photosynthesise in an atmosphere of carbon isotope (^{13}C and ^{14}C) and observed that labelled sugar moved in the phloem. This technique has also shown clearly that sugar moves in sieve elements rather than in other phloem cells.

(iv) Chemical analysis: Chemical analysis of phloem reveals that these are relatively richer in carbohydrate and organic nitrogen compounds as compared to lower percentage in xylem tissue. It is obvious, therefore that solute moves through phloem.

4.3 TRANSPORT OF ORGANIC SUBSTANCES

Translocation of solutes can also be defined as process of redistribution of, photosynthesis products and other organic compounds (metabolites, hormones) and some of the mineral nutrients. Amino acids, proteins, organic acid, mineral element like Mg, K⁺, Cl^- , PO_4 are also transported during the process of translocation along with photosynthetic product. Carbohydrates (sugars) are the most important metabolite transported during the process of translocation. Nitrogen gets translocated mainly in form of amino acid glutamate, aspartate, glutamine, asparagine. Plant hormones such as cytokinin, abscisic acid, GA_3 are also transported through

sieve tubes during phloem translocation. Proteins such as ubiquitin, chaperons, thiorodoxin, P-protein are also transported through phloem translocation. Sugars can be classified as reducing or non reducing based upon presence or absence of free aldehydic or ketonic group. Only non reducing sugars are transported during the process of translocation as they are non reactive. None of the reducing sugar like glucose, mannose, fructose are transported during translocation as they are too reactive to be transported through phloem tissue due to presence of free aldehydic or ketonic group. Sucrose is the most common (and major) sugar transported through phloem tissue from source to sink. Sucrose is a disaccharide made up of one unit of glucose and one unit of fructose. Beside sucrose other sugars transported during translocation include raffinose, stachylose, verbascose, etc. Most of these sugars contain galactose unit(s) attached to sucrose.

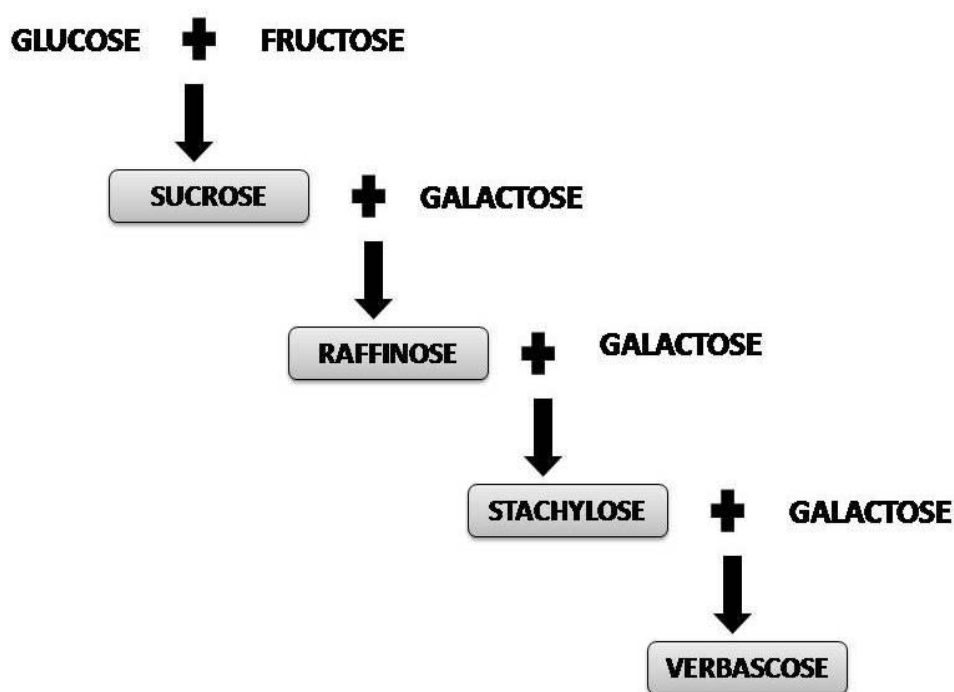


Fig. 4.2: Different types of sugar transported during phloem translocation

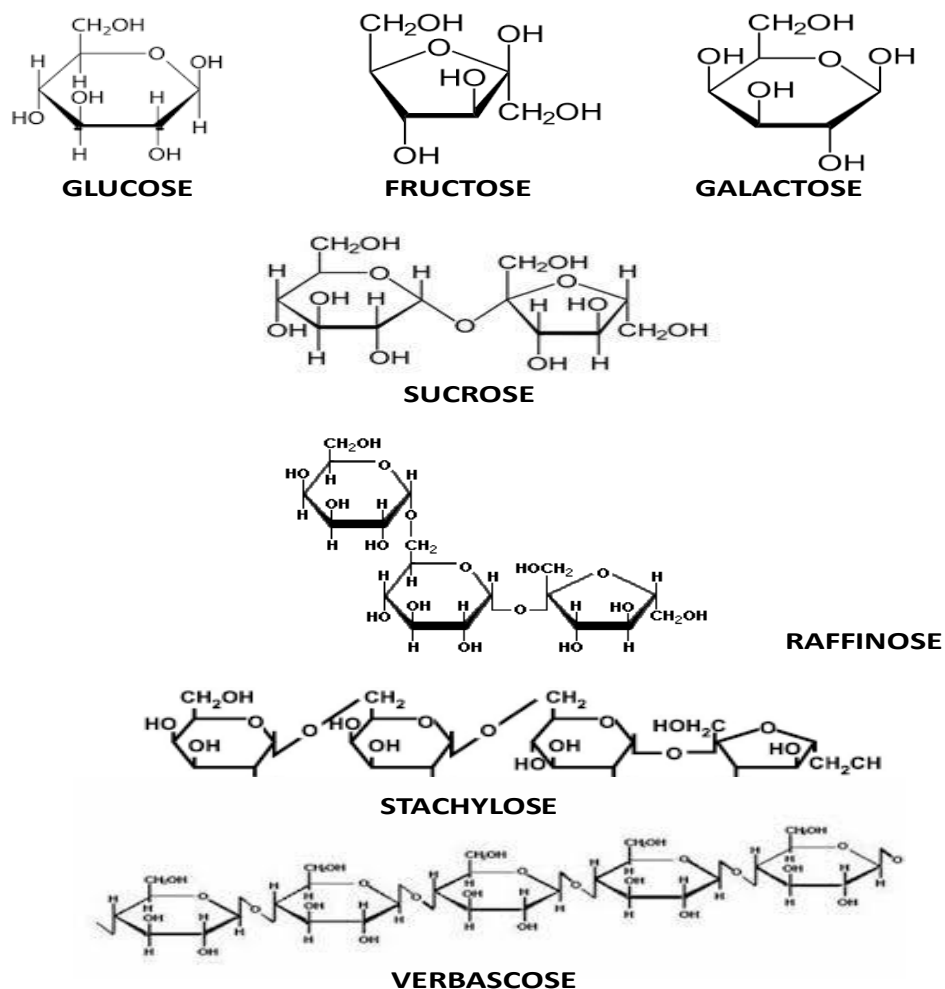


Fig. 4.3 Structure of some of reducing and non-reducing sugars

4.4 MECHANISM OF PHLOEM TRANSPORT (PHLOEM LOADING AND UNLOADING)

This transfer of sugars (photosynthetic) from mesophyll cells to sieve tube elements in the leaf is called as phloem loading. On the other hand, the transfer of sugars (photosynthetic) from sieve tube elements to the receiver cells of consumption end (i.e., sink organs) is called as phloem unloading. Both are energy requiring processes. The movement of sugars from mesophyll cells to sieve tubes of phloem may occur either through symplast (i.e., cell to cell through plasmodesmata, remaining in the cytoplasm) or the sugars may enter the apoplast (i.e., cell walls outside the protoplasts) at some point en route to phloem sieve tubes. In the latter case, the sugars are actively loaded from apoplast to sieve tubes by an energy driven transport located in the plasma membrane of these cells. The mechanism of phloem loading in such case has been called as sucrose- H^+ symport or cotransport mechanism. According to this mechanism (Fig.15.5) protons (H^+) are pumped out through the plasma membrane using the energy from ATP and an

ATPase carrier enzyme, so that concentration of H^+ becomes higher outside (in the apoplast) than inside the cell. Spontaneous tendency toward equilibrium causes protons to diffuse back into the cytoplasm through plasma membrane coupled with transport of sucrose from apoplast to cytoplasm through sucrose - H^+ symporter located in the plasma membrane

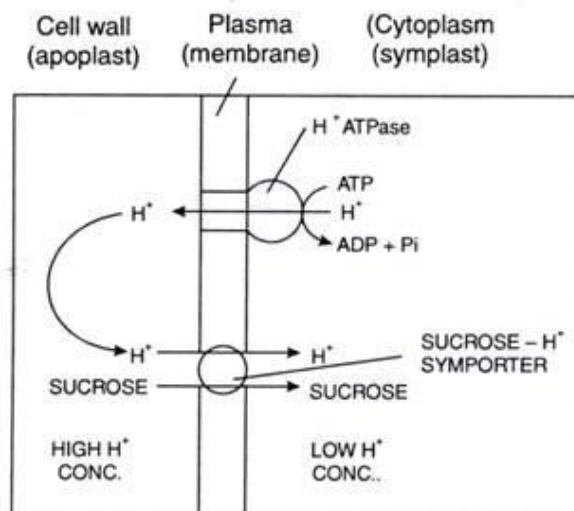


Fig.4.4: Transpore of sucrose across cell membrane

Phloem loading is specific and selective for transport sugars. Both symplastic and apoplastic pathways of phloem loading are used in plants but in different species. In some species however, phloem loading may occur through both the pathways in the same sieve tube element or in different sieve tube elements of the same vein or in sieve tubes in veins of different sizes.

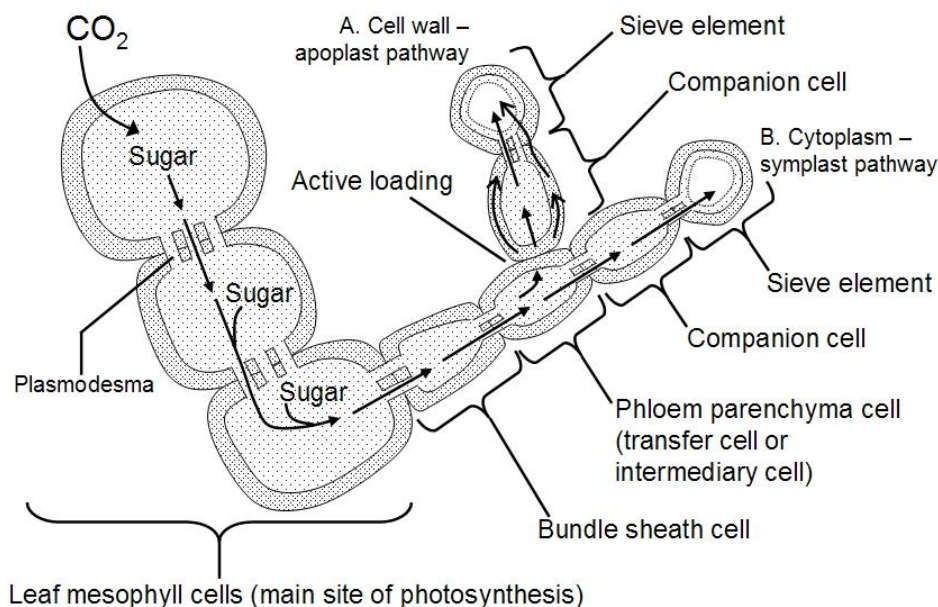


Fig.4.5 Process of phloem loading

Phloem Unloading: It occurs in the consumption end or sinks organs (such as developing roots, tubers, reproductive structures etc.)

Sugars move from sieve tubes to receiver cells in the sink involving following steps:

(i) Sieve element unloading: In this process, sugars (imported from the source) leave sieve elements of sink tissues.

(ii) Short distance transport: The sugars are now transported to cells in sink by a short distance pathway which has also been called as post-sieve element transport.

(iii) Storage and metabolism: Finally, sugars are stored or metabolized in the cells of the sink.

As with the phloem loading process, sucrose unloading also occurs through symplast via plasmodesmata or through apoplast at some point en route to sink cells. Phloem unloading is typically symplastic in growing and respiring sinks such as meristems roots, and young leaves etc. in which sucrose can be rapidly metabolized. (Young leaves act as sink until their photosynthetic machinery is fully developed, at which point they become sources).

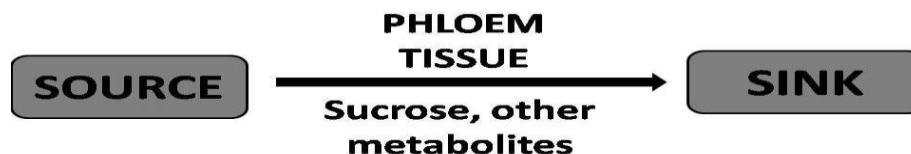
Differences between apoplast and symplast mode transport of molecules

S.No.	Apoplast pathway	Symplast pathway
1	It consists of non-living parts of plant body like cell wall, intercellular spaces.	It consists of living parts of plant body like protoplast, connected through plasmodesmata.
2	Movement through apoplast pathway faces little resistance.	Movement through symplast pathway faces comparatively more resistance.
3	The process is faster as compared to symplast movement	The process is slow as compared to apoplast movement

4.5 CONCEPT OF SOURCE AND SINK

The part or organ of plant which produces or stores photosynthetic product (photosynthate) and other metabolites are known as source. Leaves are predominant source. Sink is defined as any non photosynthetic part or organ of a plant which cannot synthesize their own metabolites (mainly sucrose) and hence needs these metabolites to be supplied to them from photosynthetic part of plant.

Source	Sink
Leaves Storage organs during exporting phase	Roots Tubers Fruits Immature leaves



PROCESS OF TRANSLOCATION

The general pattern of translocation is from source to sink, but all sources do not supply to all sink. Generally, metabolites are supplied to each sink through nearest source. Some sources specifically supply to specific sink. Upper leaves normally supply to growing shoot tips and young and immature leaves. Lower leaves predominantly supply to roots of the plant, whereas intermediate leaves transport metabolites in both (upward and downward) directions.

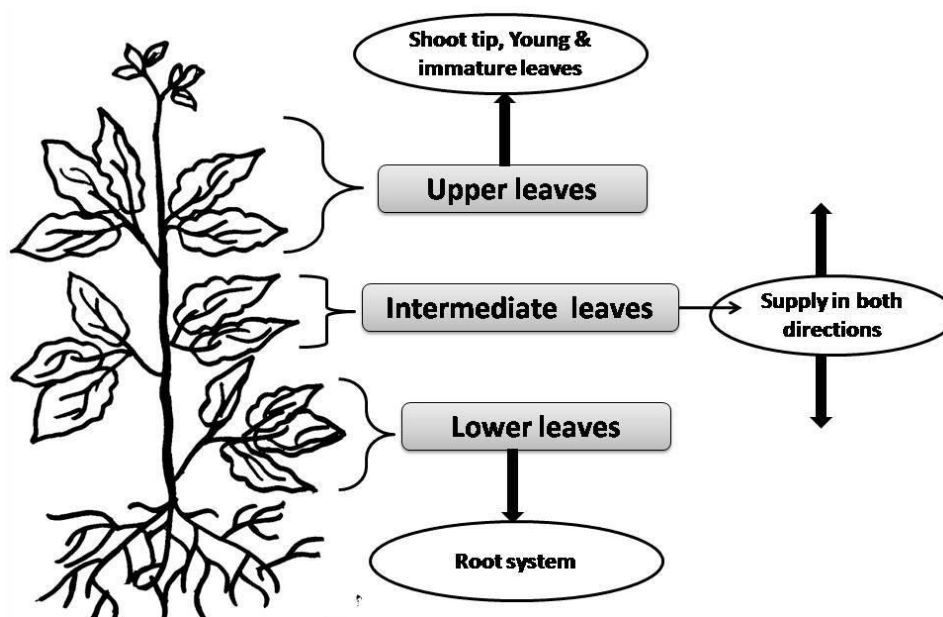


Fig.4.6 General pattern of translocation of solutes

Roots and stem are the major sink during vegetative growth (non reproductive phase) of the plant. However during flowering and fruiting season (reproductive phase) flowers, fruits and seeds become predominant sink and most of the leaves supply nutrient and metabolite to fruits and seeds. Also, the plants in which roots and underground stem act as storage organ such roots and stem act as source during growth phase and accumulate nutrients during growth phase. Such storage roots and stem act as sink and the nutrients stored in these roots and stem are utilized for growth of shoots especially during non growth phase.

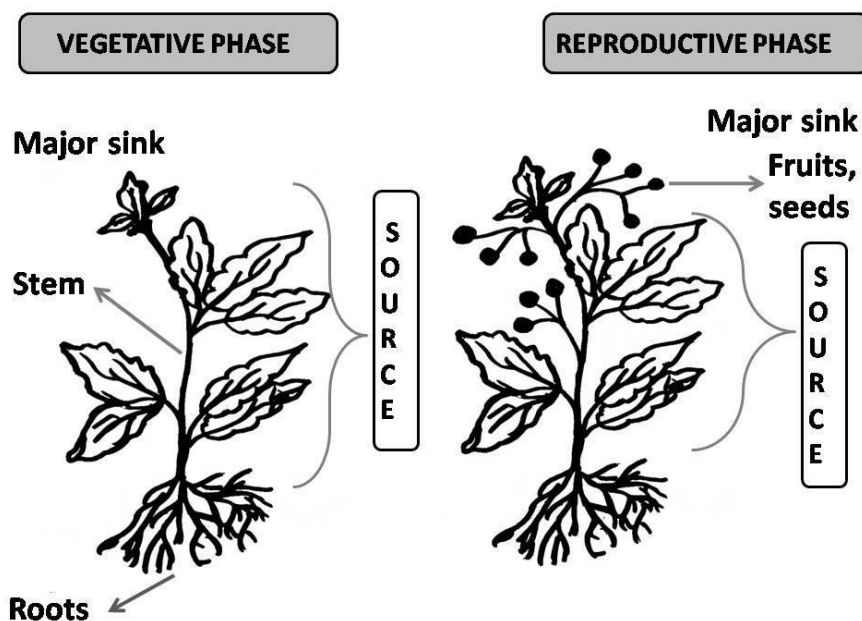


Fig.4.7: Source and sink relationship during vegetative and reproductive phase

Directions of translocation

Translocation of solutes can occur in different directions depending upon the relative positioning of source and sink. Also, direction of translocation is influenced by developmental stage of the plant.

1. Downward Translocation: The most common direction of translocation of solutes is downwards. The leaves photosynthesize food in excess (more than their own requirement). This excess food is trans-located downwards to stem (either for storage, metabolism or maintenance of its cells) and roots for their growth and development.

2. Upward Translocation: Translocation of solutes can also occur in upward direction. Two main examples of upward translocation of solutes include-

- (i) Deciduous plants, where renewal of growth and development of new leaves depend on upward transport of reserve food (nutrient) stored in the roots and stems.
- (ii) Also, for formation and growth of stem apices, formation of flowers and fruits during reproductive season is dependent upon the movement of sugars and other metabolites from leaves in upward direction.

3. Lateral Translocation: Translocation of solutes can also occur in lateral direction.

4. Bidirectional Translocation: Movement of sugars have also been reported to move from leaves in both upward as well as downward directions.

Different theories of mechanism of translocation

1. **Diffusion hypothesis:** many scientist (physiologist) proposed that translocation of solutes occurs from their higher concentration to lower concentration through the process of simple diffusion. But strongly criticised the main objection to the theory was that process of

diffusion the main objection to the theory was that process of diffusion is a slow process of whereas solute (food) molecules are transported at a much higher rate in phloem tissue.

2. **Activated diffusion hypothesis:** In 1936 Mason and Phillis proposed the concept of activated diffusion hypothesis according to which streaming protoplasm of sieve tubes possess some kind of mechanism which activates diffusion of solutes molecules.
3. **Electro-osmotic theory:** this theory proposed by Fenson Spanner (1958) . According to this theory flow of liquid through pores can occur at a faster rate under influence of electroosmotic gradient than simply under osmotic gradient. However theory lacks experimental evidence.
4. **Interfacial flow hypothesis:** Van den Honert (1932) proposed this theory according to which solute molecules can be absorbed and dispersed at interface, which is due to reduction of surface tension.
5. **Protoplasmic streaming theory:** de Vries (1885) proposed protoplasmic streaming theory. According to this theory solute molecules are transported from one end of sieve tube to another end by streaming movement of protoplasm. Transport of solute molecules from one sieve tube to next sieve tube occurs by simple diffusion through sieve pores present between two sieve tube. The theory hence accounts for movement of solute upward and downwards simultaneously.

Support of theory:

1. All living cells shows protoplasmic streaming.
2. Streaming movement is faster as compared to diffusion.
3. Solutes are known to be transported bidirectionally.
4. Rate and direction of translocation are net dependent upon concentration gradient and osmotic gradient.
5. Factors affecting protoplasm streaming are also known to affect translocation.

Objection to the theory:

1. Age old sieve tube elements do not show active protoplasmic streaming but they are actively involved in the process of translocation.
 2. Rate of protoplasmic streaming is slower as compared to the rate at which translocation of solute occurs.
-
6. **Munch mass flow hypothesis:** The hypothesis was proposed by Munch (1927-1930). This hypothesis is also known as pressure flow hypothesis protoplasm of sieve tubes is connected by plasmodesma which forms an uninterrupted permeable system known as symplast. According to this theory solute gets accumulated in leaves as a result of photosynthesis. This increase osmotic potential of leaves due to which water is absorbed from xylem elements and turgor pressure directs transport of solution (containing solute) into sieve tube (phloem).

In other region of plants such as roots and storage organs the solutes are utilized or get converted into insoluble form. This result in lowering of osmotic pressure which lowers turgor pressure overall this results in turgor pressure gradient with high pressure in leaves (source) and lower pressure in roots (sink). Hence water with solutes flows from source to sink.

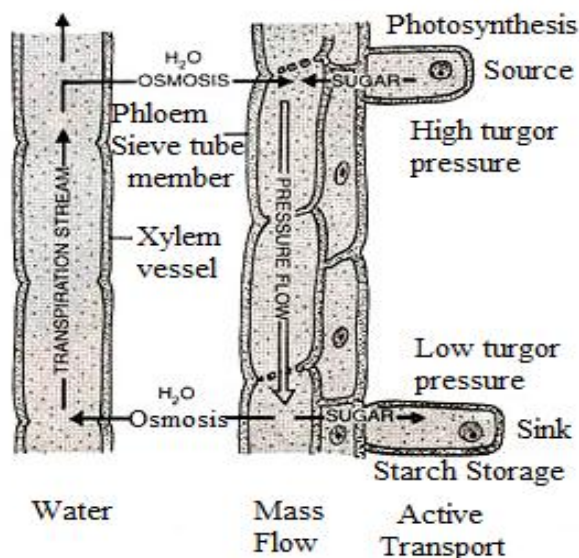


Fig.4.8: Representation of Munch mass flow hypothesis

Evidence in support of munch hypothesis

1. Mass flow hypothesis is supported by the fact that if a cut is made in phloem region of stem of plant, the sap which exudates (comes out) contains high sugar content and the flow of this exudates from the cut continues for more than 24 hours.
2. Theory is based on good physical properties along with adaptation of mature sieve elements to the mechanism.
3. Another evidence includes pressure of positive concentration gradient in many plants.

Objection to the theory

1. The theory explains only unidirectional flow whereas actually the translocation in a bidirectional process.
2. Osmotic pressure at supply end (leaves) and osmotic pressure at consumption end (roots) may not be in accordance with the principle of munch mass flow hypothesis.
3. Low temperature and pressure, metabolic inhibitors effects on translocation supports participation of cytoplasm in transport of solutes whereas the hypothesis provides only a passive role to sieve tube.
4. The hypothesis is unable to account for rapid rate of translocation in certain plants.

4.6 FACTORS INFLUENCING TRANSLOCATION

Several factors influence the movement of solutes inside the plant. The important factors are:

1. **Temperature:** Temperature influences the velocity as well as amount of transport. The rate of transport is higher between 20 to 30°C. Curtis (1929) observed that lowering of temperature decreased the transport of carbohydrates. Hewitt and Curtis (1948) observed that rate of transport increases from 4°C to 30°C and there occurs a sharp decrease in translocation at 40°C.
2. **Concentration gradient:** Translocation of carbohydrates increases with the decrease in concentration gradient. The increased rate of translocation in day time in comparison to night is due to the different concentration gradients.
3. **Light:** Light directly and indirectly both affects rate of translocation. Light wavelength 650 nm to 760 nm are more effective in increasing rate of translocation.
4. **Metabolism:** Translocation also depends on metabolic state of tissues. It is more dependent on source and consuming end tissues as compared to conducting tissues.
5. **Oxygen:** A sufficient supply of oxygen is required for the translocation of solute through phloem. Deficiency of oxygen directly decreases the rate of translocation.
6. **Water:** Because food is always translocated in form of solution, water is also essential for the process. Water supply greatly affects the turgidity of cells, their concentration gradient, hence affects the rate of translocation also.
7. **Auxin:** Amount of auxin, produced in an organ or part of plant and the continuity of its formation controls the translocation of food into the organ or part. That is why food is readily translocated in meristematic and storage regions.
8. **Minerals:** Boron is associated with translocation of solutes. Deficiency of Boron and K⁺ causes decrease in translocation.
9. **Age:** Age of plant also affects the translocation, which is more in young tissues in comparison to the old tissues.

4.7 SUMMARY

1. Translocation is defined as movement of sucrose and other organic molecules from one part of plant to another.
2. Translocation occurs through phloem tissue.
3. Translocation occurs from the part of plant which possesses higher sucrose concentration.
4. Only non reducing sugars are transported during the process of translocation because of their low reactivity.
5. Reducing sugars are not transported during the process of translocation since they have exposed aldehydic or ketonic group which makes them reactive to be transported through phloem tissue.

6. The parts of plant body (generally leaves) from which sugars are transported to other parts of plant are called source and the region, part or organ to which sugars are transported are called as sink.
7. Generally nearest source supplies to nearest sink.
8. Lower leaves supply solutes to roots, upper leaves supply solutes to growing shoot tips and young and immature leaves and intermediate leaves supply solutes to different part of plant in both the direction.
9. Sucrose is main sugar transported during translocation.
10. Some species also translocate sugars such as stachyose and raffinose.
11. Main component of phloem tissue are sieve element and companion cell.
12. Companion cells provide energy in the form of ATP for the process of translocation of solutes.
13. Sieve tube elements join to form a continuous tube.
14. A sieve tube is associated with one or more companion cells.
15. Pores present in sieve plate acts as open channel for solute transport.
16. Sieve tube cells and companion cells are linked through plasmodesmata.
17. Companion cells are of three types ordinary, transfer and intermediary.
18. Sieve elements are prone to damage by insect, wind and pollution.
19. P protein seal off the damage caused in sieve element.
20. Transport of solute during translocation is driven by pressure gradient between source and sink due to difference in concentration of solutes.
21. Transport of sugars from mesophyll cells into sieve tube element and companion cells is called phloem loading.
22. Transport of sugars and other solutes from phloem tissue into sink (roots, stem) is called phloem unloading.
23. Both phloem loading and unloading are active process.
24. Movement of solutes may occur through apoplast or symplast pathway.
25. Symplastic translocation occurs through plasmodesmata.
26. Rate of translocation is affected by several factors such as temperature, light, oxygen, water and age of plant.
27. A number of theories have been proposed for translocation of solutes.
28. These theories include diffusion hypothesis, electrodiffusion theory, protoplasmic streaming theory, mass flow hypothesis and Münch flow hypothesis.

4.8 GLOSSARY

Plasmodesmata: are microscopic channels which traverse the cell walls of plant cells and some algal cells, enabling transport and communication between them.

Reducing sugar: Reducing sugar is a sugar that is capable of acting as a reducing agent because it has a free aldehyde group or a free ketone group. All monosaccharides are reducing sugars, along with some disaccharides, oligosaccharides, and polysaccharides.

Phloem: Vasacular tissues present in plants that conduct foods made in the leaves to all other parts of the plant.

Translocation: Transport of solutes from site of synthesis or storage to other part of plant where they are required for metabolic activities.

Companion cell: Type of cell presents in phloem tissue, associated with sieve cells and provide energy for the process of translocation.

Source: Part or organ of plant where metabolites such as sucrose are synthesized.

Sink: Part or organ of plant to which metabolites and solutes are transferred from source.

Deciduous: A plant whose parts, particularly leaves, are shed at regular intervals or a given stage of development.

Vegetative growth (or phase): The period of growth between germination and flowering is known as the vegetative phase of plant development.

Reproductive growth (or phase): Phase which involves production of flowers, fruits and seeds.

Phloem loading: Phloem loading is the process whereby carbohydrates enter the sieve tubes at the source.

Phloem unloading: Photoassimilate (sugars) removal from phloem and delivery to recipient sink cells.

Apoplast: Inside a plant, the apoplast is the space outside the plasma membrane within which material can diffuse freely.

4.9 SELF-ASSESSMENT QUESTIONS

4.9.1 Multiple Choice Questions:

- The most commonly translocated sugar during translocation is
 - Sucrose
 - Galactose
 - Starch
 - Glycogen
- Phloem loading and unloading is an
 - Active process
 - Passive process
 - Facilitated diffusion
 - Both active and passive process.
- Mechanical support is provided to phloem cells by
 - Plasmodesmata
 - Phloem fibres
 - Companion cells
 - P-Proteins and callose
- Direction of translocation of solute is
 - Lateral only
 - Upward and downward

- (c) Lateral, upward and downward (d) None of the above
5. Deficiency of oxygen
(a) Decreases rate of translocation (b) Increases rate of translocation
(c) Doesn't have any effect on rate of translocation (d) None of the above
6. P Protein is found in
(a) Gymnosperms only (b) Angiosperms only
(c) Only monocots (d) Dicots and monocots
7. Which of the following statement is NOT correct
(a) Companion cells are living cells
(b) Companion cells are provided energy in form of ATP
(c) Companion cells are enucleated.
(d) Companion cells are associated with sieve elements.
8. Translocation involves transport of
(a) Reducing sugars only (b) Non reducing sugars only
(c) Reducing and non reducing sugars (d) Non sugar moieties
9. Sieve tube cells and companion cells are linked through
(a) Plasmodesmata (b) Membrane protein
(c) Cell walls (d) Cell membrane
10. Sieve elements can be damaged by
(a) Insects (b) Wind
(c) Pollution (d) All of above
11. Movements of solutes can occur through
(a) Symplast (b) Apoplast
(c) Both a and b (d) Through plasmodesmata only
12. Which of the following is not phloem unloading
(a) Transport of sucrose from phloem to roots
(b) Transport of sucrose from phloem to fruits
(c) Transport of sucrose from phloem to stem
(d) Transport of sucrose from phloem to xylem
13. Phloem loading involves transport of sugar from
(a) Companion cell to mesophyll and sieve tube
(b) Mesophyll cell to companion cell and sieve tube

- (c) Sieve tube to companion cell and mesophyll
- (d) Sieve tube to mesophyll cell only

14. Ordinary transfer and intermediate are types

- (a) Companion cells
- (b) Sieve cells
- (c) Plasmodesmata
- (d) P-Protein

15. Select the correct statement

- (a) Sucrose is the only sugar transported during translocation.
- (b) Apoplastic translocation occurs through plasmodesmata.
- (c) P-Protein seals off damage caused in sieve element.
- (d) Rate of translocation is independent of age of plant.

16. Deciduous plants require _____ transport of nutrients for renewal of growth

- (a) Downward
- (b) Upward
- (c) Lateral
- (d) None of the above

17. Which of the following is NOT translocated during translocation

- (a) Cytokinin and glutamate
- (b) Water and sucrose
- (c) Cholesterol and sucrose
- (d) None of the above

18. Roots which store food act as

- I. Source in vegetative season
- II. Sink in vegetative season
- III. Source in reproductive season
- IV. Sink in reproductive season

- (a) Both I and III are correct
- (b) only II is correct
- (c) Both II and III are correct
- (d) I, II and III are correct.

19. Which of the following is a long term solution to seal off damage caused to sieve element

- (a) P-protein
- (b) Callose
- (c) Sucrose
- (d) None of the above

4.9.2 Fill in the blanks:

1. Phloem fibres provide _____ support to phloem cells.
2. _____ cell provide energy to sieve elements .
3. Protoplasmic streaming theory was given by _____.
4. _____ is most common sugar transported during translocation.
5. _____ of oxygen decreases rate of translocation.
6. Light wavelength of _____ to _____ nm increases rate of translocation.

7. Raffinose is made up of _____ and _____.
8. _____ is made up of glucose and fructose.
9. _____ seals of damage in sieve element .
10. _____ transport occurs through plasmodesmata.
11. Pores present in _____ are open channels for transport.
12. Sieve tube cells and companion cells are joined together by _____.
13. _____ and _____ are major sink during vegetative growth.
14. Lower leaves generally supply solutes to _____.
15. Transport of sugars during translocation is an _____ process.

4.9.3 State whether the statement is true or false:

1. P- protein is present in gymnosperms
2. Activated diffusion hypothesis was given by Mason and Phillis.
3. Mass flow hypothesis is also known as pressure flow hypothesis.
4. Phloem loading is transport of solute from sink to source.
5. Mass flow hypothesis fails to account for rapid movement of solute in certain plants.
6. Ringing experiments proved that phloem tissues is responsible for translocation of solutes.
7. Deficiency of boron decreases rate of translocation.
8. Translocation of sugar decreases with decrease in concentration gradient.
9. Translocation is more in old tissue as compared to young tissues.
10. Companion cells are living and nucleated.
11. Process of phloem loading and unloading is an active process.
12. Transport of sugars through mesophyll cells occurs through symplastic diffusion.
13. Sieve cell and sieve tubes comprise main conducting tissue for translocation of solutes.
14. Lowering of temperature increases transport of sugars.
15. Sieve tube elements contains well defined nucleus.
16. Translocation is affected by availability of oxygen.
17. Callose is a long term solution for damaged sieve elements.
18. Non photosynthetic organs generally act as sink.
19. During translocation photosynthetic products are transported from sink to source.
20. High concentration of sucrose in apoplast increases phloem loading.

4.9.1 Answers Keys: 1-a, 2-a, 3-c, 4-c, 5-a, 6-d, 7-c, 8-b, 9-a, 10-a, 11-c, 12-d, 13-b, 14-a, 15-c, 16-b, 17-c, 18-c, 19-b

4.9.2 Answers Keys: 1-Mechanical, 2-Companion, 3- DeVries, 4- Sucrose, 5- Deficiency, 6- 650, 760, 7- galactose and sucrose, 8-Sucrose, 9- P-protein, 10- Symplastic, 11- Sieve plate, 12-Plasmodesmata, 13- roots, stem, 14-Roots, 15-active.

4.9.3 Answers Keys: 1-F, 2-T, 3-T, 4-F, 5-T, 6-T, 7-T, 8-F, 9-F, 10-T, 11-T, 12-T, 13-T, 14-F, 15-F, 16-T, 17-T, 18-T, 19-F, 20-T

4.10 REFERENCES

- Plant Physiology: Lincoln Taiz and Ederordo Zeiger Sinauev Associates, Inc. publishers.
- Plant Physiology: V Verma, Ane Books India.
- Plant Physiology: SN Pandey and B K Sinha , Vikas publishing House PVT.Limited.
- Text book of Plant Physiology C.P. Malik, S.K.Srivastava, Kalyani Publications New Delhi

4.11 SUGGESTED READING

- Text book of Plant Physiology C.P. Malik, S.K.Srivastava, Kalyani Publications New Delhi.
- Plant Physiology: H S Srivastava, Rastogi and Company.
- Plant Physiology: G Ray Noggle and George J Fritz Practice Hall of India . private Limited New Delhi.
- Plant Physiology: Frank B Salisbury and deonw Ross Wadsworth Biology Series.
- Plant Physiology: Lincoln Taiz and Ederordo Zeiger Sinauev Associates, Inc. publishers.
- Plant Physiology: V Verma, Ane Books India.
- Plant Physiology : SN Pandey and B K Sinha, Vikas publishing House PVT.Limited

4.12 TERMINAL QUESTIONS

4.12.1 Very short answer type questions:

1. Define translocation?
2. Mention the role of companion cells in translocation of solutes?
3. Which molecules are transported during translocation
4. What is plasmodesmata?
5. What are P-protein.
6. What is the function of callose.
7. What is symplastic transport?
8. Name any four factors affecting rate of translocation?
9. What do you understand by source and sink?
10. What are reducing sugars?
11. Give two examples of sugar transported during translocation?
12. Mention the monomer components of raffinose and sucrose?
13. Name different types of companion cells?
14. State protoplasmic streaming theory?
15. Why reducing sugars are not transported during phloem translocation?

4.12.2 Short answer type questions:

1. What is protoplasmic streaming theory? Mention evidences in support and objection to the theory?
2. Briefly describe about phloem loading and unloading.
3. Mention about different directions of translocations of solutes?
4. Write short note on Munch hypothesis?
5. Define translocation. Which cells are involved in the process of translocation?
6. Mention about the theory which best explains the mechanism of translocation.
7. Mention about role of plasmodesmata in translocation of solute.
8. How does symplastic and apoplastic transport differ from one another?
9. Mention about different cells present in phloem tissue?
10. Describe about ringing experiment?
11. Giving examples with structure mention which type of sugars are transported during translocation?
12. Differentiate between
 - (a) Reducing and non reducing sugars
 - (b) Phloem loading and unloading
 - (c) Symplastic and apoplastic transport

4.12.3 Long answer type questions:

1. Describe the process of phloem loading and unloading?
2. What is translocation of solutes? Mention about different factors affecting translocation?
3. Describe about various theories of translocation?
4. What do you understand by source and sink. How do they vary according to developmental stage of plant?
5. With well labelled diagram describe about structure of phloem tissue?
6. What are reducing and non reducing sugars? Why only non reducing sugars are transported during the process of translocation?
7. What is Munch mass flow hypothesis? Also mention evidence in favour and objections to mass flow hypothesis?
8. How does apoplastic pathway differ from symplastic pathway of transport? Support your answer with well labelled diagram?

BLOCK -2: METABOLISM

UNIT-5 PHOTOSYNTHESIS

Content

- 5.1 Objectives
- 5.2 Introduction
- 5.3 Significance
- 5.4 Historical Aspects
- 5.5 Photosynthetic Pigments
- 5.6 Concept of Two Photosystems
- 5.7 Photophosphorylation
- 5.8 Calvin Cycle / Dark Reaction
- 5.9 C₄ Pathways
- 5.10 CAM Plants
- 5.11 Photorespiration
- 5.12 Summary
- 5.13 Glossary
- 5.14 Self Assessment Question
- 5.15 References
- 5.16 Suggested Readings
- 5.17 Terminal Questions

5.1 OBJECTIVES

After reading this unit students will be able to-

- Understand the significance and mechanism of photosynthesis.
- Learn about different photosynthetic pigments.
- Understand the mechanism of photophosphorylation.
- Distinguish between light and dark reactions.

5.2 INTRODUCTION

The autotrophic plants synthesize enormous amounts of organic food with the help of the light energy available from Sun. Carbohydrates produced through photosynthesis constitute the basic raw materials, which directly or indirectly give rise to all the organic components of virtually all plants and animals. The entire humanity depends upon the prepared food of plants. Every year some 200 billion tons of carbon go through the photosynthetic process. It is one of the most massive chemical events going on the earth. It has been estimated that plants take up 7×10^{11} tons of CO_2 to produce roughly 5×10^{11} tons of solid plant material. Approximately 90 per cent of the world's photosynthesis is carried out of marine and freshwater algae.

5.3 SIGNIFICANCE

Photosynthesis is a crucial energy converting process by which plants produce molecular oxygen and carbohydrates by the use of photons present in the light. The natural source of light, the Sun, helps the green colored plants to fix the atmospheric carbon dioxide into usable molecular oxygen that we humans happen to breathe.

They help in maintaining a balanced level of oxygen and carbon dioxide in the atmosphere. Almost all the oxygen present in the atmosphere can be attributed to the process of photosynthesis, which also means that respiration and photosynthesis go together. Also, the chemical energy stored in plants is transferred to animal and humans when they consume plant matter. Photosynthesis can therefore be considered the ultimate source of life for nearly all plants and animals by providing the source of energy that drives all their metabolic processes.

In a nutshell, the process of photosynthesis benefits us in the following ways.

1. Photosynthesis converts inorganic raw materials into food that provides our ecosystem with energy.
2. Green plants provide organic food to all the animals and humans.
3. Rare fossil fuels like coal, petroleum and natural gas are formed through the degradation of the past plant and animal parts which were originally formed by photosynthesis.

4. Plant products like timber, rubber, herbs, medicines, resin and oils are derived from photosynthesis.
5. Photosynthesis helps in providing oxygen in the atmosphere required by all living organisms.

5.4 HISTORICAL ASPECTS

From the time of **Aristotle** until the 17th century it was generally believed that plants derived all their nutrition from the plant and animal debris of the soil. In the early seventeenth century **J.B. van Helmont** (1577 – 1644), a Belgian physician who cultivated a willow plant in a container for five years with enough watering concluded that it was water and soil which contributed to the growth of the plant. In 1699, **Woodward** propounded the view that vegetables were not formed of water but of a certain peculiar terrestrial matter, which was absorbed along with water.

It was left to **Stephen Hales** (1727), an English clergyman, an illustrious contemporary of **Issac Newton**, often referred to as ‘father of plant physiology’ to point out that green plants may get part of their nourishment through their leaves from the air and sunlight.

In 1779, **Ingenhousz**, who was a physician to the emperor of Austria, got interested in Priestley’s papers and reported that plants ‘purified’ the air only in the presence of light. He found that the same tissue made air ‘impure’ in dark. He also wrote that only the green parts of the plant produced the purifying agent (oxygen), while non-green tissue contaminated the air. Thus, Ingenhousz recognized the participation of chlorophyll and light in the photosynthetic process. He performed about 500 experiments to show that the plants purified the air.

In 1804, **de Saussure** confirmed the finding of Ingenhousz regarding the gas exchange of the two types: one in light (photosynthesis) and another in darkness (respiration). He also discovered that water was also utilized in the process.

Dutrochet (1837) confirmed that the green part (chlorophyll) was essential for photosynthesis.

Liebig (1840), German agricultural chemist, reported that the sole source of carbon in plants was carbon dioxide of the air and that the oxygen was released from CO₂.

Boussingault (1860-65) conducting an experiment on volumetric equality of carbon dioxide absorbed and oxygen given out in light showed that plants obtained their total requirements of carbon from carbon dioxide of air.

Englemann (1888) gave the action spectrum of photosynthesis.

Warburg (1919) was the first to use the green alga *Chlorella* for study of photosynthesis.

5.5 PHOTOSYNTHETIC PIGMENTS

The Photosynthetic products are energy rich organic compounds. The potential chemical energy of these compounds comes from the light energy.

The light energy to be effective in photosynthesis must be absorbed by a suitable pigment. This vital role is performed by the green pigment, chlorophylls, in plants.

5.5.1 Chlorophyll

There are at least seven types of chlorophylls known: **chlorophylls a, b, c, d and e**, **bacteriochlorophyll** and **bacterioviridin**. All these chlorophyll molecules contain a tetrapyrrole skeleton formed into a ring with an atom of magnesium in the centre of the ring. A so-called pyrrole molecule contains a skeleton of five atoms, four of carbon and one nitrogen and the five are arranged in a ring. Four such pyrroles arranged in a ring form the head of a chlorophyll molecule. Attached to this porphyrin ring at one point is an alcohol (phytol) “tail”, a long chain of linked carbons. Relatively minor variations in the kinds and groupings of other atoms joined to this head and tail skeleton account for the differences among different kinds of chlorophylls.

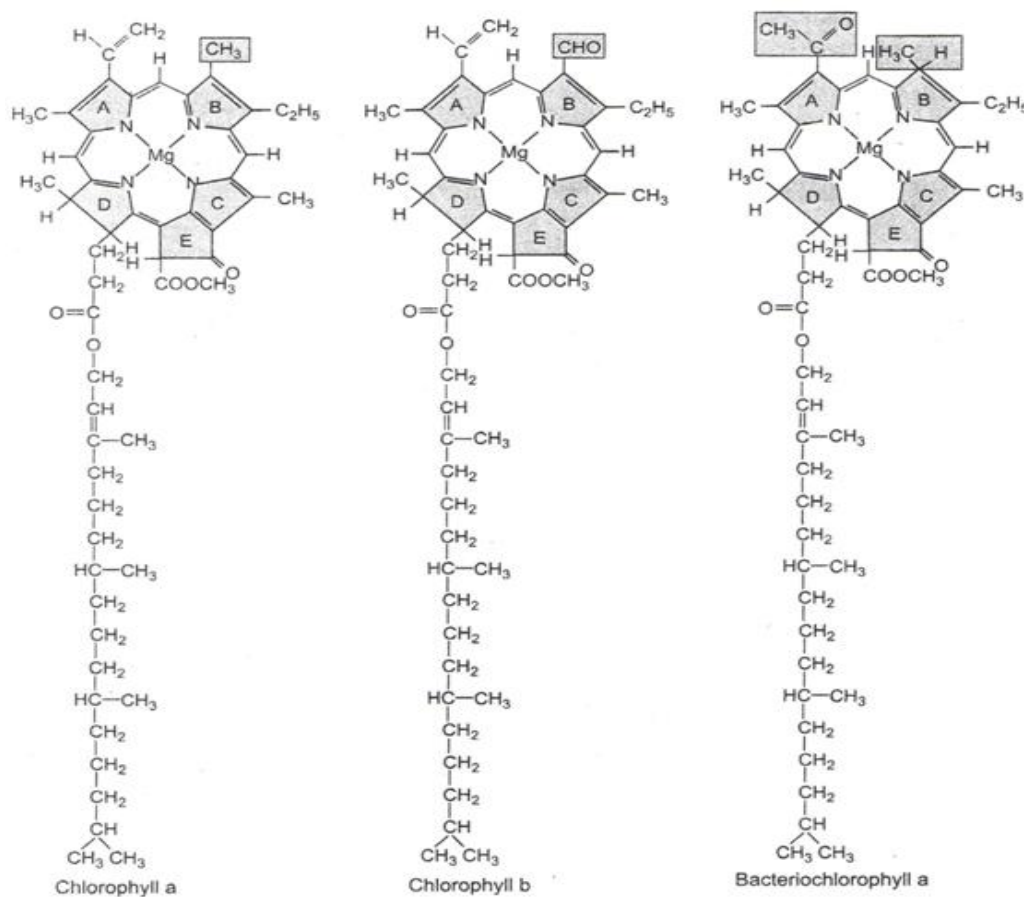


Fig.5.1 Structures of Chlorophyll a, chlorophyll b and bacteriochlorophyll

Chlorophylls 'a' and 'b' are the two most abundant chlorophylls. Chlorophyll a is found in all the autotrophic plants except the photosynthetic bacteria. Chlorophyll b is absent in the blue green, brown and red algae. The other chlorophylls c, d, e are found only in algae and in combination with chlorophyll a. Chlorophyll a possess $-\text{CH}_3$, a methyl group which is replaced by $-\text{CHO}$, an aldehyde group in chlorophyll b. The structures of chlorophyll a, chlorophyll b and bacteriochlorophyll are given in Fig.5.1.

The molecular formulae of the chlorophylls are given below:

Chlorophyll a : $\text{C}_{55}\text{H}_{72}\text{O}_5\text{N}_4\text{Mg}$

Chlorophyll b : $\text{C}_{55}\text{H}_{70}\text{O}_6\text{N}_4\text{Mg}$

Both the chlorophylls a and b have hydrophilic Mg – Porphyrin head and a lipophilic phytol tail.

The chlorophylls are primarily located within the grana thylakoids. The chlorophyll molecules form a monolayer between the protein and lipid layers of the membranes of the thylakoids. The hydrophilic heads of the chlorophyll molecules are embedded within the protein layer while the lipophilic tails are located within the lipid layer.

5.5.2 The Absorption Spectrum

The portion of the electromagnetic spectrum which participates in photosynthesis is from 300 to 900 nm. In green plants only the visible spectrum (400-750 nm) is effective in photosynthesis. Photosynthetic green bacteria can absorb wavelengths from 375-800 nm while purple photosynthetic bacteria absorb 300 to 950 nm.

The chlorophyll pigments chiefly absorb in the violet blue and red parts of the spectrum. The absorption band in the violet blue region is called Soret band.

The chlorophyll a has two prominent absorption peaks at 430 nm and 662 nm. There are reports of different forms of chlorophyll a with absorption peaks at 660, 670, 680, 685, 690 and 695 to 720 nanometers. These variations are perhaps due to the environmental changes. The absorption peaks for chlorophyll b occur at 453 and 642 nm as shown in Fig.5.2.

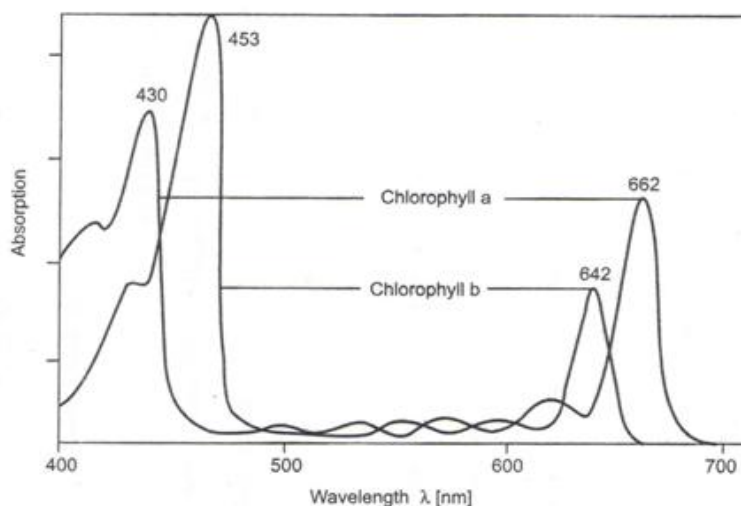


Fig.5.2 Absorption spectrum of chlorophyll a and chlorophyll b

The solubility properties of the two pigments differ. Chlorophyll a dissolves very well in petroleum ether while chlorophyll b in methyl alcohol.

The distribution of pigments and their absorption peaks are given in Table-1

S.No.	Type of Pigments	Distribution in Plant Kingdom	Absorption peaks in cells in nm
1	Chl a	All green Plants	435 670-680
2	Chl b	All green plants except diatoms brown, red and blue green algae	480 650
3	Chl c	Diatoms and brown algae	- 645
4	Chl d	Some red algae	- 740
5	Protochlorophyll	Etiolated Plants	- -
6	Bacterioviridin	Green Sulphur Bacteria	- 750 or 760
7	Bacteriochlorophyll	Purple sulphur bacteria	- 800, 850 and 890

5.5.3 Carotenoids

The carotenoids are the main accessory pigments in photosynthesis. They transfer the light energy to chlorophyll for photosynthesis. The carotenoids are widely distributed in plants. They occur in bacteria, algae and higher plants. They include orange carotenes and yellow xanthophylls. They absorb wavelength 400 nm to 500 nm because of which they are orange in colour. Of the carotenes β -carotene is the abundant type. It absorbs blue light, and, therefore, appears yellow in colour.

α -carotene is present in very small amounts in certain species. The carotenoids perform two types of functions in the green plants. They trap light energy and transfer it to the chlorophyll a particularly in algae and to some extent in higher plants. In higher plants this function is performed by lutein of the xanthophylls and β -carotene.

At high light intensities the entire cell apparatus is oxidized by atmospheric oxygen into carbon dioxide. This process is termed photo oxidation which is as good as combustion. The carotenoids (β -carotene) protect the photosynthetic apparatus from this type of destruction by trapping and dissipating the excess excitation energy which would have otherwise converted molecular oxygen to a highly reactive and mutagenic superoxide O_2 . The dissipation of excess energy in the form of heat is facilitated by xanthophylls cycle.

Carotenoids consist of long chains of carbon atoms linked by conjugated single and double bonds with six carbon rings as each end Fig.5.3.

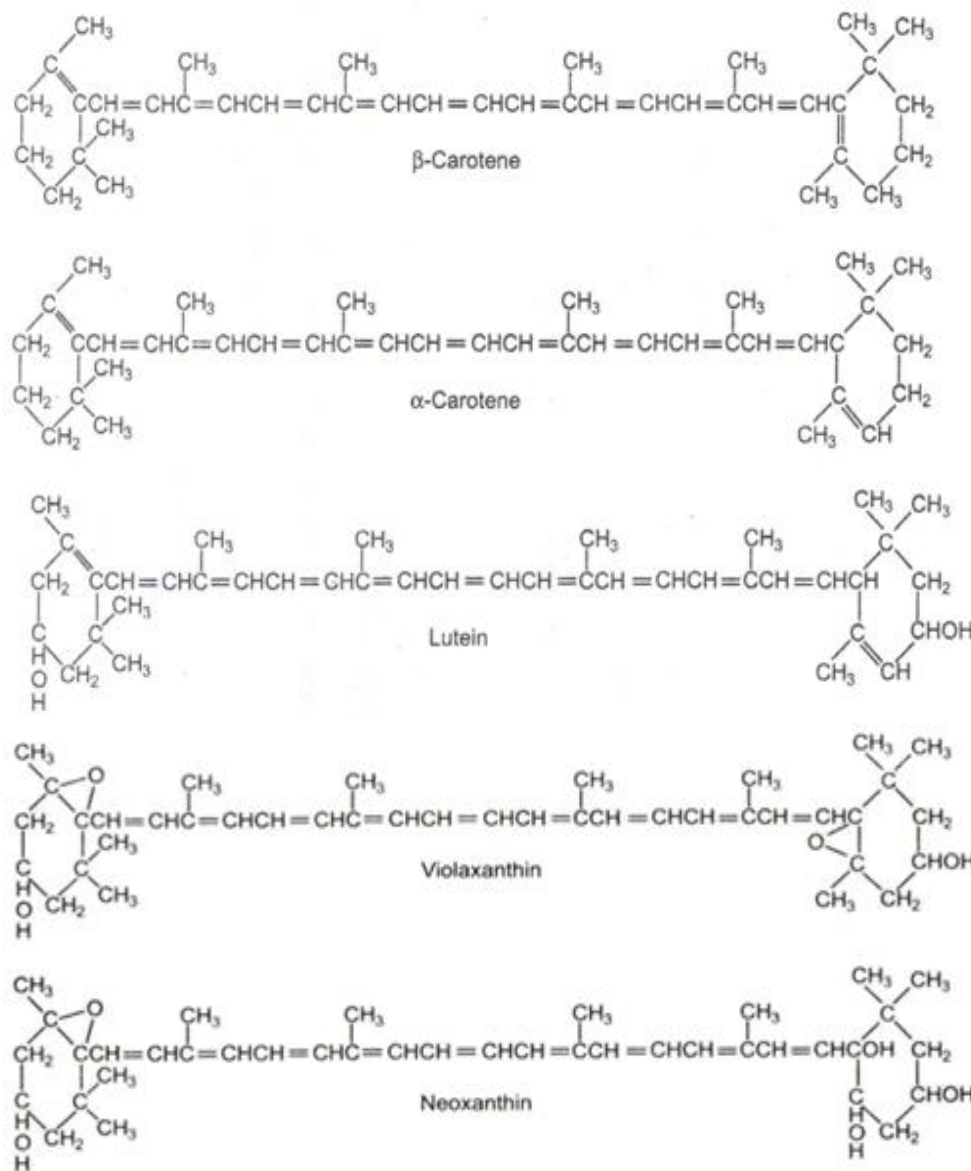


Fig.5.3 The molecular structures of the major carotenoids of higher plants

The carotenes are hydrocarbons i.e. they contain carbon and hydrogen. The xanthophylls (also known as carotenols) are alcohols and ketones and contain oxygen, carbon and hydrogen. Like chlorophylls they are located in chloroplasts and also in chromoplasts. Carotenes are named after carrot in which they are abundant. The distribution of the carotenoids in the plant kingdom is shown in the Table 2.

Table 2: The Carotenoids

Carotenes		
Types of Carotenoids	Distribution in Plant Kingdom	Characteristic absorption peaks (nm)
α – Carotene	Many leaves and certain algae. In red algae and a group of green algae called <i>siphonales</i> .	In hexane, at 420, 440, 470
B-Carotene	Main carotene of all other plants.	In hexane, at 425, 450, 480
γ – Carotene	Major carotene of green sulphur bacteria; traces in some plants.	In hexane, at 440, 460, 495
Carotenols (Also called Xanthophylls)		
Luteol	Major carotenol of green leaves, green algae and red algae	In ethanol at 425, 445, 475
Violaxanthol	Second major carotenol of leaves	In ethanol, at 425, 450, 475
Fucoxanthol	Major carotenol of diatoms and brown algae	In hexane, at 425, 450, 475
Spirilloxanthol	Common in purple bacteria	In hexane, at 464, 490, 524

5.5.4 Phycobilins

Englemann found blue green light to be very effective in increasing the rate of photosynthesis of brown and red algae. In fact the red algae gave the best result in green light. Since chlorophylls hardly absorb green light it became obvious that some other accessory pigment was involved. Several workers have since then demonstrated the role of phycobilins and carotenoids in photosynthesis. The irradiation of carotenoids causes fluorescence of chlorophyll suggesting the transfer of energy by accessory pigments to chlorophyll a during photosynthesis.

In blue-green and red algae some additional pigments known as phycobilins are present. They are also tetrapyrroles like chlorophylls but the four joined pyrrole rings form a straight chain. Like anthocyanins they also mask the green color of the chlorophylls. They are, however, intimately associated with the chlorophylls. The light absorbed by them can be used in photosynthesis. Phycobilins include red coloured phycoerythrins and blue coloured phycocyanins found in red and blue-green algae respectively. Phycoerythrin absorbs the green light the best. Phycocyanin absorbs blue light the best.

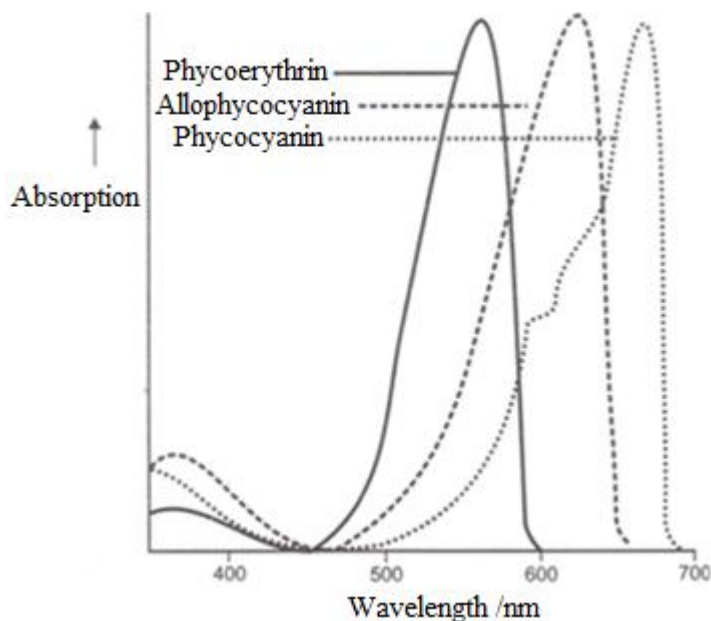


Fig.5.4 Absorption spectrum of three phycobilins

The distribution of phycobilins in the plant kingdom is shown in the given table. (Table-3)

Table-3

Type of Phycobilins	Distribution in plant kingdom	Absorption peaks in cells in nm
Phycocyanins	Mostly in blue green algae	In water and in vivo, at 618
Phycoerythrins	Mostly in red algae	In water and in vivo, at 490, 546 and 576
Allophycocyanins	Blue green and red algae	In phosphate buffer at PH 6.5 at 654

5.5.5 Anthocyanins

The colour of leaves is modified in certain plants due to the presence of purple pigment called anthocyanins. They are formed by several rings of atoms, the rings being joined in complex ways. Anthocyanins are soluble in water; hence they occur in the vacuolar sap of the cells. This pigment does not take any part in photosynthesis. Anthocyanin is not present in the cytoplasm.

5.6 CONCEPT OF TWO PHOTOSYSTEMS

There are two reactions involved in photosynthesis. The first reaction requires light and is called the light or Hill reaction. The second reaction does not require light and is called the dark or Blackman reaction.

1. The light reaction is a photochemical reaction, while the dark reaction is a thermochemical reaction. The unit of photosynthesis is believed to consist of two types of centres,

photosystem I and photosystem II. These are excited at different wavelengths of light. The two systems are linked by redox catalysts. The light reaction involves two processes, photophosphorylation and photolysis of water. In photophosphorylation there is conversion of light energy into chemical energy. Photophosphorylation is of two types, cyclic photophosphorylation and noncyclic photophosphorylation.

2. The dark reaction takes place through a series of steps known as the Calvin-Benson cycle. The details of different stages of photosynthesis will now be taken up.

In higher plants and algae two pigment systems are involved in photophosphorylation. These are called photosystems I and II. The pigments of the two systems are known as pigment system I (PS I) and pigment system II (PS II), respectively. PS I and PS II are structurally distinct. PS I and PS II both contain chlorophyll a, chlorophyll b and carotenoids. The distribution of the two pigments however, varies in the two systems. PS I contain more carotenes than PS II. Xanthophyll predominates in PS II. The primary photosynthetic pigment of both systems is chlorophyll a. In blue-green and red algae phycobiliproteins (formerly phycoerythrin and phycocyanin) are present as accessory pigments.

5.6.1 The Photosynthetic Apparatus

The membranes of the thylakoids are the seat for the light reactions of photosynthesis. The reactions are accomplished with the involvement of a large variety of proteins. They are all intrinsic proteins. They project on one side into the lumen of the thylakoid and on the other side into the stroma. They include some of the enzymes of the electron transport chain, the reaction centres and the antenna pigment protein complexes. The chlorophylls and carotenoids are linked to these proteins in a highly specific manner. Fig.5.5 shows the organization of the protein complexes within the membranes of the thylakoids. Park and sane (1971) assumed that the stromal lamellae has PS I whereas granal lamellae has both PS I and PS II. The stromal lamella can carry on cyclic photophosphorylation while the granal lamellae carry on the non cyclic photophosphorylation. The PS II occurs in the appressed region of grana thylakoids and PS I occurs in the stroma thylakoids and non-appressed regions of grana thylakoids.

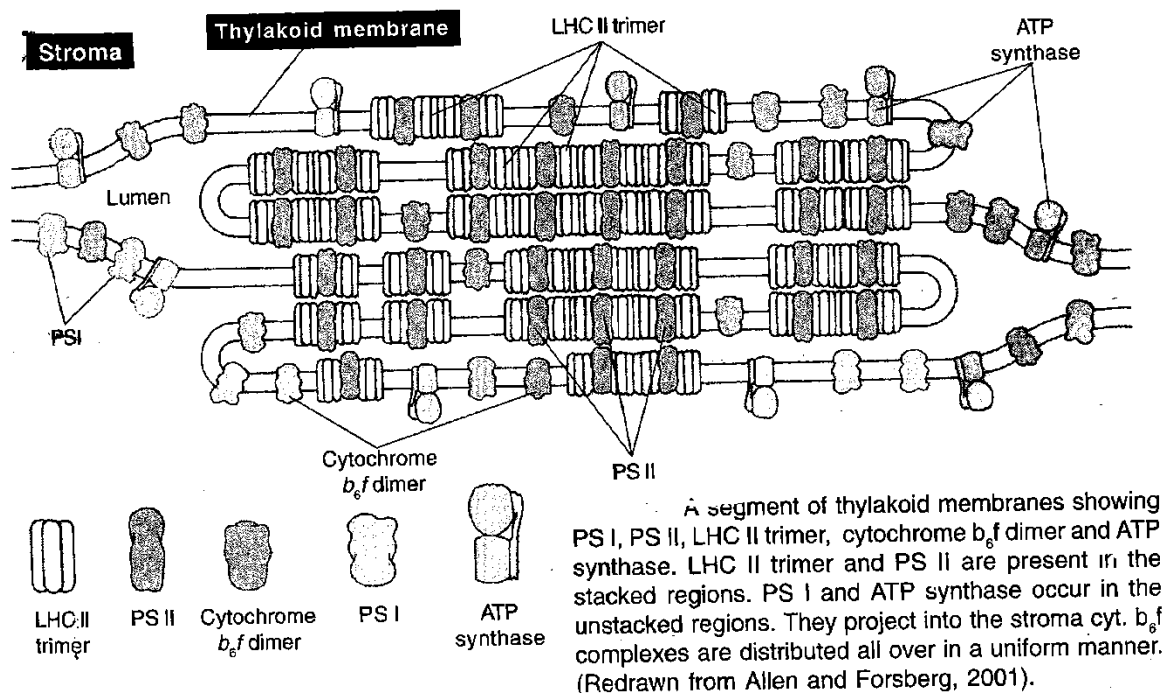


Fig. 5.5 A segment of thylakoid membranes

Anderson and Anderson (1988) have reported that PS II reaction center and antenna chlorophylls as well as electron transport proteins are concentrated in the stacked regions of grana lamellae. The PS I reaction center, its associated antenna pigments, electron transfer proteins as well as ATP synthase occur only in the stroma lamellae and at the edges of the grana lamellae. The cytochrome b_6f complex of the electron transport chain are present all over the grana and stroma lamellae. The electron carriers are placed in such a manner that they transfer the electron lost by PS II in the grana to the stroma region where PS I is situated.

PS II is present in large amount in the thylakoids of grana. The ratio of PS II and PS I has been found to be 1.5:1 in majority of the species. The PS I is, however, in plenty in cyanobacteria and the cells of the bundle sheath of C_4 plants.

5.7 PHOTOPHOSPHORYLATION

There are two types of photophosphorylations in the chloroplasts of plants:

1. Non cyclic photophosphorylation
2. Cyclic photophosphorylation.

1. Non-Cyclic Photophosphorylation

The famous Z scheme of non-cyclic photophosphorylation was developed as a result of work of several research workers. According to Hill and Bendall (1960), Rabinowitch and Govindjee (1965) and others the photochemical reactions occur in series, the product of one being used up by the other. It has undergone modifications from time due to discovery of new electron carriers.

When the chlorophyll is photoexcited the electron gets dislodged from the chlorophyll molecule leaving a 'hole' in it. The 'hole' left by displaced electron is soon filled up by the return of the same electron or another one (cyclic and non-cyclic electron transport). In pigment system I there are 200 chlorophyll a molecules and just one P700 molecule. Any one or all of the chlorophyll a molecules can absorb red or blue photons of light and can pass on the excitation energy to P700 molecule which alone can lose the electron. Due to very tight arrangement of the chlorophyll a 683 molecules within PS I the singlet excitation energy resulting from the absorption of a light quantum by Chl a 683 migrates from one molecule to another by resonance transfer until it reaches a chlorophyll a 683 molecule which is adjacent to the long wave absorbing pigment (P700). The energy is then passed as an excitation to the P700 molecule which is the reaction centre. P700 attains the first excited singlet state and loses an electron to an electron acceptor. The PSI function has been compared to an antenna which causes the 'funneling' of photons into an 'energy trap' or 'sink' (P700) or to a lens which concentrates light into a focal point (P700).

The sequential steps in the electron transport chain are discussed under the following headings:

a) PS II AND Photolysis of water: One of the two primary photochemical reactions in photosynthesis is the absorption of red light by PS II. According to Barber et al. (1999) PS II is a multi subunit protein super complex which has two unique chlorophyll a P680 reaction centers (dimer) and some antenna complexes. After receiving light the P680 reaction center gets photoexcited as a result of which an electron is ejected. The excited form of P680 known as P680* loses its electron to pheophytin, which is the primary electron acceptor. Pheophytin is a type of chlorophyll a in which magnesium is substituted by two hydrogens. According to Okamura et al. (2000) the electron is transferred from pheophytin to plastoquinones Q_A and then to Q_B . The two are bound to the reaction center. The second plastoquinone Q_B after receiving two electrons is reduced to Q_B^{-2} which reacts with two protons of the stroma to form QH_2 (plastohydroquinone). QH_2 which is a small nonpolar molecule was earlier bound to PS II is freed from it. It moves into the non-polar part of the phospholipids and transfers the electrons to cytochrome b_6f complex. (Fig 5.6)

b) The Cytochrome Complex : The cytochrome b_6f complex is also a large multi subunit protein. It has many prosthetic groups. There are two b type hemes, a c type heme (cytochrome f), and a Rieske iron sulphur protein (FeS) present in the complex.

In the non-cyclic or linear type of electron transfer QH_2 transfers one electron to Rieske FeS protein which is present on the side of the lumen and transfers another electron to one of the cytochrome b. The Rieske FeS protein transfers its electron to cytochrome f, which is also present on the luminal side of the thylakoid membrane. In the process two H^+ ions are released into the lumen.

In another mechanism for the transfer of protons and electrons in the cytochrome b_6f complex, called cyclic process, the other QH_2 electron received by cytochrome b is transferred which after

passing through the two b type cytoquinone reduces the semiquinone to plastohydroquinone. In the process two protons are taken up from the stroma so that a total of 4 protons are released in the lumen. (Fig 5.6).

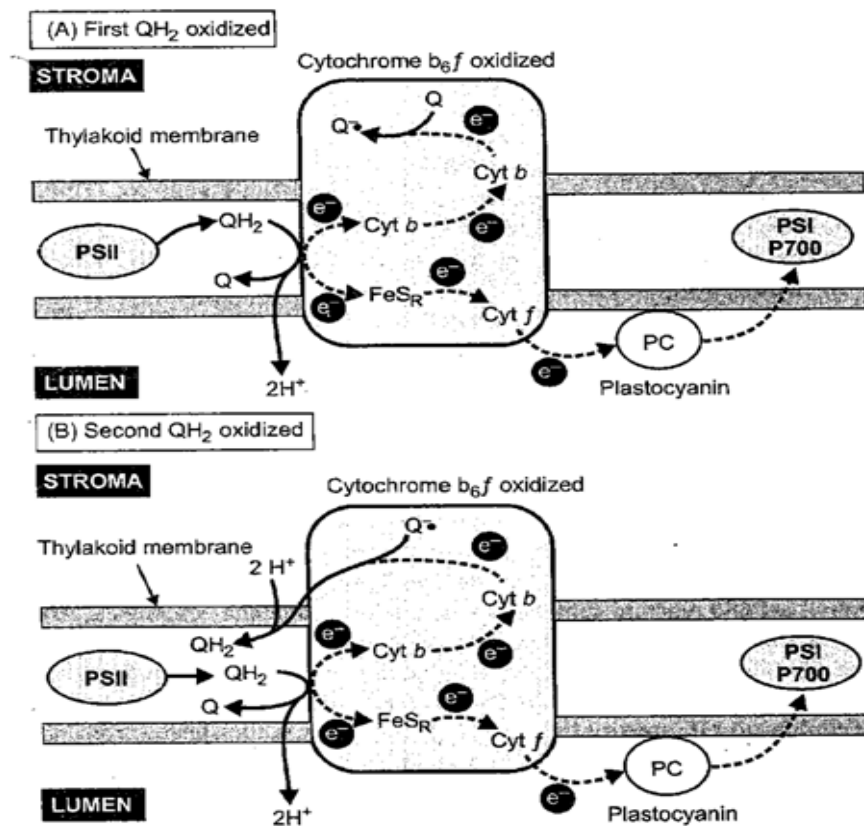


Fig.5.6 Diagram to show the non-cyclic type of electron transport in the cytochrome b_6f complex. A. Linear or cyclic transport; B. Cyclic transport (Redrawn from Taiz and Zeiger, 2002)

- c) **Plastocyanin, the Electron Donor to PS I:** The plastocyanin accepts electrons from cytochrome b_6f complex and donates them to PS I. It is the only copper containing electron carrier in the electron transport chain of photosynthesis. It is a small water soluble protein and is present on the side of the lumen. Plastocyanin does not appear to be indispensable since in some algae non-cyclic electron transport chain occurs even in its absence.
- d) **The PS I:** According to Jordan *et al.*, the PS I is also a large multi subunit complex. It contains not only the reaction center P700 but also a number of components which participate in the transfer of electrons. They are all present around two big sized proteins called PsA and PsB and some small proteins. The P700 is positioned in such a manner in the membrane that the electron is easily ejected. The primary electron acceptor is A_0 which is a type of chlorophyll. The next electron acceptor is A_1 which is a quinone. The electron is then transferred to soluble ferredoxin (Fd) through a series of iron sulphur proteins namely $Fe\ S_x$, $Fe\ S_A$ and $Fe\ S_B$. The electrons are then transferred from ferredoxin to ferredoxin-NADP⁺ reductase (FNR) which donates them to NADP⁺ to produce NADPH (Vishniac and Ochoa, 1951).

e) The ATP Synthase: It is a large complex enzyme and is known by several names such as ATPase, the coupling factor, and CF_0 - CF_1 . This enzyme is situated only at the edges of granal thylakoids and in the stroma lamella and therefore, the protons coming from photolysis of H_2O and from cytochrome b_6f complex have to move great distances laterally to reach it. One of the two components of the enzyme called CF_0 is hydrophobic and is bound to the membrane. The other component termed CF_1 projects into the stroma.

According to Peter Mitchell's chemiosmotic theory the energy required for the synthesis of ATP is provided by proton motive force. The proton motive force is created by the proton chemical potential and the transmembrane electrical potential. The H^+ ions released during photolysis of water accumulate in the lumen of the thylakoids. The accumulated H^+ ions in the lumen try to leak back into the stroma through the ATP synthase. The protons enter the channel formed by CF_0 and when they cross through the catalytic sites of β -polypeptide of CF_1 , the proton motive force breaks, releases energy as a result of which ATP is generated. The latest detailed Z scheme is given in Fig. 5.7.

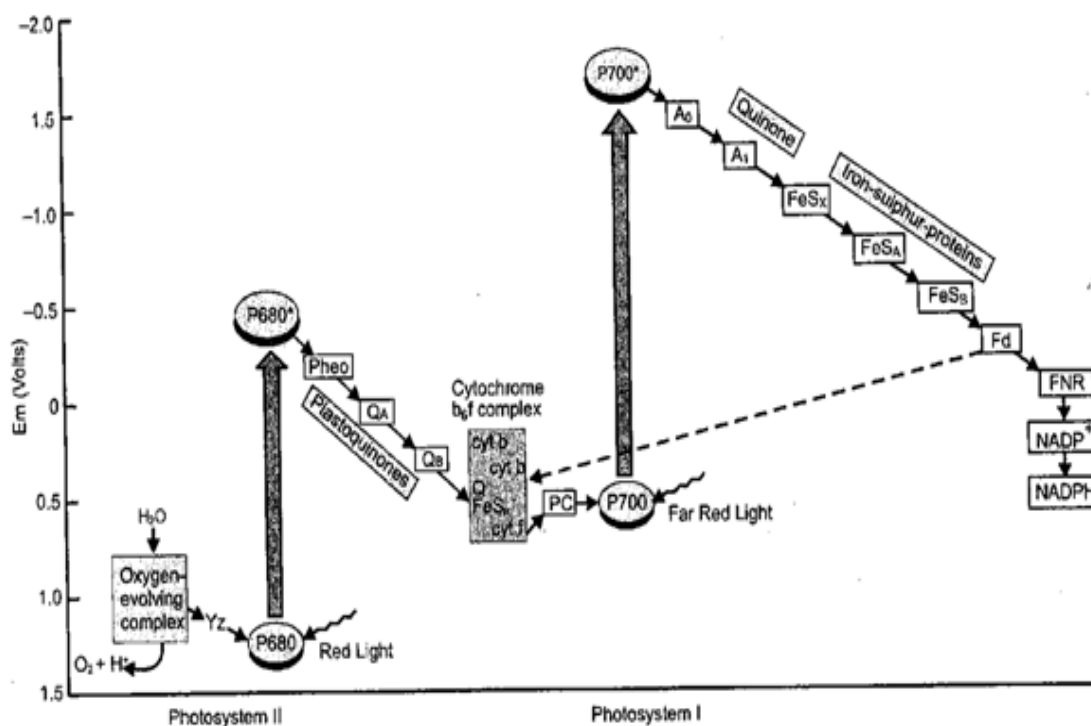


Fig. 5.7 Current concept of the Z-scheme of light phase of photosynthesis. (After Blankenship and Prince, 1985). The cyclic electron transport is indicated by dotted line.

2. Cyclic Photophosphorylation

Another type of photophosphorylation can also take place under certain conditions e.g. when the amount of available NADP^+ is low or PS II is absent or monochromatic light beyond 680 nm is given to the plant in the laboratory. This process involves only pigment system I and, therefore, photolysis of water and the consequent evolution of oxygen does not take place. Non cyclic electron transfer does not take place and NADPH is not formed. The CO_2 assimilation is retarded (red drop). The photosynthetic enhancement can, however, take place if PS II wavelength of light is also given to bring into action the noncyclic photophosphorylation.

In cyclic electron transfer the electron flows from photoexcited P700 to X, A and B, FRS and then to ferredoxin, which unable to pass electrons to NADP^+ transfers them to cytochrome b_6 ($E^0 = -0.06 \text{ V}$). The electron is ultimately cycled back to P700 via PQ, FeS, cytochrome f and plastocyanin. ATP molecule is formed either between ferredoxin and cytochrome b_6 or between cytochrome b_6 and cytochrome f or at both steps.

It is however, doubtful whether cyclic process occurs in normal photosynthesis (VAN Niel, 1962), According to Ramirex et al. (1968) it may serve as the source of ATP for biosynthetic processes occurring in chloroplasts that are not on the main photosynthetic path of carbohydrate synthesis but branch off from this path for the synthesis of protein, DNA, RNA, starch, lipids, pigments etc. Part of the ATP requirement of the dark phase of photosynthesis is also met with by this process.

The cyclic electron transport chain was studied by Arnon. It is not inhibited by 3(3), 4-dichlorophenyl 1, 1-dimethylurea: DCMU). This inhibitor, however, inhibits non-cyclic transport of electrons. Cytochrome b_6 is an electron carrier which participates exclusively in cyclic electron transport chain.

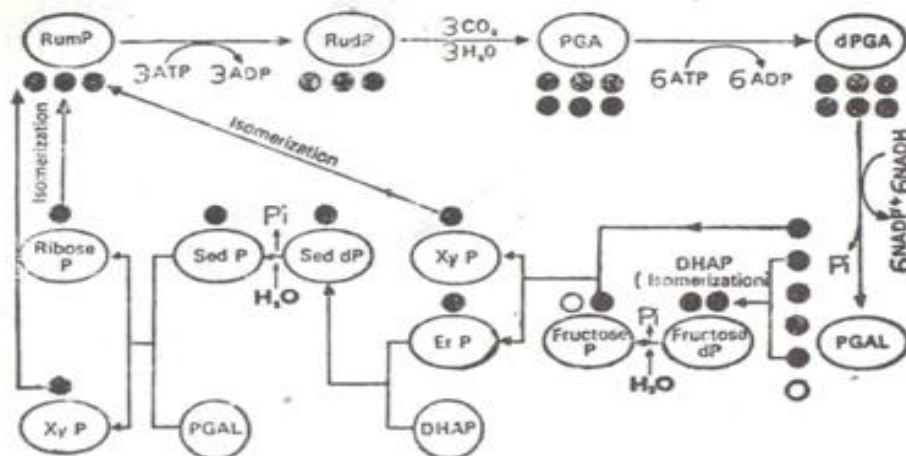
Table 4: Difference between cyclic and non cyclic photophosphorylation

S.No.	Cyclic Photophosphorylation	Noncyclic Photophosphorylation
1	Only photosystem I functions in cyclic photophosphorylation.	Both photosystem I and II function in noncyclic photophosphorylation.
2	Cyclic photophosphorylation functions in a closed loop. Electrons released from chlorophyll to acceptor return to chlorophyll.	An independent electron donor is necessary. Water is the ultimate source of electrons. NADP^+ is the final electron acceptor.
3	There is no net production of reduced compounds. NADPH_2 is not formed and assimilation of CO_2 is retarded.	NADPH_2 is the reduced compound formed. It is utilized in carbon assimilation.
4	Oxygen is not evolved.	ATP formation is coupled to evolution of oxygen.
5	In bacteria only cyclic	In green plants noncyclic

	photophosphorylation takes place.	photophosphorylation also occurs.
6	Cyclic photophosphorylation is not sensitive to either antimycin or dichlorophenyl-dimethyl-urea (DCMU).	DCMU inhibits flow of electrons from water to NADP ⁺ and thus stops noncyclic photophosphorylation.
7	The flow of electrons is as follows Chl (PS-I)-FRS-Fd-Cyt. b ₆ -Cyt. f-PC-Chl (PS-I)	The flow of electrons is as follows: PS-II-Q-PQ-Cyt.f-PC-PS-I-FRS-NADP ⁺

5.8 CALVIN CYCLE / DARK REACTION

During the dark reactions of photosynthesis carbon dioxide is reduced to form carbohydrates. The term dark reaction implies that the reaction is not dependent on light. Synthesis of carbohydrates proceeds in the dark. The dark reaction has been worked out mainly by Calvin, Bensen and Bassham. The pathway by which carbon dioxide is fixed into carbohydrates is called the Calvin-Benson cycle or Calvin-Bassham cycle. Carbon dioxide and water are used to generate carbohydrate in the presence of ATP and NADPH. (Fig.5.8).



Outline of the dark reaction.

Outline of the dark reactions

- 1) $6 \text{ Ribulose-1-5-diphosphate} + 6 \text{ CO}_2 + 6 \text{ H}_2\text{O} \rightarrow 12 \text{ 3-Phosphoglyceric acid (3 PGA)}$
(RudP)
- 2) $12 \text{ 3-phosphoglyceric acid} + 12 \text{ ATP} \rightarrow 12 \text{ 1-3-Diphosphoglyceric acid} + 12 \text{ ADP}$
(3 PGA) acid
 $12 \text{ 1-3-Diphosphoglyceric acid} + 12 \text{ NADPH} \rightarrow 12 \text{ Phosphoglyceraldehyde (PGAL)} + 12 \text{ NADP} + 12 \text{ Pi}$
- 3) $5\text{-Phosphoglyceraldehyde (PGAL)} \rightarrow 5\text{-Dihydroxyacetone phosphate (DHAP)}$
 $3 \text{ PGAL} + 3 \text{ Dihydroxyacetone phosphate} \rightarrow 3 \text{ Fructose-1-6 diphosphate (DHAP)}$
 $3 \text{ Fructose-1-6-diphosphate} + 3 \text{ H}_2\text{O} \rightarrow 3 \text{ Fructose-6-phosphate} + 3 \text{ Pi}$
- 4) $2 \text{ Fructose-6-phosphate} + 2 \text{ PGAL} \rightarrow 2 \text{ Xylulose-5-phosphate} + 2 \text{ Erythrose-4-phosphate}$
- 5) $2 \text{ Erythrose-4-phosphate} + 2 \text{ DHAP} \rightarrow 2 \text{ Sedoheptulose-1-7-diphosphate}$
 $2 \text{ Sedoheptulose-1-7-diphosphate} + 2 \text{ H}_2\text{O} \rightarrow 2 \text{ Sedoheptulose-7-phosphate} + 2 \text{ Pi}$
- 6) $2 \text{ Sedoheptulose-7-phosphate} + 2 \text{ PGAL} \rightarrow 2 \text{ Ribose-5-phosphate} + 2 \text{ Xylulose-5-Phosphate}$
 $2 \text{ Ribose-5-phosphate} \rightarrow 2 \text{ Ribulose-5-phosphate}$
 $4 \text{ Xylulose-5-phosphate} \rightarrow 4 \text{ Ribulose-5-phosphate (Ribose monophosphate) (RumP)}$
 $6 \text{ Ribulose-5-phosphate} + 6 \text{ ATP} \rightarrow 6 \text{ Ribulose-1-5-diphosphate} + 6 \text{ ADP}$

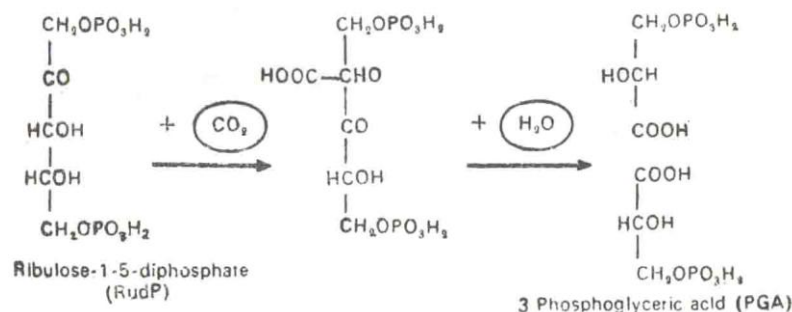
Sum : $6 \text{ CO}_2 + 18 \text{ ATP} + 12 \text{ NADPH} + 12 \text{ H}^+ + 11 \text{ H}_2\text{O} \rightarrow \text{Fructose-6-phosphate} + 18 \text{ ADP} + 12 \text{ NADP}^+ + 17 \text{ H}_3\text{PO}_4 \text{ (Pi)}$

Fig.5.8 Outline of the dark reactions

1. Production of PGA

Calvin and his co-workers found that the first product to accumulate during photosynthesis was phosphoglyceric acid (PGA). This arises as follows.

Carbon dioxide is first attached to ribulose-1-5-diphosphate (RudP) a 5-carbon atom compound, to form an intermediate 6-carbon compound. Each molecule of this compound then splits to form two molecules of PGA. Radioactive carbon dioxide ($^{14}\text{CO}_2$) was used in the experiment, and this CO_2 contributed one carbon atom of the two PGA molecules formed only one has radioactive carbon. Thus only one free molecule of PGA is formed per molecule of CO_2 entering the cycle. Actually, 6 molecules of RudP and 6 molecules of CO_2 react to produce 12 molecules of PGA.

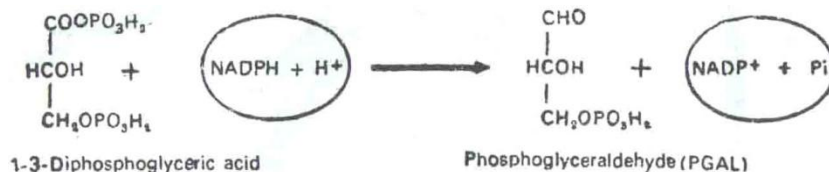


2. Production of PGAL

Phosphoglyceric acid (PGA) is reduced to phosphoglyceraldehyde (PGAL). This Process is the reverse of the oxidation step in glycolysis when PGA is oxidized to PGAL. In all 12 molecules of PGAL are produced from the 6 molecules of RudP. The reaction takes place in two steps. Firstly, PGA is phosphorylated by ATP to 1, 3-diphosphoglyceric acid.

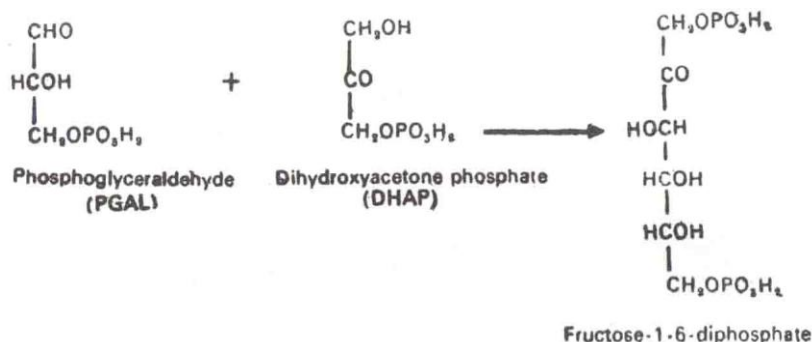


Secondly, 1, 3- diphosphoglyceric acid is reduced by $\text{NADPH} + \text{H}^+$ to Phosphoglyceraldehyde (PGAL)



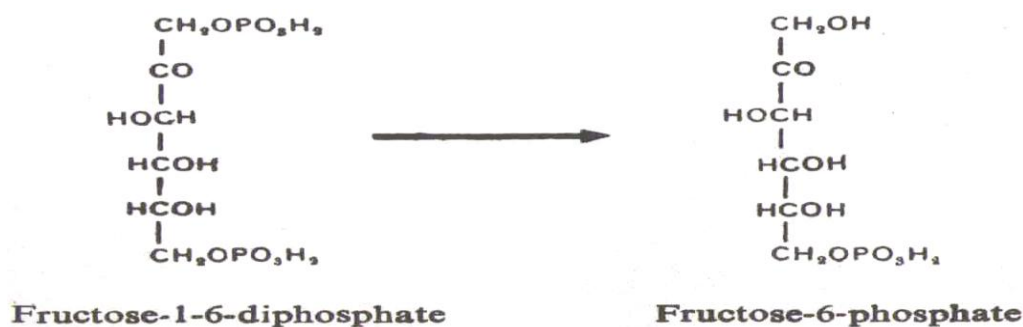
3. Production of Fructose-6- phosphate

PGAL is converted into its isomer dihydroxyactone phosphate (DHAP), as in glycolysis. DHAP condense with PGAL to form fructose-1-6-diphosphate.



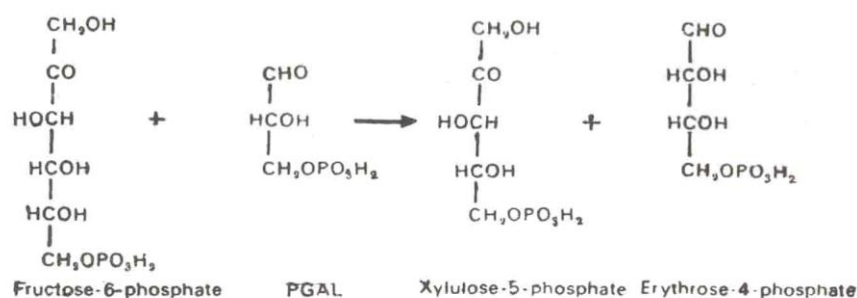
This process is the reverse of the breakdown of fructose-1-6-diphosphate in glycolysis.

One phosphate group is removed from fructose 1-6 diphosphate (dephosphorylation) resulting in the formation of fructose-6-phosphate.



4. Production of Xylulose Phosphate

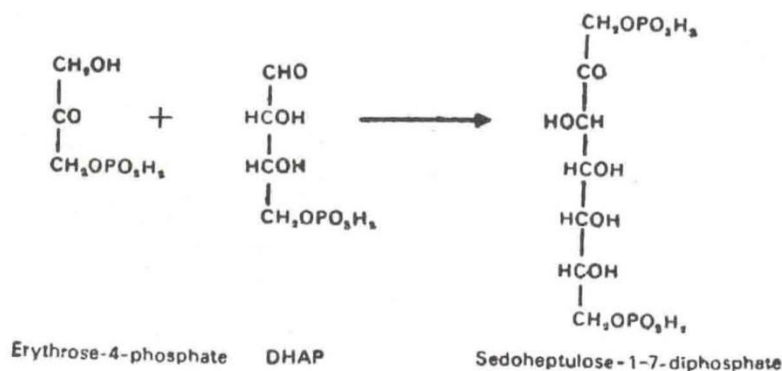
Fructose-6-phosphate reacts with PGAL to yield a pentose (5C), xylulose-5-phosphate, and a tetrose (4C), erythrose-4-phosphate the reaction is catalysed by the enzyme transketolase.



Xylulose-5-phosphate readily isomerizes to ribulose-5-phosphate.

5. Formation of Sedoheptulose-1, 7-diphosphate

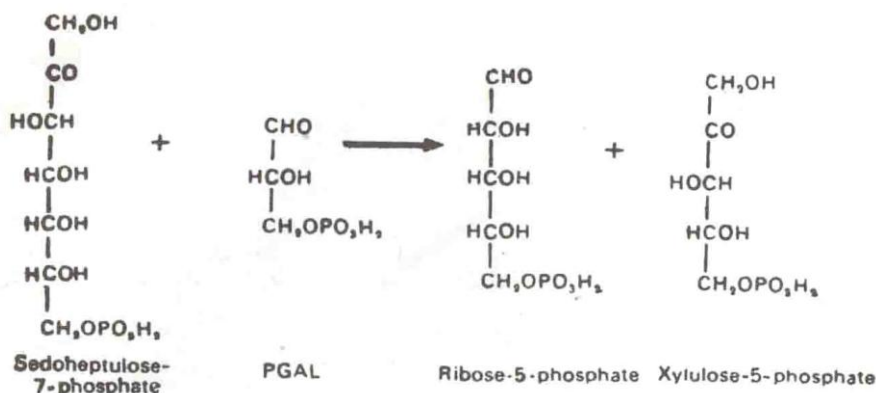
Erythrose-4-phosphate reacts with DHAP to form sedoheptulose-1-7 diphosphate, the reaction being catalysed by the enzyme transaldolase.



Sedoheptulose 1-7-diphosphate loses one phosphate (dephosphorylation) and becomes sedoheptulose-7-diphosphate.

6. Formation of Ribose-5-phosphate

Sedoheptulose-7-phosphate reacts with another molecule of PGAL to form ribose-5-phosphate and xylulose-5-phosphate. Both these products readily isomerize to ribulose-5-phosphate or ribulose monophosphate (RumP)



5.9 C₄ PATHWAYS

Most of the C₄ plants are monocots. There are also about 300 species of C₄ plants in dicots. About 900 species belonging to 19 families are C₄ plants. The primary objective of the Hatch Slack cycle is to trap atmospheric CO₂ in the maximum amount and to transport it into the bundle sheath cells so that the rate of Calvin cycle is enhanced.

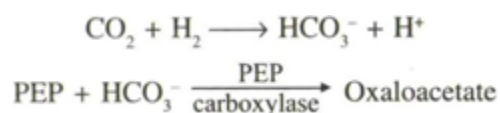
Till 1965 the mechanism of the photosynthetic CO₂ fixation was believed to occur only by means of what is popularly known as Calvin cycle. In 1965 H.P. Kortschak, C.E. Hart and G.O. Burr working with C¹⁴O₂ on sugar cane leaves found highly efficient photosynthesis and C₄ dicarboxylic acid, malate and aspartate to be the major labeled products (80% of radio activity) in very short periods of photosynthesis. Working on grasses this observation was confirmed by

M.D. Hatch and C.R. Slack of David North Plant Research Centre, Queensland, Australia in 1967. The Hatch Slack pathway, as this alternative CO₂ fixation is called, has been found to occur in tropical and subtropical grasses and some dicotyledons. Some of the important C₄ plants are sugarcane, maize, sorghum etc. It is interesting to note that even within a single genus a subtropical species *Atriplex rosea* exhibits Hatch Slack pathway whereas the temperate species *Atriplex hastata* has only the Calvin cycle.

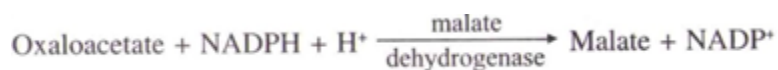
There are 4 distinct stages in the C₄ cycle:

1. CO₂ fixation in the mesophyll by phosphoenol pyruvate to form C₄ acids: malic and aspartic acids.
2. The transfer of C₄ acids into the bundle sheath through plasmodesmata.
3. Decarboxylation of the C₄ acids in the bundle sheath.
4. The diffusion of pyruvate or alanine back into the mesophyll for the regeneration of CO₂ acceptor pyruvate.

In Hatch Slack cycle the first step occurs in the cytoplasm of the mesophyll where PEP Carboxylase (PEPcase) brings about the following reaction in the presence of water.



The oxaloacetate then moves into the chloroplast where it is reduced to malate by the enzyme malic acid dehydrogenase which is present inside the chloroplast.



The oxaloacetate is also transaminated into aspartate. The malate diffuses into the cells of bundle sheath through plasmodesmata. (Fig.5.9).The malate is decarboxylated within the cells of the bundle sheath by NADP⁺ dependent malic enzyme to produce CO₂ and pyruvate. While CO₂ is taken up by RuBP to accelerate the calvin cycle the pyruvate diffuses back to the mesophyll cell. The pyruvic acid reacts with ATP and an inorganic phosphate in the presence of an enzyme present in the chloroplast known as pyruvate phosphate dikinase to produce phosphoenolpyruvic acid, AMP and pyrophosphate.



The number of ATP molecules required to synthesis one hexose is, therefore, much more than that of the Calvin cycle. The enzyme pyruvate orthophosphate dikinase brings about the conversion of pyruvic acid into phosphoenol pyruvic acid by breaking up of two energy rich bonds of ATP. The conversion of AMP back into ATP requires expenditure of two ATP

molecules. While in Calvin cycle the requirement is 18 ATP in C_4 plants, 12 extra ATP are required because two additional ATP per CO_2 are essential for regenerating ATP from AMP. The reactions of the Hatch-Slack cycle are given in Table 5.

Phosphoenolpyruvate carboxylase, pyruvate phosphate dikinase and the $NADP^+$ specific malate dehydrogenase are present in the chloroplasts of the mesophyll cells, whereas RUBP carboxylase $NADP$ specific malic enzyme, and the remaining Calvin cycle enzymes have been found to be present in the chloroplasts of a layer of paranchymatous cells which form a sheath around the vascular bundle. Plasmodesmata have been observed to connect adjacent cells of the bundle sheath and mesophyll layer in maize and sugarcane.

Table: 5
Reactions of C_4 photosynthetic carbon cycle.

S.N.	Reaction	Enzyme
1.	$\text{Phosphoenolpyruvate} + \text{HCO}_3^- \rightarrow \text{oxaloacetate} + \text{P}_i$	Phosphoenolpyruvate(PEPcase) carboxylase
2.	$\text{Oxaloacetate} + \text{NADPH} + \text{H}^+ \rightarrow \text{malate} + \text{NADP}^+$	NADP^+ : malate dehydrogenase
3.	$\text{Oxaloacetate} + \text{glutamate} \rightarrow \text{aspartate} + \alpha\text{-ketoglutarate}$	Aspartate aminotransferase
4.	$\text{Malate} + \text{NAD(P)}^+ \rightarrow \text{pyruvate} + \text{CO}_2 + \text{NAD(P)H} + \text{H}^+$	NAD(P) malic enzyme
5.	$\text{Oxaloacetate} + \text{ATP} \rightarrow \text{phosphoenolpyruvate} + \text{CO}_2 + \text{ADP}$	Phosphoenolpyruvate carboxykinase
6.	$\text{Pyruvate} + \text{glutamate} \rightarrow \text{alanine} + \alpha\text{-ketoglutarate}$	Alanine aminotransferase
7.	$\text{AMP} + \text{ATP} \rightarrow 2 \text{ADP}$	Adenylate kinase
8.	$\text{Pyruvate} + \text{P}_i + \text{ATP} \rightarrow \text{phosphoenolpyruvate} + \text{AMP} + \text{PP}_i$	Pyruvate-orthophosphate dikinase
9.	$\text{PP}_i + \text{H}_2\text{O} \rightarrow 2\text{P}_i$	Pyrophosphatase

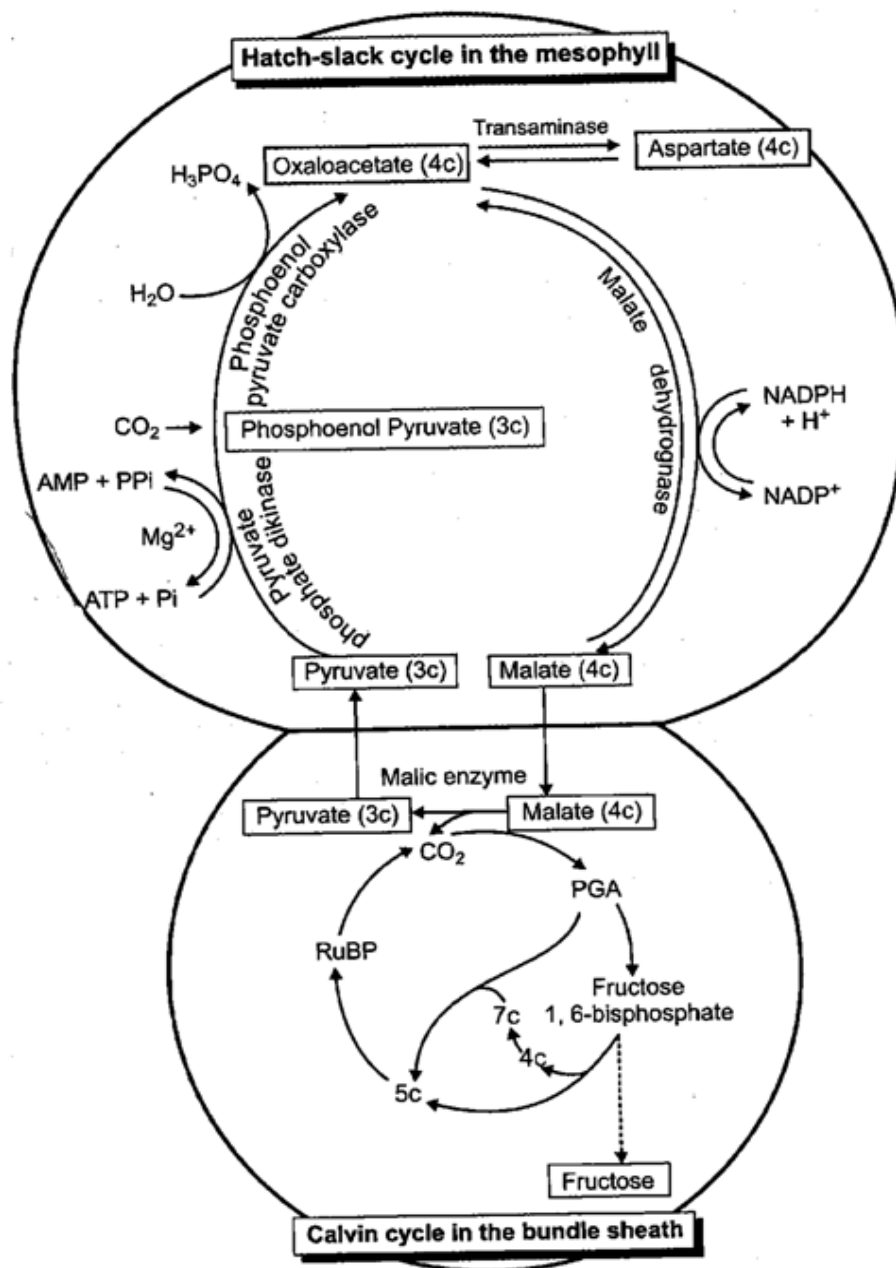


Fig.5.9 Hatch-Slack cycle and Calvin cycle as they occur within the mesophyll and bundle sheath of the C₄ plants

5.10 CAM PLANTS

The members of the family Crassulaceae have a special type of metabolism called Crassulacean acid metabolism (CAM) which is also exhibited by member of other families. CAM plant are succulents with thick fleshy leaves, or, when leaves are absent, a swollen photosynthetic stem. CAM plant can synthesize large amounts of malic and isocitric acids at night. Photosynthesis occurs during the day and these acids disappear. The stomata of the leaves remain closed during

the day and open only at night. This is an adaptation to conserve water, since succulents exhibiting CAM are found in dry habitats. During the night CO_2 is taken into the leaves through the open stomata. Because photosynthesis is limited by the storage pool of organic acid and carbohydrate, CAM plants are generally slow growing. (Fig.5.10)

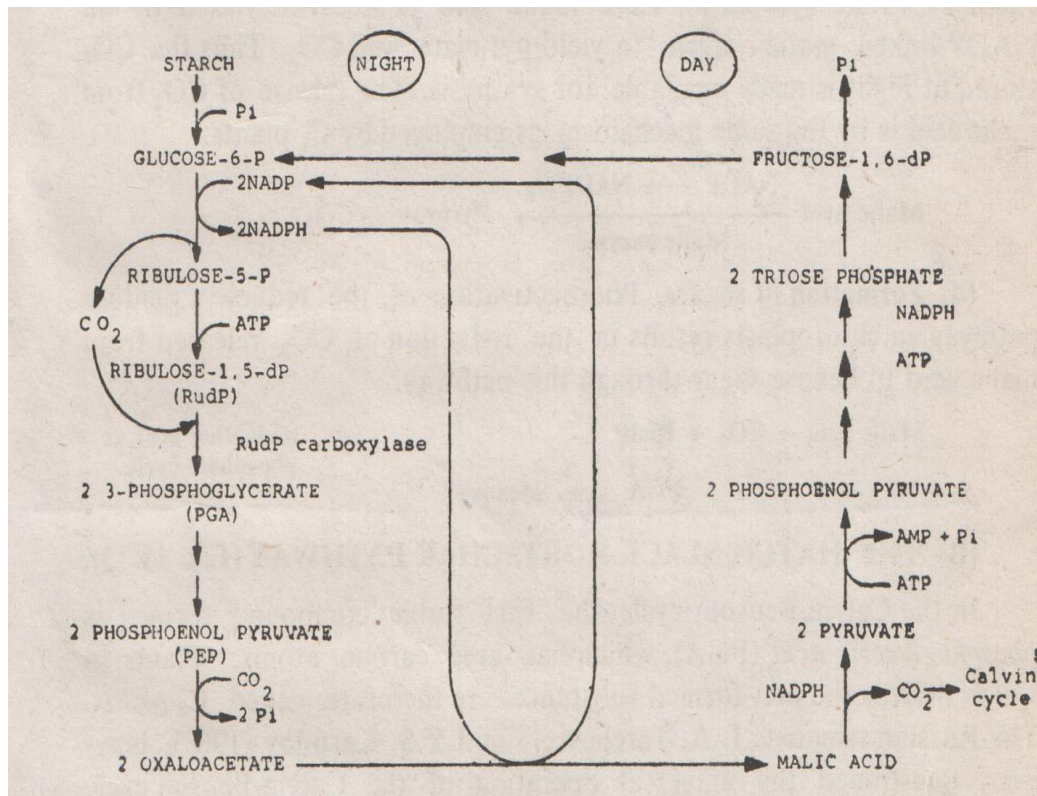
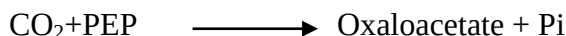


Fig.5.10 Crassulacean acid metabolism

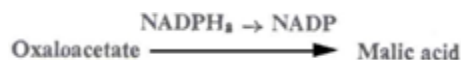
The CAM mechanism shows various modifications. Well watered *Agave americana* shows some normal daytime photosynthesis along with some CO_2 fixation at night. In watered *Agave deserti*, however, dark carboxylation stops and is replaced by normal C_3 daytime photosynthesis.

5.10.1 Reactions of CAM

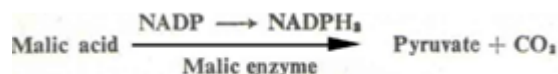
(1) **Formation of oxaloacetate.** The requirements for formation of oxaloacetate are CO_2 and phosphoenol pyruvic acid (PEP). PEP is formed from stored carbohydrate in leaves, particularly starch. In CAM species the stomata remain open at night and CO_2 entering the leaves are fixed by PEP to form oxaloacetate.



(2) **Formation of Malic acid-** Oxaloacetate is reduced by malic dehydrogenase to malic acid, which accumulates in the vacuoles of leaf cells. During this step NADPH_2 formed in the pentose phosphate pathway is utilized, and the NADP formed enters the pentose phosphate pathway.



(3) **Release of CO₂ from malic acid.** – During the day ATP and NADPH are abundantly available from the photosynthesis reactions. The stomata of the leaves are closed and malate is transported back out of the vacuoles to the cytoplasm. Here malic acid is decarboxylated by an NADP-linked malic enzyme to yield pyruvate and CO₂. Thus the CO₂ stored at night is made available for synthesis. The release of CO₂ from malic acid is by the same mechanism as employed by C₄ plants.



(4) **Formation of sugar** – Photoactivation of the reductive pentose pathway in chloroplasts results in the reduction of CO₂ released from malic acid to hexose sugar through this pathway.



5.11 PHOTORESPIRATION

Although C₃ plants respire in the dark, the rate of oxygen utilization increases markedly when the plants are illuminated. Photorespiration is a light driven efflux of CO₂ which proceeds alongside with net CO₂ influx during photosynthesis. Photorespiration may attain 50% of the net rate of photosynthesis. Photorespiration results in CO₂ evolution in light. This has the net effect of decreasing photosynthesis which takes up CO₂ in light. It is therefore a wasteful process which prevents plants from achieving a maximum yield in photosynthesis. In crop species the yield would be greater if photorespiration did not occur. The substrate for photorespiration is glycolate. Breeding of plants with lower photorespiration rates, or inhibiting glycolate synthesis, would be means of increasing crop yields.

Photorespiration is exhibited by crop plants like wheat, rice, other cereals, many legumes and sugar beets, while crops like corn, sorghum and sugarcane do not have photorespiration. (Fig. 5.11)

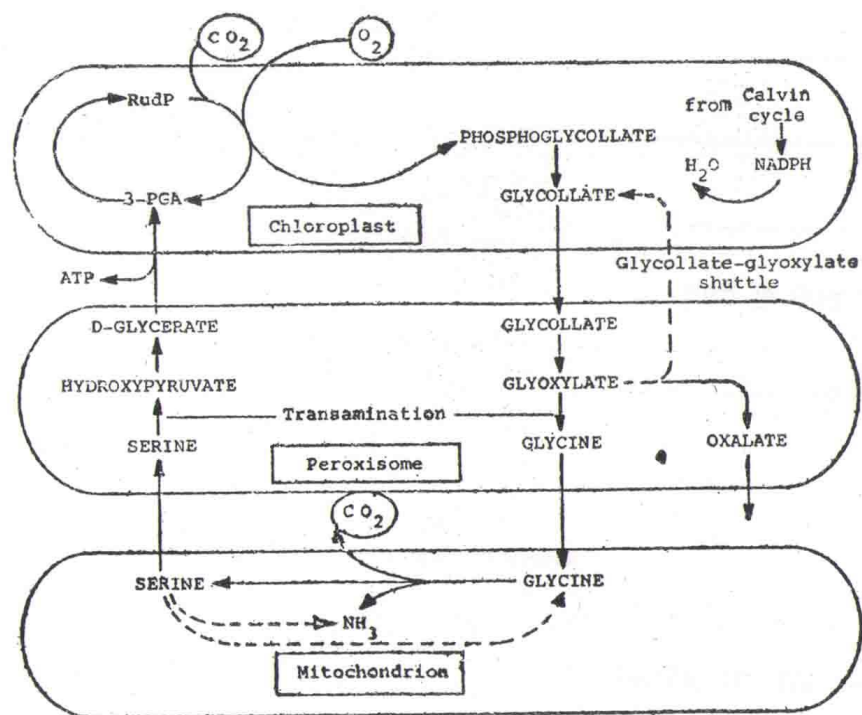


Fig.5.11 Some pathways in glycollate metabolism

The CO_2 compensation point is the CO_2 concentration at a given constant light intensity at which there is a balance between photosynthetic assimilation and respiration. The enzymes of the photorespiratory pathways are present in both C_3 and C_4 plants. The carbondioxide compensation point for common C_3 crop plants is about 40-60 ppm at 25°C , while that for C_4 plants is often less than 10 ppm. The CO_2 generated in C_4 plants during photorespiration is trapped and re-cycled internally by cytoplasmic PEP carboxylase of mesophyll cells. Thus CO_2 efflux is prevented.

Glycollic acid is a 2-carbon compound which is formed in large quantities in the chloroplasts of C_3 plants, from where it moves out into the cytosol.

5.12 SUMMARY

In this unit you have learnt about the mechanisms of photosynthesis occurring in plants. So let us sum it up.

1. Photosynthesis is the chemical change which happens in the leaves of green plants. It is the first step towards making food - not just for plants but ultimately every animal on the planet.
2. All green parts of a plant have chloroplasts.
3. However, the leaves are the major site of photosynthesis for most plants. There are about half a million chloroplasts per square millimeter of leaf surface.
4. The color of a leaf comes from chlorophyll, the green pigment in the chloroplasts. Chlorophyll plays an important role in the absorption of light energy during photosynthesis.

5. Powered by light, the green parts of plants produce organic compounds and O_2 from CO_2 and H_2O .
6. The equation describing the process of photosynthesis is:
 - a. $6CO_2 + 12H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$
 - b. $C_6H_{12}O_6$ is glucose.
7. Water appears on both sides of the equation because 12 molecules of water are consumed, and 6 molecules are newly formed during photosynthesis.
8. We can simplify the equation by showing only the net consumption of water:
 - a. $6CO_2 + 6H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2$
9. Photosynthesis is two processes, each with multiple stages.
10. The light reactions (photo) convert solar energy to chemical energy.
11. The Calvin cycle (synthesis) uses energy from the light reactions to incorporate CO_2 from the atmosphere into sugar.
12. In the light reactions, light energy absorbed by chlorophyll in the thylakoids drives the transfer of electrons and hydrogen from water to $NADP^+$ (nicotinamide adenine dinucleotide phosphate), forming NADPH.
 - a. NADPH, an electron acceptor, provides reducing power via energized electrons to the Calvin cycle.
 - b. Water is split in the process, and O_2 is released as a by-product.
13. The light reaction also generates ATP using chemiosmosis, in a process called photophosphorylation.
14. Thus light energy is initially converted to chemical energy in the form of two compounds: NADPH and ATP.
15. The cycle begins with the incorporation of CO_2 into organic molecules, a process known as carbon fixation.
16. The fixed carbon is reduced with electrons provided by NADPH.
17. ATP from the light reactions also powers parts of the Calvin cycle.
18. Thus, it is the Calvin cycle that makes sugar, but only with the help of ATP and NADPH from the light reactions.
19. The metabolic steps of the Calvin cycle are sometimes referred to as the light-independent reactions, because none of the steps requires light directly.
20. Nevertheless, the Calvin cycle in most plants occurs during daylight, because that is when the light reactions can provide the NADPH and ATP the Calvin cycle requires.
21. While the light reactions occur at the thylakoids, the Calvin cycle occurs in the stroma.
22. There are two types of photosystems in the thylakoid membrane.
 - a. Photosystem I (PS I) has a reaction center chlorophyll a that has an absorption peak at 700 nm.
 - b. Photosystem II (PS II) has a reaction center chlorophyll a that has an absorption peak at 680 nm.

- c. The differences between these reaction centers (and their absorption spectra) lie not in the chlorophyll molecules, but in the proteins associated with each reaction center.
 - d. These two photosystems work together to use light energy to generate ATP and NADPH.
23. During the light reactions, there are two possible routes for electron flow: cyclic and noncyclic.
24. Noncyclic electron flow, the predominant route, produces both ATP and NADPH.
25. Under certain conditions, photoexcited electrons from photosystem I, but not photosystem II, can take an alternative pathway, cyclic electron flow.
- a. Excited electrons cycle from their reaction center to a primary acceptor, along an electron transport chain, and return to the oxidized P700 chlorophyll.
 - b. As electrons flow along the electron transport chain, they generate ATP by cyclic photophosphorylation.
 - c. There is no production of NADPH and no release of oxygen.
26. Certain plant species have evolved alternate modes of carbon fixation to minimize photorespiration.
27. C_4 plants first fix CO_2 in a four-carbon compound.
- a. Several thousand plants, including sugarcane and corn, use this pathway.
28. A unique leaf anatomy is correlated with the mechanism of C_4 photosynthesis.
29. In C_4 plants, there are two distinct types of photosynthetic cells: bundle-sheath cells and mesophyll cells.
- a. Bundle-sheath cells are arranged into tightly packed sheaths around the veins of the leaf.
 - b. Mesophyll cells are more loosely arranged cells located between the bundle sheath and the leaf surface.
30. C_4 photosynthesis minimizes photorespiration and enhances sugar production.
31. A second strategy to minimize photorespiration is found in succulent plants, cacti, pineapples, and several other plant families.
- a. These plants are known as CAM plants for crassulacean acid metabolism.
 - b. They open their stomata during the night and close them during the day. Temperatures are typically lower at night, and humidity is higher.
 - c. During the night, these plants fix CO_2 into a variety of organic acids in mesophyll cells.
 - d. During the day, the light reactions supply ATP and NADPH to the Calvin cycle, and CO_2 is released from the organic acids.
32. Both C_4 and CAM plants add CO_2 into organic intermediates before it enters the Calvin cycle.

5.13 GLOSSARY

ADP- Adenosine diphosphate, product of the Calvin cycle that is used in the light-dependent reactions.

ATP- Adenosine triphosphate. ATP is a major energy molecule in cells. ATP and NADPH are products of the light-dependent reactions in plants. ATP is used in reduction and regeneration of RuBP.

Autotrophs- Photosynthetic organisms which convert light energy into the chemical energy they need to develop, grow, and reproduce.

Calvin cycle- Set of chemical reactions of photosynthesis that does not necessarily require light. The Calvin cycle takes place in the stroma of the chloroplast. It involves the fixing of carbon dioxide into glucose using NADPH and ATP.

Carbon fixation- ATP and NADPH are used to fix CO₂ into carbohydrates. Carbon fixation takes place in the chloroplast stroma.

Chemical equation of photosynthesis - $6 \text{CO}_2 + 6 \text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2$

Chlorophyll- Primary pigment used in photosynthesis. Plants contain two main forms of chlorophyll: a & b. Chlorophyll has a hydrocarbon tail that anchors it to an integral protein in the thylakoid membrane of the chloroplast. Chlorophyll is the source of the green color of plants and certain other autotrophs.

Chloroplast - Organelle in a plant cell where photosynthesis occurs.

G3P - Isomer of PGA formed during the Calvin cycle

Glucose (C₆H₁₂O₆) - Sugar that is the product of photosynthesis. Glucose is formed from 2 PGAL's.

Granum - Stack of thylakoids (plural: grana)

Light - Electromagnetic radiation; the shorter the wavelength the greater amount of energy. Light supplies the energy for the light reactions of photosynthesis.

Light harvesting complexes (photosystems complexes) - Multi-protein unit in the thylakoid membrane that absorbed light to serve as energy for reactions

Light reactions (light dependent reactions) - Chemical reactions requiring electromagnetic energy (light) that occur in the thylakoid membrane of the chloroplast to convert light energy into chemical forms ATP and NADPH.

Lumen - Region within the thylakoid membrane where water is split to obtain oxygen. The oxygen diffuses out of the cell, while the protons remain inside to build positive electrical charge inside the thylakoid.

Mesophyll cell - A type of plant cell located between the upper and lower epidermis that is the site for photosynthesis

NADPH - A high-energy electron carrier used in reduction

Oxidation - The loss of electrons

Oxygen (O₂) - Gas that is a product of the light-dependent reactions

Palisade mesophyll - Area of the mesophyll cell without many air spaces

PGAL - Isomer of PGA formed during the Calvin cycle.

Photosynthesis - The process by which organisms convert light energy into chemical energy (glucose).

Photosystem – A cluster of chlorophyll and other molecules in a thylakoid that harvest the energy of light for photosynthesis

Pigment - Colored molecule. A pigment absorbs specific wavelengths of light. Chlorophyll absorbs blue and red light and reflects green light, so it appears green.

Reduction - The gain of electrons

Rubisco - An enzyme that bonds carbon dioxide with RuBP

Thylakoid - Disc-shaped portion of chloroplast, found in stacks

5.14 SELF ASSESSMENT QUESTIONS

5.14.1 Very Short Answer Type Questions

1. RUBISCO enzyme is also called as?
2. Which photosynthetic pigments contains open pyrrole ring?
3. What is the visible product of photosynthesis?
4. What is the source of CO₂ during calvin cycle in C₄ plant ?
5. Absorption spectrum of chlorophyll is maximum in which light?

5.14.2 Multiple Choice questions:

1. During light phase of photosynthesis _____ is oxidized and _____ is reduced.
(a) CO₂ and Water (b) Water and CO₂
(c) Water and NADP (d) NADPH₂ and CO₂
2. During dark phase of photosynthesis _____ is oxidized and _____ is reduced.
(a) CO₂ and Water (b) Water and CO₂
(c) Water and NADP (d) NADPH₂ and CO₂
3. The visible product of photosynthesis is _____
(a) glucose (b) cellulose
(c) starch (d) fructose
4. Glycolytic reversal is a part of _____
(a) aerobic respiration (b) anaerobic respiration
(c) light phase of photosynthesis (d) dark phase of photosynthesis
5. The source of CO₂ during calvin cycle in C₄ plant is
(a) Malic acid (b) OAA
(c) PEP (d) RuDP
6. Absorption spectrum of chlorophyll is maximum in _____ light.
(a) red (b) blue

- (c) yellow (d) blue-violet
7. The oxygen molecule in glucose formed during photosynthesis comes from
(a) Water (b) Organic acids
(c) CO₂ (d) atmosphere
8. Light reaction of photosynthesis results in formation of
(a) O₂ (b) NADPH +H⁺
(c) ATP (d) All of these
- 9.Reduction of co-enzyme NADP depends on
(a) Reduction of CO₂ (b) Evolution of O₂
(c) Photolysis of water (d) Formation of ATP
- 10.Calvin cycle involves
(a) Oxidative phosphorylation (b) Oxidative carboxylation
(c) Reductive carboxylation (d) Reductive phosphorylation

5.14.3 True/ False

1. Photosynthesis takes place only in high intensity of light.
2. During photosynthesis the reaction when PGA is converted into PGAL, is called reduction.
3. During light reaction CO₂ reacts with H₂.
4. In C₃ pathway, the first stable compound is PGA.
5. The source of oxygen evolved during photosynthesis is H₂O.
6. In C₄ plants, synthesis of glucose occurs in bundle sheath cells.
7. In cyclic and non-cyclic photophosphorylation, the first acceptor of electron are ferridoxin and plastoquinone respectively.
8. Release of oxygen occurs during both cyclic and non-cyclic photophosphorylation.
9. During Photosynthesis water is oxidized and CO₂ is reduced.
10. Calvin was given Nobel prize in 1961 for his discovery of photolysis of water.

5.14.1 Answer keys:

1. RUBISCO is called marker enzyme of chloroplast since it can perform carboxylation as well as oxidation, therefore also called **carboxydimutase**.
2. Chlorophylls are made of closed pyrrole, whereas Phycobilins are made of open pyrrole, Carotenoids are carbon hydrogen derivatives.
3. Starch is visible product since it can be stained with iodine.
4. During C₄ pathway malic acid undergo decarboxylation to release CO₂.
5. Absorption spectrum of chlorophyll is maximum in blue light whereas action spectrum is maximum in red light.

5.14.2 Answer Keys: 1- (c), 2-(d), 3-(c), 4-(d), 5-(a), 6-(b), 7-(c), 8-(d), 9-(c), 10-(c)

5.14.3 Answer Keys: 1-False, 2-True, 3-False, 4-True, 5-True, 6-True, 7-True, 8-False, 9-True, 10-False

5.15 REFERENCES

- Bryant DA, Frigaard NU (Nov 2006). "Prokaryotic photosynthesis and phototrophy illuminated". Trends in Microbiology. **14** (11): 488–96. doi:10.1016/j.tim.2006.09.001. PMID 16997562.
- Plants: Diversity and Evolution, page 14, Martin Ingrouille, Bill Eddie
- Campbell NA, Williamson B, Heyden RJ (2006). Biology Exploring Life. Upper Saddle River, NJ: Pearson Prentice Hall. ISBN 0-13-250882-6.
- Smith, Alison (2010). Plant biology. New York, NY: Garland Science. p. 5. ISBN 0815340257.
- Whitmarsh J, Govindjee (1999). "The photosynthetic process". In Singhal GS, Renger G, Sopory SK, Irrgang KD, Govindjee. Concepts in photobiology: photosynthesis and photomorphogenesis. Boston: Kluwer Academic Publishers. pp. 11–51. ISBN 0-7923-5519-9.
- "Photosynthesis". McGraw-Hill Encyclopedia of Science & Technology. **13**. New York: McGraw-Hill. 2007. ISBN 0-07-144143-3.
- Whitmarsh J, Govindjee (1999). "Chapter 2: The Basic Photosynthetic Process". In Singhal GS, Renger G, Sopory SK, Irrgang KD, Govindjee. Concepts in Photobiology: Photosynthesis and Photomorphogenesis. Boston: Kluwer Academic Publishers. p. 13. ISBN 978-0-7923-5519-9.

5.16 SUGESSTED READINGS

- Photosynthesis.(Sixth Edition). David O Hall Krishna Rao. Cambridge University Press.
- Plant Physiology and Biochemistry. H.S Srivastava. Rastogi Publications.Meerut.
- Botany for Degree students. B.P Pandey. S.Chand Publications.
- Fundamentals of plant physiology. V.K Jain. S.Chand Publications.
- A textbook of botany. V.Singh, P.C Pande and D.K Jain. (2008)
- Plant Physiology. S.N Pandey.
- Textbook of Plant Physiology and Biochemistry. Mohit Verma and Pooja Gupta.
- A textbook of Plant Physiology, Biochemistry and Biotechnology. Dr. S.K Verma and Mohit Verma.

5.17 TERMINAL QUESTIONS

5.17.1 Long Answer Type Questions:

1. What is photosynthesis? Describe the role of light and chlorophyll in photosynthesis.
2. Give an account of the mechanism of CO_2 fixation, explaining major steps and the end products in photosynthesis.
3. Comment on major chemical events occurring in the dark reactions of photosynthesis.
4. Write a concise account of photophosphorylation.
5. Give an account on Calvin cycle.
6. Explain why does photolysis of water take place only in pigment system II and not in pigment system I.
7. What is photorespiration and how is it related to photosynthesis?
8. Give evidences for the existence of two photosystems in light reaction.
9. Write a short account on factors which affect photosynthesis.
10. Schematically represent the Z scheme of photosynthesis electron transport.

UNIT-6 RESPIRATION

Contents

- 6.1 Objectives
- 6.2 Introduction
- 6.3 ATP -the biological energy currency
- 6.4 Types of respiration
- 6.5 Aerobic respiration
 - 6.5.1 Glycolysis
 - 6.5.2 Krebs Cycle
 - 6.5.3 Terminal Oxidation
- 6.6 Anaerobic respiration
 - 6.6.1. Ethyl Alcohol Fermentation
 - 6.6.2. Lactic Acid Fermentation
 - 6.6.3. Alternate Anaerobic Respiration
 - 6.6.3.1 Pentose Phosphate Pathway
 - 6.6.3.2. Entner Duodoroff Pathway
- 6.7 Summary
- 6.8 Glossary
- 6.9 Self Assessment Question
- 6.10 References
- 6.11 Suggested Readings
- 6.12 Terminal Questions

6.1 OBJECTIVES

After reading this unit students will be able to-

- Understand the significance and mechanism of respiration.
- Learn the mechanism of aerobic and anaerobic respiration.
- Understand Kreb's cycle and electron transport mechanism and fermentation mechanism.
- Explain how actually energy is released and stored in the form of ATP in the cell.
- Account for 38 ATP molecules that are released during aerobic respiration.

6.2 INTRODUCTION

You know that all living organisms respire in order to release energy from glucose and make it available in the form of ATP for chemical, osmotic and other work. Plants are no exception. They need to respire virtually all the time in order to supply their energy needs. They are not able to use the ATP generated in photosynthesis for these purposes. Plants respire in the normal way using glycolysis, Krebs cycle, oxidative phosphorylation etc.

Often, the respiration is masked by the fact that photosynthesis produces oxygen faster than respiration takes it up and photosynthesis uses carbon dioxide faster than respiration produces it. It is only in the dark that the full effects of respiration become apparent when photosynthesis is brought to a halt.

Plants need energy to take in mineral salts from the soil where they are present in very low concentration - this needs work (energy) to concentrate the mineral inside the plant. Plants growing in waterlogged soils (which are short of oxygen) cannot respire in their roots and soon show the symptoms of shortage of minerals (like yellow leaves). (Rice is interesting because it has a pithy stem through which it enables oxygen from above the water to get down to the roots and therefore rice thrives in "paddy fields".)

Respiration is one of the many processes needed for survival. It is the process by which energy is released from food by oxidizing the organic molecules. Respiration may occur in the presence of oxygen, in which case it is called aerobic respiration or it may occur in the absence of oxygen and is called anaerobic respiration. The main organic molecules used in respiration are carbohydrates, such as the monosaccharide glucose and fructose, and fats. Proteins may also be oxidized however it is a secondary source as protein is needed for other things such as cell growth and repair.

6.3 ATP- THE BIOLOGICAL ENERGY CURRENCY

Adenosine triphosphate (ATP) is a nucleoside triphosphate, a small molecule used in cells as a coenzyme. It is often referred to as the "molecular unit of currency" of intracellular energy transfer. ATP transports chemical energy within cells for metabolism.

ATP transports chemical energy within cells for metabolism. Most cellular functions need energy to be carried out: synthesis of proteins, synthesis of membranes, movement of the cell, cellular division etc.

The ATP is the molecule that carries energy to the place where the energy is needed. When ATP breaks into ADP (Adenosine diphosphate) and Pi, the breakdown of the last covalent link of phosphate (a simple -P₀₄) liberates energy that is used in reactions where it is needed.

It is one of the end products of photophosphorylation, aerobic respiration, and fermentation, and is used by enzymes and structural proteins in many cellular processes, including biosynthetic reactions, motility, and cell division. One molecule of ATP contains adenosine, ribose, and three phosphate groups, and it is produced by a wide variety of enzymes, including ATP synthase, from adenosine diphosphate (ADP) or adenosine monophosphate (AMP) and various phosphate group donors. Substrate-level phosphorylation, oxidative phosphorylation in cellular respiration, and photophosphorylation in photosynthesis are three major mechanisms of ATP biosynthesis.

The structure of this molecule consists of a purine base (adenine) attached by the 9' nitrogen atom to the 1' carbon atom of a pentose sugar (ribose). Three phosphate groups are attached at the 5' carbon atom of the pentose sugar. It is the addition and removal of these phosphate groups that inter-convert ATP, ADP and AMP. When ATP is used in DNA synthesis, the ribose sugar is first converted to deoxyribose by ribonucleotide reductase.

Respiration results in the formation of ATP (adenosine triphosphate) (Fig.6.1) through a process called the Krebs's cycle which takes place on the membranes of the mitochondrion. ATP is the form of energy used in the body and provides cells with all the energy needed to carry out their daily functions such as drive metabolic reactions which must occur fast enough if life is to be sustained. If there is an excess of ATP then this will be stored in the mitochondrion for later use.

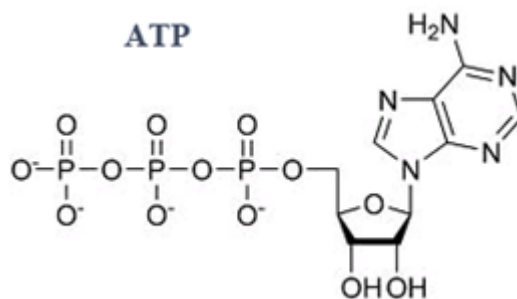


Fig. 6.1 Adenosine triphosphate

6.4 TYPES OF RESPIRATION

Respiration starts with glucose (usually). In aerobic and anaerobic respiration initial reactions are common as a result of which pyruvic acid is formed by breakdown of glucose. The process is called Glycolysis or EMP Pathway (Embden-Meyerhof-Parnas Pathway). This process does not require O_2 although this can take place in the presence of oxygen. After this stage, the fate of pyruvic acid is different depending upon the presence or absence of oxygen. (Fig.2).

If oxygen is present there is complete oxidation of pyruvic acid into H_2O and CO_2 and chemical reactions through which this occurs is called Tri-Carboxylic Acid cycle (TCA Cycle) or Krebs Cycle. This cycle occurs in mitochondria. If oxygen is absent, pyruvic acid forms ethyl alcohol (C_2H_5OH) and CO_2 without the help of any cell organelle. This process is called anaerobic respiration.

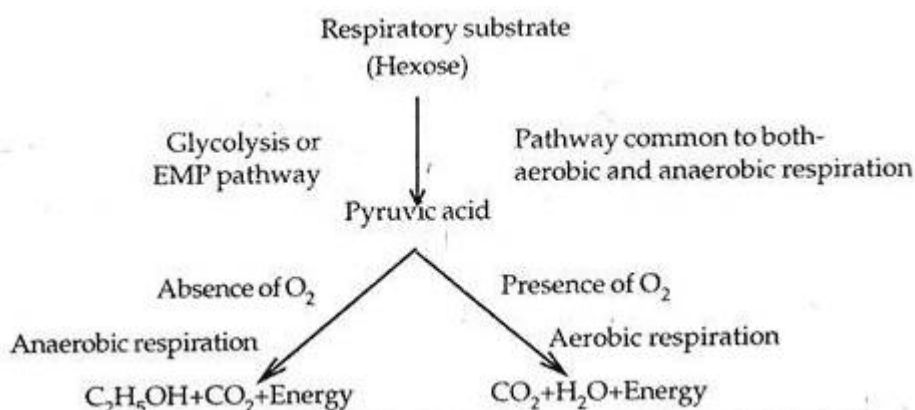


Fig.6.2 Schematic representation of interrelationship between aerobic and anaerobic respiration

6.5 AEROBIC RESPIRATION

Aerobic respiration is an enzymatically controlled release of energy in a stepwise catabolic process of complete oxidation of organic food into carbon dioxide and water with oxygen acting as terminal oxidant. The common mechanism of aerobic respiration is also called common pathway because its first step, called glycolysis, is common to both aerobic and anaerobic modes of respiration. The common aerobic respiration consists of three steps—glycolysis, Krebs cycle and terminal oxidation.

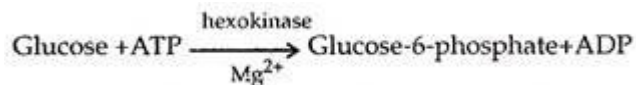
6.5.1 GLYCOLYSIS

It is also called EMP pathway because it was discovered by three German scientists Embden, Meyerhof and Parnas. Glycolysis is the process of breakdown of glucose or similar hexose sugar to molecules of pyruvic acid through a series of enzyme mediated reactions releasing some

energy (as ATP) and reducing power (as NADH₂) (Fig 6.3). It occurs in the cytoplasm. It takes place in the following sub steps.

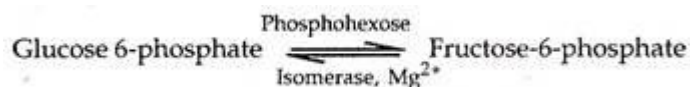
a. Phosphorylation:

Glucose is phosphorylated to glucose-6-phosphate by ATP in the presence of enzyme hexokinase (Meyerhof, 1927) or glucokinase (e.g., liver) and Mg²⁺.

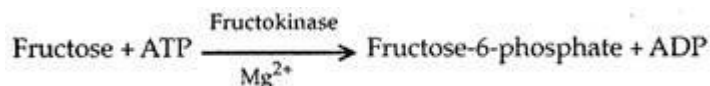


b. Isomerization:

Glucose-6-phosphate is changed to its isomer fructose-6-phosphate with the help of enzyme phosphohexose isomerase.

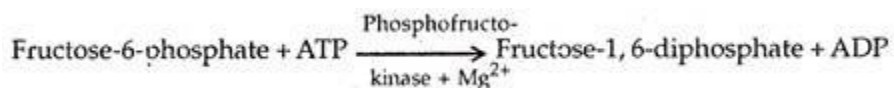


Fructose-6-phosphate can also be produced directly by phosphorylation of fructose with the help of enzyme fructokinase.



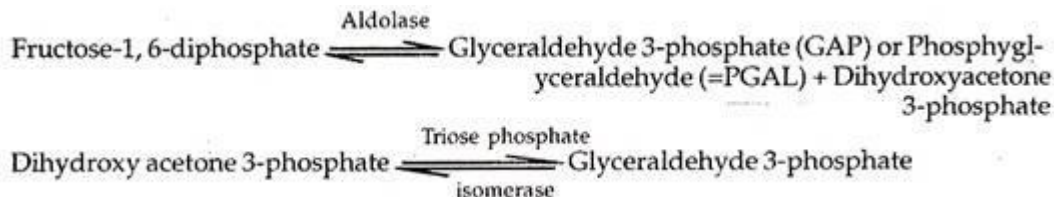
c. Phosphorylation:

Fructose-6-phosphate is further phosphorylated by means of ATP in presence of enzyme phosphofructo-kinase and Mg²⁺. The product is Fructose-1, 6 diphosphate.



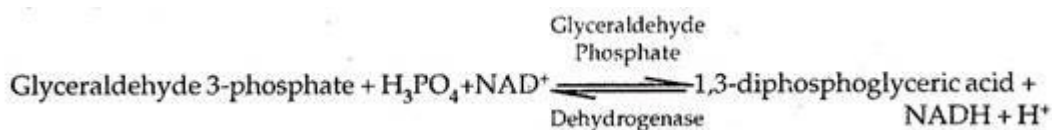
d. Splitting:

Fructose-1, 6-diphosphate splits up enzymatically to form one molecule each of 3- carbon compounds, glyceraldehyde 3-phosphate (= GAP or 3-phosphoglyceraldehyde = PGAL) and dihydroxy acetone 3-phosphate (DIHAP). The latter is further changed to glyceraldehyde 3-phosphate by enzyme triose phosphate isomerase (= phosphotriose isomerase).



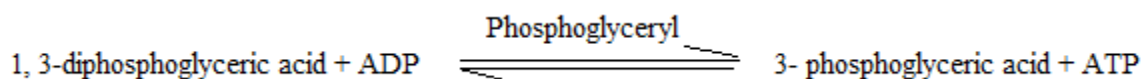
e. Dehydrogenation and Phosphorylation:

In the presence of enzyme glyceraldehyde phosphate dehydrogenase, glyceraldehyde 3-phosphate loses hydrogen to NAD to form NADH₂ and accepts inorganic phosphate to form 1, 3-diphosphoglyceric acid.



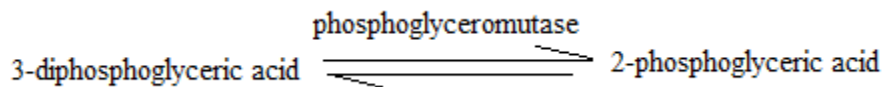
f. Formation of ATP:

One of the two phosphates of diphosphoglyceric acid is linked by high energy bond. It can synthesise ATP and form 3-phosphoglyceric acid. The enzyme is phosphoglyceryl inase. The direct synthesis of ATP from metabolites is called substrate level phosphorylation.



g. Isomerization:

3-phosphoglyceric acid is changed to its isomer 2-phosphoglyceric acid by enzyme phosphoglyceromutase.



h. Dehydration:

Through the agency of enzyme enolase, 2-phosphoglyceric acid is converted to phosphoenol pyruvate (PEP). A molecule of water is removed in the process. Mg²⁺ is required.

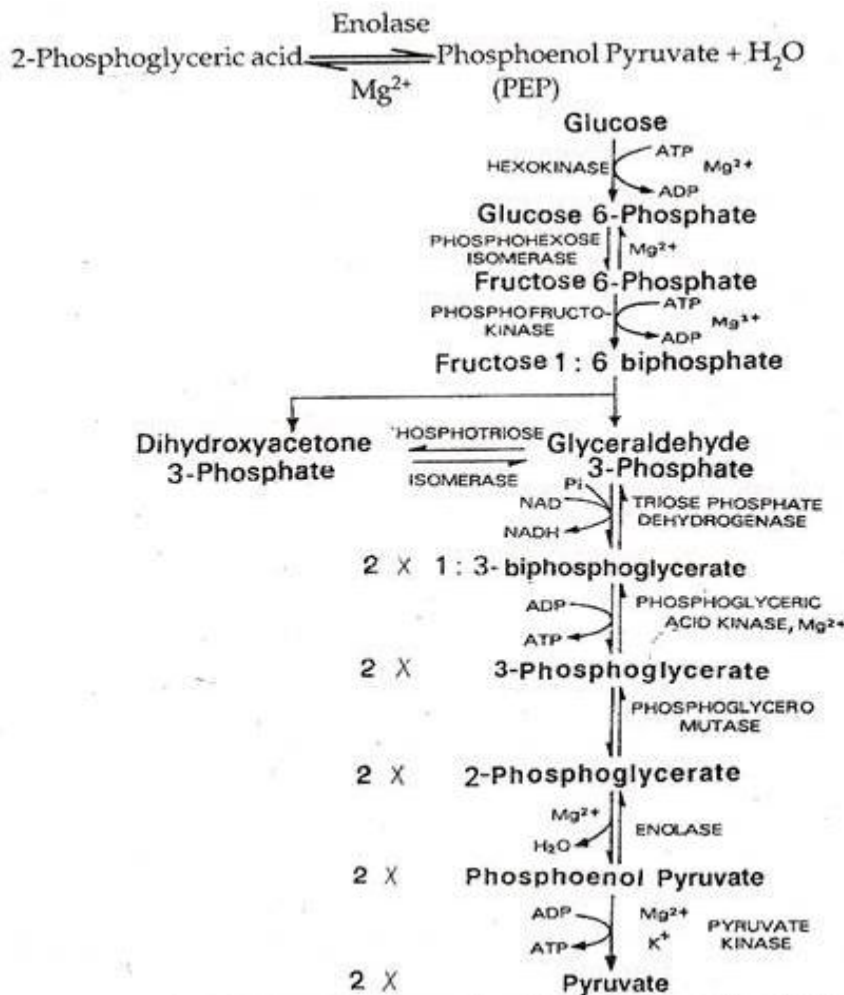
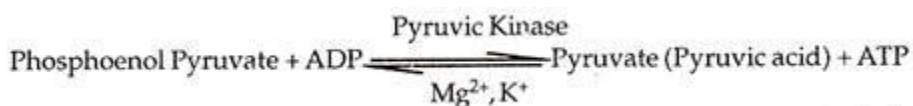


Fig. 6.3 Schematic representation of glycolysis or EMP pathway

i. Formation of ATP:

During formation of phosphoenol pyruvate the phosphate radical picks up energy. It helps in the production of ATP by substrate level phosphorylation. The enzyme is pyruvic kinase. It produces pyruvate from phosphoenol pyruvate.



NET PRODUCTS OF GLYCOLYSIS:

In glycolysis two molecules of ATP are consumed during double phosphorylation of glucose to form fructose-1, 6 diphosphate. In return four molecules of ATP are produced by substrate level phosphorylation (conversion of 1, 3 diphosphoglyceric acid to 3-phosphoglyceric acid and phosphoenol pyruvate to pyruvate). Two molecules of NADH₂ are formed at the time of oxidation of glyceraldehyde 3-phosphate to 1, 3-diphosphoglyceric acid. The net reaction is as follows:

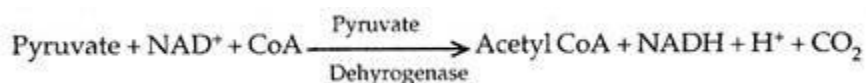


6.5.2 KREBS CYCLE

The cycle was discovered by Hans Krebs (1937, 1940, Nobel Prize 1953). It occurs inside mitochondria. The cycle is also named as citric acid cycle or tricarboxylic acid (TCA) cycle after the initial product. Krebs cycle is stepwise oxidative and cyclic degradation of activated acetate derived from pyruvate (Fig. 6.4).

Oxidation of Pyruvate to Acetyl-CoA:

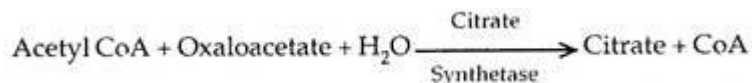
Pyruvate enters mitochondria. It is decarboxylated oxidatively to produce CO_2 and NADH. The product combines with sulphur containing coenzyme A to form acetyl CoA or activated acetate. The reaction occurs in the presence of an enzyme complex pyruvate dehydrogenase (made up of a decarboxylase, lipoic acid, TPP, transacetylase and Mg^{2+}).



Acetyl CoA functions as substrate entrant for Krebs cycle. The acceptor molecule of Krebs cycle is a 4-carbon compound oxaloacetate. Krebs cycle involves two decarboxylations and four dehydrogenations. The various components of Krebs cycle are as follows.

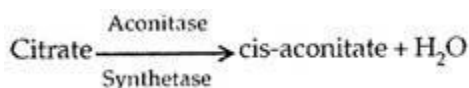
a. Condensation:

Acetyl CoA (2-carbon compound) combines with oxaloacetate (4-carbon compound) in the presence of condensing enzyme citrate synthetase to form a tricarboxylic 6-carbon compound called citric acid. It is the first product of Krebs cycle. CoA is liberated.



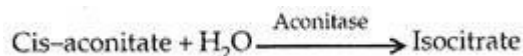
b. Dehydration:

Citrate undergoes reorganisation in the presence of aconitase forming cis aconitate releasing water.

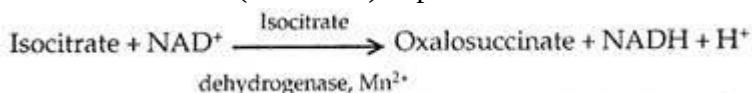


c. Hydration:

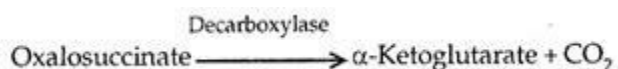
Cis-aconitate is converted into isocitrate with the addition of water in the presence of iron containing enzyme aconitase.

**d. Dehydrogenation:**

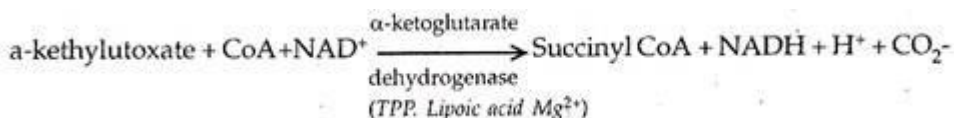
Isocitrate is dehydrogenated to oxalosuccinate in the presence of enzyme isocitrate dehydrogenases and Mn^{2+} . NADH_2 (NADPH_2) is produced.

**e. Decarboxylation:**

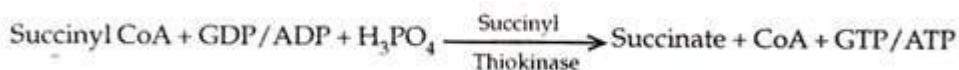
Oxalosuccinate is decarboxylated to form α -ketoglutarate through enzyme decarboxylase. Carbon dioxide is released.

**f. Dehydrogenation and Decarboxylation:**

α -Ketoglutarate is both dehydrogenated (with the help of NAD^+) and decarboxylated by an enzyme complex α -ketoglutarate dehydrogenase. The enzyme complex contains TPP and lipoic acid. The product combines with CoA to form succinyl CoA.

**g. Formation of ATP/GTP:**

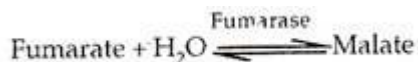
Succinyl CoA is acted upon by enzyme succinyl thiokinase to form succinate. The reaction releases sufficient energy to form ATP (in plants) or GTP (in animals).

**h. Dehydrogenation:**

Succinate undergoes dehydrogenation to form fumarate with the help of a dehydrogenase. FADH_2 (reduced flavin adenine dinucleotide) is produced.

**i. Hydration:**

A molecule of water gets added to fumarate to form malate. The enzyme is called fumarase.



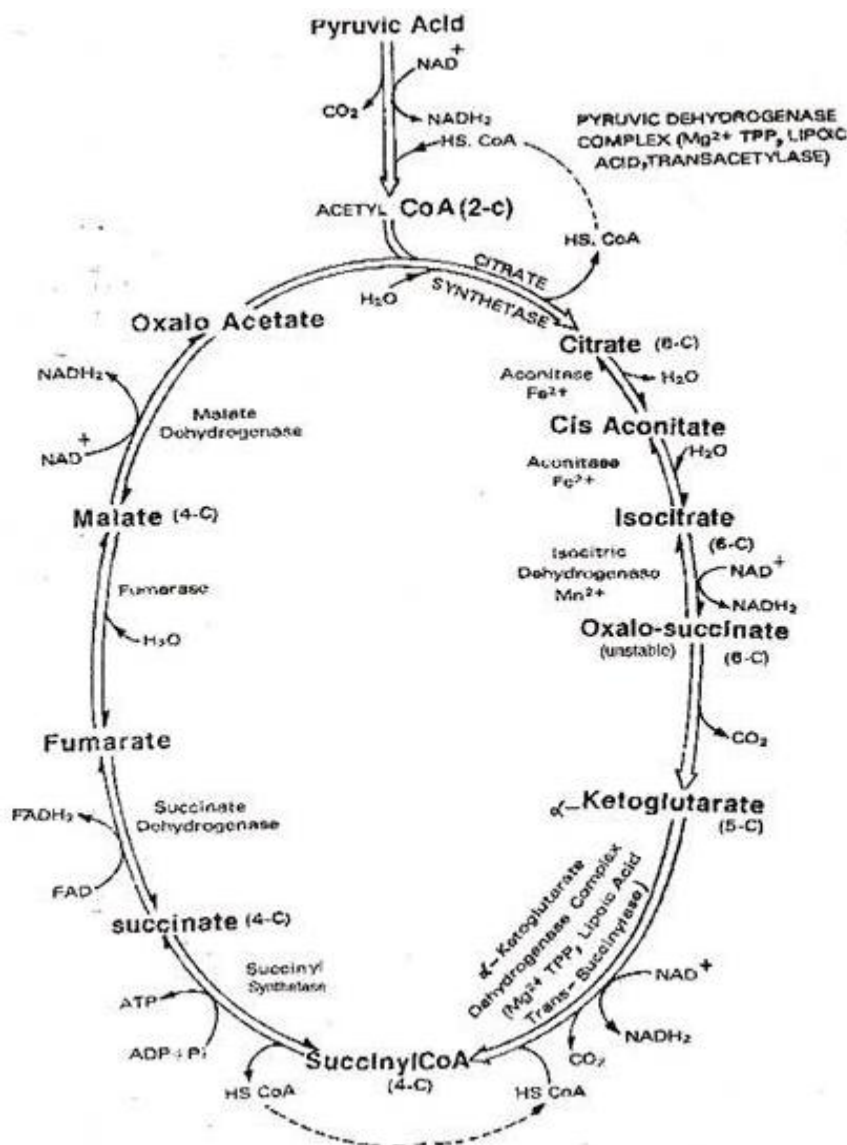
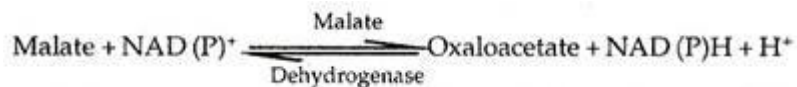


Fig.6.4 Schematic representation of Krebs cycle or TCA cycle

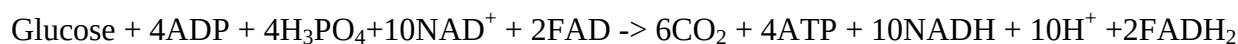
j. Dehydrogenation:

Malate is dehydrogenated or oxidised through the agency of malate dehydrogenase to produce oxaloacetate. Hydrogen is accepted by NADP^+ NAD^+



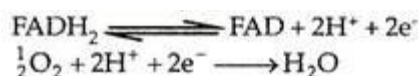
Oxaloacetate picks up another molecule of activated acetate to repeat the cycle.

A molecule of glucose yields two molecules of NADH_2 , 2ATP and two pyruvate while undergoing glycolysis. The two molecules of pyruvate are completely degraded in Krebs cycle to form two molecules of ATP, 8NADH_2 , and 2FADH_2 .



6.5.3 TERMINAL OXIDATION

It is the name of oxidation found in aerobic respiration that occurs towards the end of catabolic process and involves the passage of both electrons and protons of reduced coenzymes to oxygen.



Terminal oxidation consists of two processes—electron transport and oxidative phosphorylation.

6.5.3.1. ELECTRON TRANSPORT CHAIN:

Inner mitochondrial membrane contains groups of electron and proton transporting enzymes. In each group the enzymes are arranged in a specific series called electron transport chain (ETC) or mitochondrial respiratory chain or electron transport system (ETS). (Fig.6.5)

An electron transport chain or system is a series of coenzymes and cytochromes that take part in the passage of electrons from a chemical to its ultimate acceptor. The passage of electrons from one enzyme or cytochrome to the next is a downhill journey with a loss of energy at each step. At each step the electron carriers include flavins, iron sulphur complexes, quinones and cytochromes.

Most of them are prosthetic groups of proteins. Quinones are highly mobile electron carriers. Four enzymes are involved in electron transport—(i) NADH-Q reductase or NADH-dehydrogenase (ii) Succinate Q-reductase complex (iii) QH_2 -cytochrome c reductase complex (iv) Cytochrome c oxidase complex. NADH-Q reductase (or NADH-dehydrogenase) has two prosthetic groups, flavin mononucleotide (FMN) and iron sulphur (Fe-S) complexes. Both electrons and protons pass from NADH_2 to FMN. The latter is reduced.

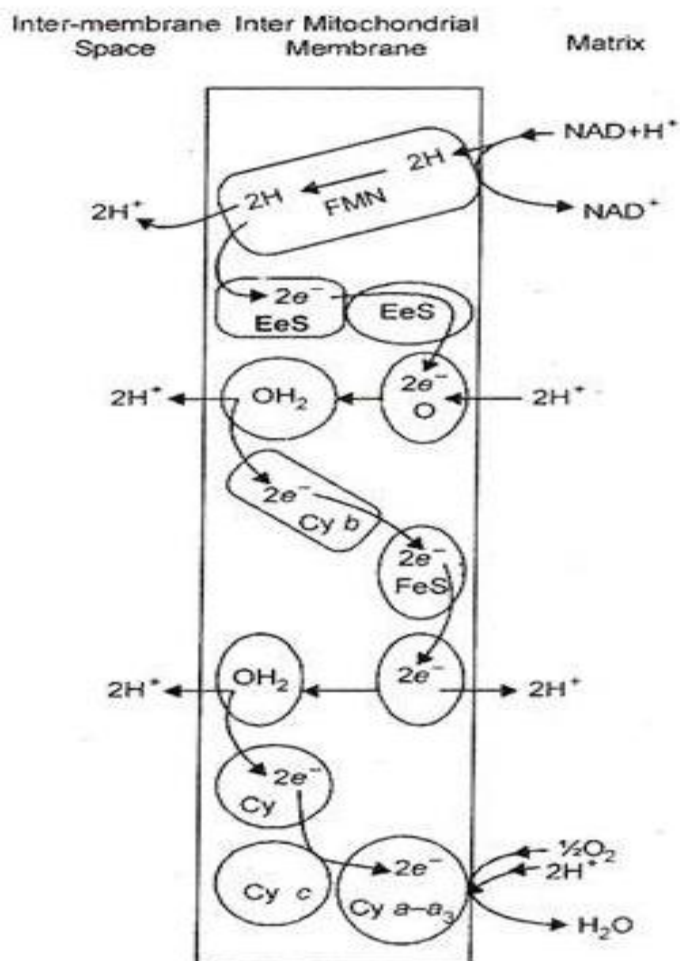
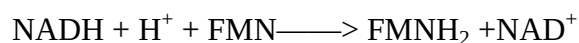
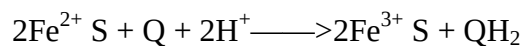
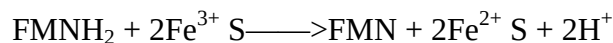


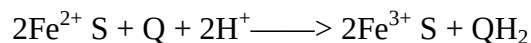
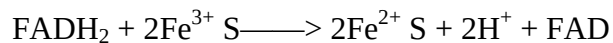
Fig.6.5 Electron Transport System (ETS)



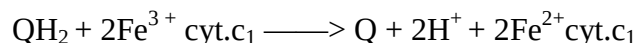
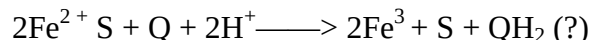
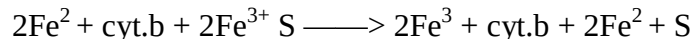
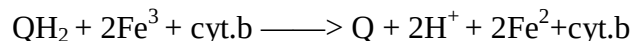
Electron now moves to the FeS complex and from there to a quinone. The common quinone is co-enzyme Q, also called ubiquinone (UQ).



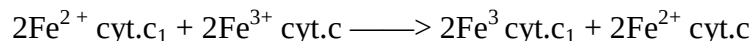
FADH₂ produced during reduction of succinate also hands over its electrons and protons to co-enzyme Q through FeS complex. The enzyme is succinate-Q reductase complex.



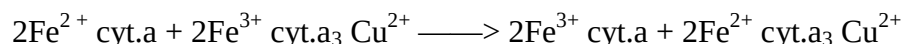
QH₂-cytochrome c reductase complex has three components—cytochrome b, FeS complex and cytochrome c₁. Coenzyme Q may also be involved between FeS complex and cytochrome c₁.



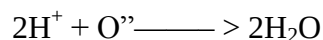
Cytochrome c₁ hands over its electron to cytochrome c. Like co-enzyme Q, cytochrome c is also mobile carrier of electrons.



Cytochrome c oxidase complex comprises cytochrome a and cytochrome a₃. Cytochrome a₃ also possesses copper. The latter helps in transfer of electron to oxygen.



Oxygen is the ultimate acceptor of electrons. It becomes reactive and combines with protons to form metabolic water.



Energy released during passage of electrons from one carrier to the next is made available to specific transmembrane complexes, which pump protons (H^+) from the matrix side of the inner mitochondrial membrane to the outer chamber. There are three such sites corresponding to three enzymes present in the electron transport chain (NADH-Q reductase, QH₂-cytochrome c reductase and cytochrome c-oxidase).

This increases proton concentration in the outer chamber or outer surface of the inner mitochondrial membrane. The difference in the proton concentration on the outer and inner sides of the inner mitochondrial membrane is known as proton gradient. (Fig 6.6)

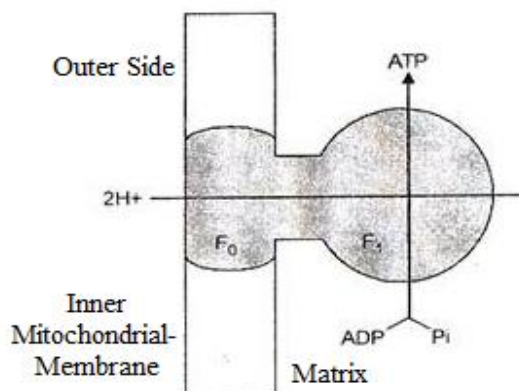


Fig.6.6 Diagrammatic representation of ATP synthesis in mitochondria

6.5.3.2. Oxidative Phosphorylation:

Oxidative phosphorylation is the synthesis of energy rich ATP molecules with the help of energy liberated during oxidation of reduced co-enzymes (NADH_2 , FADH_2) produced in respiration. The enzyme required for this synthesis is called ATP synthetase.

It is located in F_1 or head piece of F_0 - F_1 or elementary particles present in the inner mitochondrial membrane. ATP-synthetase becomes active in ATP formation only where there is a proton gradient having higher concentration of H^+ or protons on the F_0 side as compared to F_1 side (chemiosmotic hypothesis of Peter Mitchel, 1961).

Increased proton concentration is produced in the outer chamber or outer surface of inner mitochondrial membrane by the pushing of protons with the help of energy liberated, by passage of electrons from one carrier to another.

Transport of the electrons from NADH_2 over ETC helps in pushing three pairs of protons to the outer chamber while two pairs of protons are sent outwardly during electron flow from FADH_2 (as the latter donates its electrons further down to the ETC).

Higher proton concentration in the outer chamber causes the protons to pass inwardly into matrix or inner chamber through the inner membrane. The latter possesses special proton channels in the region of F_Q (base) of the F_0 - F_1 particles.

The flow of protons through the F_0 channel induces F_1 particles to function as ATP-synthetase. The energy of the proton gradient is used in attaching a phosphate radicle to ADP by high energy bond. This produces ATP. Oxidation of one molecule of NADH_2 produces 3 ATP molecules while a similar oxidation of FADH_2 forms 2 ATP molecules.

2 ATP molecules are produced during glycolysis and 2 ATP (GTP) molecules during double Krebs cycle. Glycolysis also forms 2NADH_2 . Its reducing power is transferred to mitochondria

for ATP synthesis. For this a shuttle system operates at the inner mitochondrion membrane. (i) $\text{NADH}_2 \rightarrow \text{NAD} \rightarrow \text{NADH}_2$. (ii) $\text{NADH}_2 \rightarrow \text{FAD} \rightarrow \text{FADH}_2$.

The former operates in liver, heart and kidney cells. No energy is spent. The second method occurs in muscle and nerve cells. It lowers the energy level of 2NADH_2 by 2ATP molecules. A total of 10NADH_2 and 2FADH_2 molecules are formed in aerobic respiration.

They help in formation of 34ATP molecules. The net gain from complete oxidation of a molecule of glucose in muscle and nerve cells is 36ATP molecules ($10\text{NADH}_2 = 30\text{ATP}$, $2\text{FADH}_2 = 4\text{ATP}$, four formed by substrate level phosphorylation in glycolysis and Krebs cycle and two consumed in transport of the NADH_2 molecules into mitochondria). (Fig 6.7)

In procaryotes, heart, liver, and kidneys, 38ATP molecules are produced per glucose molecules oxidised. Passage of ATP molecules from inside of mitochondria to cytoplasm is through facilitated diffusion.

Since, one ATP molecule stores 8.9 kcal/mole (7 kcal/mole according to early estimates) the total energy trapped per gm mole of glucose is 338.2 kcal (266 kcal) or an efficiency of 49.3% (38.8% according to older estimates). The rest of the energy is lost as heat.

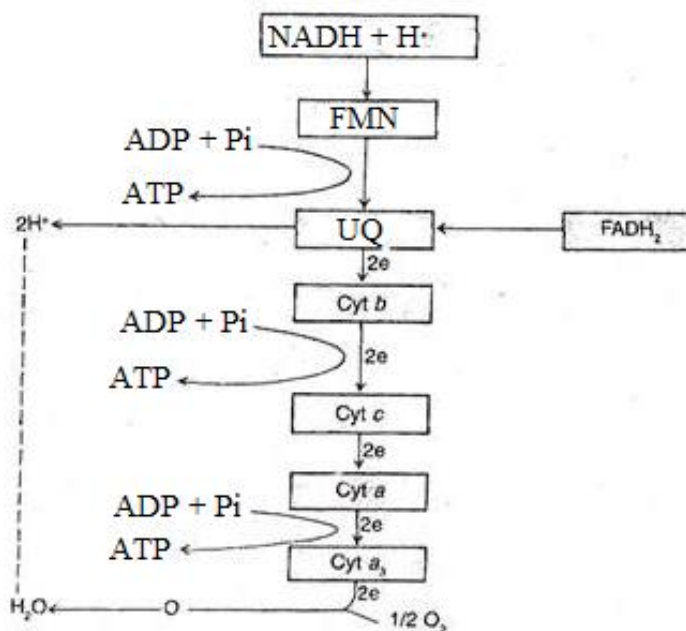


Fig.6.7 A simplified system of terminal oxidation and oxidative phosphorylation

Significance of Krebs cycle:

1. Apart from serving as an energy-generating system, Krebs cycle yields several substances that figure as starting points for a number of biosynthetic reactions. Ordinarily Krebs cycle of respiration is considered catabolic in nature, but it provides a number of intermediates for

anabolic pathways. Therefore Krebs cycle is amphibolic (both catabolic and anabolic). A few examples are cited below:

(a) The synthesis of sucrose by way of glyoxylytic acid cycle is an instance in point. A slightly modified Krebs cycle leads to the formation of glyoxylate, malate, oxaloacetate, phosphoenol pyruvate and then by a reversed glycolytic pathway, sucrose is formed.

(b) There are two keto acids in Krebs cycle and on amination they yield the respective amino acids- Pyruvic acid $\rightarrow$ alanine; Oxaloacetic acid $\rightarrow$ aspartic acid; and α -ketoglutaric acid $\rightarrow$ glutamic acid.

The last of these opens up new pathways leading to the synthesis of glutamine, ornithine, proline, hydroxyproline, citrulline and arginine.

(c) Succinyl-CoA is the starting point for the biosynthesis of several porphyrins.

2. Krebs cycle is a common pathway of oxidative breakdown of carbohydrates, fatty acids, and amino acids.

6.6 ANAEROBIC RESPIRATION

Anaerobic respiration is synonymous with fermentation. It is also called intermolecular respiration.

Here the carbohydrates are degraded into two or more simple molecules without oxygen being used as oxidant. In anaerobic respiration (fermentation) the carbon-skeleton of glucose molecule is never completely released as CO_2 and in some it may not appear at all. It does not require mitochondria and is completed in cytoplasm.

The reason for believing that the two processes, fermentation and anaerobic respiration are identical, are:

1. Hexose sugar is respiratory substrates in both.
2. The principal end products are same (CO_2 and $\text{C}_2\text{H}_5\text{OH}$) in both the cases.
3. The same enzyme systems drive both the processes.
4. Pyruvic acid and acetaldehyde are formed as intermediates in both the processes.
5. Both the processes are accelerated by addition of phosphate.

But it must be mentioned that fermentation is an in vitro process, referring to an occurrence outside of a living system while anaerobic respiration is a cellular process, occurring in vivo. Also the energy produced during fermentation is totally lost as heat but the energy produced during anaerobic respiration, some of it at least, is trapped into ATP (Fig.6.8).

The term anaerobic respiration is often used in connection with higher organisms where it occurs in the roots of water-logged plants, muscles of animals and as supplementary mode of respiration in massive tissues. Anaerobic respiration is the exclusive mode of respiration in some parasitic worms and micro-organisms (e.g., bacteria, moulds). In micro-organisms, the term fermentation is more commonly used where anaerobic respiration is known after the name of product like alcoholic fermentation, lactic acid fermentation.

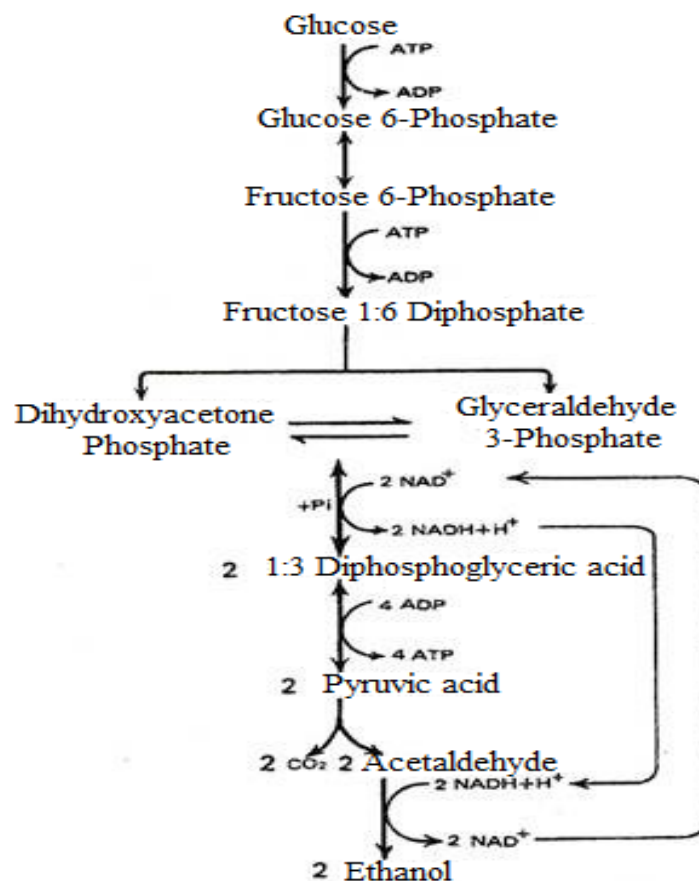
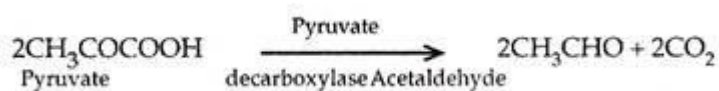


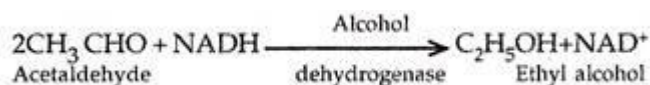
Fig.6.8 Schematic representation of alcoholic fermentation or anaerobic respiration yielding ethyl alcohol

6.6.1. Ethyl Alcohol Fermentation

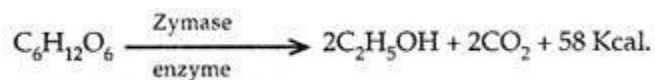
It is quite common in fungi (e.g., Rhizopus, Yeast) and bacteria. Yeast can respire both aerobically and anaerobically. Anaerobic respiration occurs in sugary solution if the fungus is not in contact with atmosphere. It causes fermentation. In the presence of pyruvate decarboxylase and TPP (thiamine pyrophosphate), pyruvate is broken down to form acetaldehyde. Carbon dioxide is released.



In the second step, acetaldehyde is reduced to alcohol by alcohol dehydrogenase. Hydrogen is obtained from NADH-, produced during oxidation of glyceraldehyde 3-phosphate to 1,3-diphospho- glyceric acid in glycolysis.

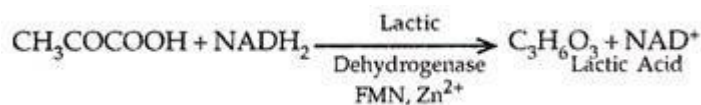


Thus from 1 molecule of glucose, 2 molecules of pyruvic acid are formed and from 2 molecules of pyruvate (pyruvic acid) 2 molecules of ethyl alcohol and 2 molecules of CO_2 are produced. The overall equation is as follows:



6.6.2. Lactic Acid Fermentation

Less familiar in higher plants but quite common in animal tissue, this pathway leads to the formation of lactic acid. A NADH-requiring lactic dehydrogenase brings about this reaction. The NADH required for the reaction is produced in glycolysis.



6.6.3 Alternate Anaerobic Respiration

The EMP pathway of glycolysis is no doubt the main anaerobic process but not the only channel of glucose metabolism. There are other pathways by which glucose is metabolised anaerobically in both plants and animal tissues. Two such systems discovered working in cells are Pentose phosphate pathway and Entner Duodoroff pathway.

6.6.3.1 Pentose Phosphate Pathway:

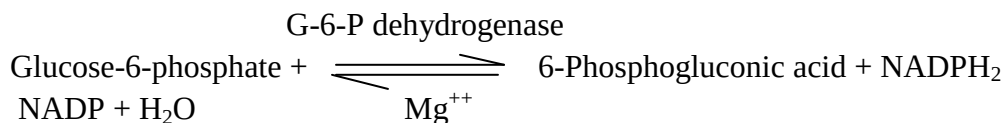
Variously called Direct Oxidation Pathway, Pentose Phosphate Pathway, Warburg-Dickens Pathway and Hexose Monophosphate Shunt, this metabolic pathway had been discovered through a number of experiments of Lippman, Warburg (1935) and Dickens (1938). Later Horacker (1955) and Racker (1954) worked out the sequence of events in the pathway.

Pentose Phosphate Pathway could be considered to proceed in two phases, a decarboxylative phase and a subsequent regenerative phase, in the first phase, the hexose is converted into a pentose. Two reactions, a decarboxylation and two dehydrogenations bring this about.

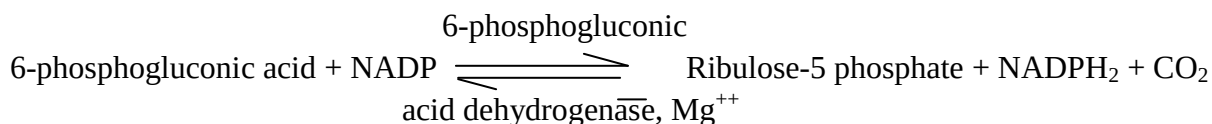
NADP functions as the coenzyme in both these reactions. In the second phase, there is reorganisation of the pentoses formed in phase I to produce a hexose. Therefore, in this pathway there is no cleavage of hexose to trioses as in glycolysis, and in PPP, NADP serves as the coenzyme and not NAD as in glycolysis.

Phase I. Decarboxylative phase:

a. Glucose-6-phosphate, which is the starting point for the operation of this pathway, is oxidised to phospho-gluconic acid by the mediation of NADP-linked glucose-6-phosphate dehydrogenase. Magnesium serves as an activator for this enzyme. The production of NADPH_2 marks the first dehydrogenation in this reaction.



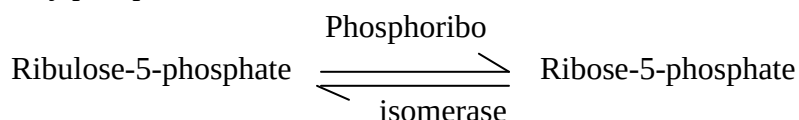
b. The 6-phosphogluconic acid is oxidised and decarboxylated by the NADP-linked 6-phosphogluconic acid dehydrogenase. The reaction is activated by Mg^{++}



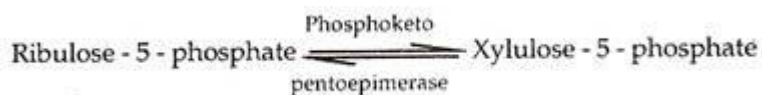
The production of NADPH_2 is the final dehydrogenation occurring along this pathway.

Phase II. Regenerative Phase:

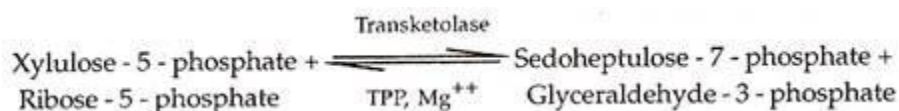
c. Here, ribulose-5-phosphate is converted to its aldopentose isomer, ribose-5-phosphate and is mediated by phosphoriboisomerase.



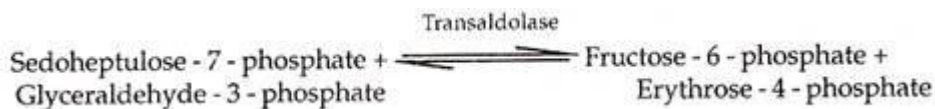
d. Some of the ribulose-5-phosphate formed in reaction 2 is isomerised to xylulose-5-phosphate, ketopentose. This is effected by phosphoketopento-epimerase.



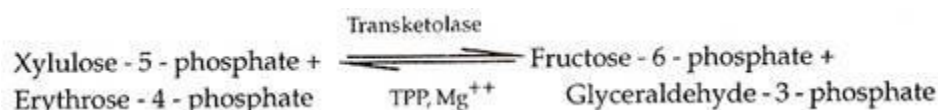
e. Ribose-5-phosphate and Xylulose-5-phosphate produced in reaction 3 and 4 form the substrates for this reaction. The enzyme, transketolase, transfers the ketol group from xylulose-5-phosphate to ribose-5-phosphate. As a result, a seven-carbon keto sugar, sedoheptulose-7-phosphate and a triose, glyceraldehyde-3-phosphate are formed. TPP and Mg^{++} serves as cofactors for this enzyme.



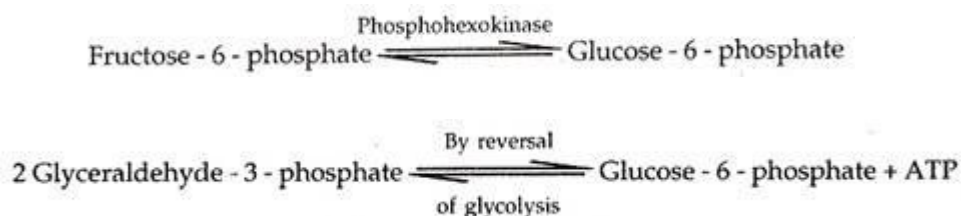
f. Sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate are converted into fructose-6-phosphate and erythrose-4-phosphate by the mediation of transaldolase.



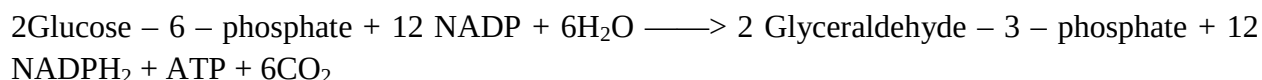
g. The erythrose-4-phosphate of reaction 6 and xylulose-5-phosphate of reaction 4 react through the agency of transketolase, forming fructose-6-phosphate and glyceraldehyde-3-phosphate.



Eventually, the fructose-6-phosphate formed in reactions f and g and glyceraldehyde-3-phosphate from reactions e and g, are converted into glucose-6-phosphate and this is further utilised to promote the pathway, until all of its carbon go off as CO₂. These reactions do not form part of the pathway.



Thus the PPP may be summarized as follows:



Significance of Pentose Phosphate Pathway

- (a) The NADPH₂ produced drive a number of reactions leading to the conversion of glucose to sorbitol pyruvic acid to malic acid and phenylalanine to tyrosine.
- (b) NADH₂ also plays a key role in the production of fatty acid and steroids.
- (c) In this cycle several metabolically important intermediates such as ribose-5-phosphate and erythrose-4-phosphate are generated.

6.6.3.2 Entner Duodoroff Pathway:

In some bacteria like Azotobacter, the enzyme phosphofructokinase is absent. Such organisms naturally cannot phosphorylate glucose in the usual EMP pathway. They dissimilate glucose by a combination of pentose phosphate pathway and an aldolase type of reaction as in glycolysis.

Here, glucose is oxidized to 6-phosphogluconic acid, in the same manner as in reaction 1 of PPP. In the next step, 6-phosphogluconic acid undergoes a dehydration and a conformational change, resulting in a α -keto deoxysugar phosphate which is then cleaved into pyruvate and glyceraldehyde phosphate. GAP is converted to pyruvic acid. This pathway also produces 2 pyruvic acids from one molecule of glucose.

6.7 SUMMARY

In this unit you have learnt about the mechanisms of aerobic and anaerobic respiration occurring in plants. So let us sum it up.

1. Respiration is a cellular catabolic process where in glucose is oxidized to produce ATP, carbon dioxide and water.
2. It is of two types- aerobic and anaerobic.
3. Aerobic respiration is seen in higher plant and animal cells and it involves complete oxidation of glucose producing 38 ATP molecules.
4. Aerobic respiration takes place in three stages- Glycolysis, Krebs's cycle and Electron transport system (ETS).
5. Glycolysis is common to both aerobic and anaerobic respirations and it takes place in the cytoplasm of the cell. The other two steps are seen only in aerobic respiration and takes place in the mitochondrial matrix and the cristae present in the inner membrane of mitochondria.
6. Anaerobic respiration is also called fermentation. It is seen in microorganisms and incomplete oxidation of glucose yields only 2 ATP molecules.
7. Aerobic respiration is more efficient than anaerobic in terms of ATP production.
8. Value of Respiratory quotient (RQ) indicates the type of respiratory substrate used.

6.8 GLOSSARY

Acetyl Coenzyme A - A small molecule that carries acetyl functional groups in cells. Composed of an acetyl group attached to a coenzyme A molecule. The starting product of the citric acid cycle.

Adenosine Triphosphate (ATP) - The molecule from which cells derive energy. Comprised of an adenosine molecule bonded to three phosphates, each phosphate bond contains energy, especially the third bond. By breaking that one bond and reducing ATP to adenosine diphosphate (ADP), the cell can get the energy to carry out its various processes.

Aerobic Respiration - A metabolic process involving oxygen in the breakdown of glucose.

Anaerobic Respiration - A metabolic process that does not involve oxygen in the breakdown of glucose.

Carbohydrate - A molecular compound containing carbon, hydrogen, and oxygen. Subunits are sugars.

Citric acid cycle - Also known as the Krebs Cycle; a metabolic pathway found in aerobic organisms that oxidizes acetyl coA groups to carbon dioxide and water.

Coenzyme - A molecule that participates in an enzyme-catalyzed reaction and functions to transfer atoms or electrons between itself and various molecules.

Elimination reaction - A reaction that involves the ejection of a specific group from a molecule, often resulting in the formation of a carbon-carbon double bond.

Glycolysis - A metabolic pathway occurring in the cell cytosol that during a series of reactions converts glucose to pyruvate and synthesizes ATP.

Isomerization - A reaction that does not change the atomic make-up of a molecule, but rather changes its geometric conformation, yielding a slightly different molecule.

Metabolism - All the reactions occurring in an organism that participate in the acquisition or conversion of energy for use in the organism.

Nicotinamide Adenine Dinucleotide - A coenzyme that participates in oxidation and reduction reactions. An important electron carrier in oxidative phosphorylation.

Oxidation - A reaction that involves the overall loss of electrons from a specific molecule or atom. Can occur with the addition of an oxygen or by the removal of a hydrogen.

Oxidative Phosphorylation - A process occurring in the mitochondria that results in the formation of ATP from the flow of electrons to oxygen.

Photosynthesis - A process in which plants convert sunlight into energy sources that can be used inside the cell to sustain life.

Reduction - A reaction that results in the overall gain of electrons to a specific molecule or atom. Can occur with the addition of a hydrogen atom or by the removal of an oxygen atom.

Respiration - A process that occurs in cells in which cells breakdown food molecules to yield ATP. Can be either aerobic or anaerobic.

6.9 SELF ASSESSMENT QUESTIONS

6.9.1 Very short answer type:

1. Name the most common substrate used for respiration.
2. Name the two types of respirations.
3. What is the product of glycolysis?
4. How many ATP are formed for complete oxidation of one molecule of glucose.
5. Name the hydrogen acceptors in respiratory cycle.
6. Where does ETS operate?

6.9.2 Objective type questions:

1. The process of respiration in green plants occurs
 - (a) only when stomata are open
 - (b) only when photosynthesis ceases
 - (c) only when photosynthesis is in progress
 - (d) At all times

2. Respiratory enzymes are located in
(a) mitochondrial matrix (b) cristae
(c) perimitochondrial space (d) outer membrane
3. The net gain of ATP produced during the oxidation of one glucose molecule in a plant cell
(a) 38 ATP molecules (b) 30 ATP molecules
(c) 36 ATP molecules (d) 24 ATP molecules
4. The ultimate respiratory substrate, yielding maximum number of ATP molecules, is
(a) glycogen (b) glucose
(c) amylose (d) ketogenic amino acid
5. End product of fermentation are
(a) O_2 and C_2H_5OH (b) CO_2 and O_2
(c) CO_2 and C_2H_5OH (d) CO_2 and acetaldehyde
6. Out of 36 ATP molecules produced per glucose molecule during respiration
(a) 2 are produced outside glycolysis and 34 during respiratory chain
(b) 2 are produced outside mitochondria and 34 inside the mitochondria
(c) all the formed inside mitochondria
(d) 2 during glycolysis and 34 during Krebs cycle
7. Incomplete oxidation of glucose into pyruvic acid with several intermediate steps is known as:
(a) TCA pathway (b) Glycolysis
(c) HMS pathway (d) Glycolysis
8. When one glucose molecule is completely oxidised, it changes
(a) 36 ADP molecules into 36 ATP molecules
(b) 38 ADP molecules into 38 ATP molecules
(c) 30 ADP molecules into 30 ATP molecules
(d) 32 ADP molecules into 32 ATP molecules
9. Link between glycolysis, Krebs's cycle and β oxidation of fatty acid or carbohydrate and fat metabolism is
(a) citric acid (b) succinic acid
(c) acetyl CoA (d) Oxaloacetic acid

6.9.3 Fill in the blanks:

1. Conversion of pyruvic acid into ethyl alcohol is facilitated by enzymes.

2. End product of citric acid/ Kreb's cycle is
3. is the common immediate source of energy in cellular activity.
4. The first phase in the breakdown of glucose, in animal cell, is
5. Kreb's cycle take place in

6.9.4 True/ False

1. The site of glycolysis in a cell is cytoplasm.
2. Respiration is endothermic process
3. The final acceptor of electrons in the electron transport chain is oxygen
4. Photorespiration involves calvin cycle.
5. During respiration yeast converts glucose to ethanol and CO₂

6.9.1 Answer Keys:

1. Glucose.
2. Aerobic respiration and Anaerobic respiration
- 3 Two molecules of 3C pyruvic acid.
4. 38 ATP molecules.
5. NAD (Nicotinamide adenine dinucleotide) and FAD (Flavin adenine dinucleotide)
6. On the F₀- F₁ particles present on the cristae of mitochondria.

6.9.2 Answer Keys: 1. (d), 2. (b), 3. (c), 4. (b), 5. (c), 6. (b), 7. (b), 8. (b), 9. (c).

6.9.3 Answer keys:

- 1) carboxylase and dehydrogenase
- 2) Carbon Dioxide and water
- 3) ATP
- 4) Glycolysis
- 5) Mitochondrial matrix

6.9.4 Answer keys: 1. True, 2. False, 3. True, 4. False, 5. True

6.10 REFERENCES

- Advances in photosynthesis and respiration: Focus of plant respiration. Photosynthesis research (2005) 85:255-259
- Plant physiology and Biochemistry. Dr.M.S Rawat.
- Cellular Respiration. Dr.Howaida Nounou.
- Plant Respiration. Roges Gifford.CRS for Greenhouse Accounting CSIRO. Plant industry Nee Workshop Proceedings. 18-20. April 2001.
- Cannell MGR and Thornley JHM (2000) Modelling the components of plant respiration: Some guiding principles. Annals of Botany 85: 45-54.
- Rich, P. R. (2003). "The molecular machinery of Keilin's respiratory chain". Biochemical

Society Transactions 31 (Pt 6): 1095–1105.

- Ryan MG (1991) Effects of climate change on plant respiration. *Ecological Applications* 1:157–167.
- Beevers H (1970) Respiration in plants and its regulation. pp209-214 in *Prediction and measurement of photosynthetic productivity : proceedings of the IBP/PP. Technical Meeting, Trebon, 14-21 September 1969.*

6.11 SUGGESTED READINGS

- A textbook of botany. V.Singh, P.C Pande and D.K Jain. (2008)
- Fundamentals of Plant Physiology. V.K Jain
- Plant Physiology. S.N Pandey.
- Textbook of Plant Physiology and Biochemistry. Mohit Verma and Pooja Gupta.
- Botant for Degree Students. Dr. B.P Pandey.
- A textbook of Plant Physiology, Biochemistry and Biotechnology. Dr. S.K Verma and Mohit Verma.

6.12 TERMINAL QUESTIONS

6.12.1 Short Answer Type Questions:

1. Define respiration.
2. Write the equation for aerobic and anaerobic respiration.
3. List the three steps of aerobic respiration.
4. What is glycolysis? Where does it take place in the cell?
5. What is oxidative phosphorylation?
6. What is fermentation? Name the different types.
7. Why is anaerobic respiration less efficient than aerobic respiration?
8. Write a note on the application of fermentation process.
9. Define RQ.
10. Why is respiration called an amphibolic pathway?

6.12.2 Long answer type Questions:

1. Oxygen is critical for aerobic respiration. Explain its role with respect to electron transport system.
2. Give an account on glycolysis. Where does it occur? What are the end products? Trace the fate of these production both aerobic and anaerobic respiration.
3. What is the significance of pentose phosphate pathway? Write the mechanism of respiration.
4. Differentiate between aerobic and anaerobic respiration.
5. How are glycolysis, Krebs TCA cycle and electron transport chain linked.

6.12.1 Answer Key:

1. The process of harvesting chemical energy for metabolic activities in the form of ATP by oxidising the food molecules is called 'respiration
2. Aerobic respiration-
 $\text{Glucose (C}_6\text{H}_{12}\text{O}_6) + 6\text{O}_2 \longrightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} + 38\text{ATP}$
Anaerobic respiration-
 $\text{Glucose (C}_6\text{H}_{12}\text{O}_6) \longrightarrow 2\text{C}_2\text{H}_5\text{OH} + 2\text{ATP} + 2\text{CO}_2$ (alcohol fermentation)
OR
 $\text{Glucose (C}_6\text{H}_{12}\text{O}_6) \longrightarrow 2\text{CH}_3\text{CHOHCOOH} + 2\text{ATP}$ (Lactic acid fermentation)
3. The three steps of aerobic respiration are glycolysis, Krebs' cycle and Electron transport System.
4. Glycolysis is the first step in aerobic respiration in which, glucose (6C) is broken in a stepwise manner into two molecules of 3C Pyruvic acid, without utilizing oxygen. It takes place in the cytoplasm of the cell.
5. Synthesis of ATP during the oxidation of NADH and FADH₂ in electron transport system is called oxidative phosphorylation.
6. The anaerobic respiration that takes place in microorganisms, where glucose is partially oxidized to form ethyl alcohol or lactic acid and 2 ATP molecules is called fermentation. The two types of fermentation are ethyl alcohol fermentation and lactic acid fermentation.
7. Because in anaerobic respiration only 2 ATP are produced per glucose molecule as against 38 in aerobic respiration.
8. Fermentation process has wide application in food, pharmaceutical and chemical industries. The process is used in the production of alcohol and in bakeries.
9. Respiratory quotient is defined as the ratio of volume of CO₂ evolved to the volume of Oxygen consumed during respiration
10. During aerobic respiration the organic substrates are broken down into simple substances such as carbon dioxide and water (Catabolism). However it also produces many intermediate organic compounds/acids during Krebs' cycle which form the starting compound for the synthesis of other complex substances, needed for the cell. Hence respiration is said to be both catabolic and anabolic and is referred to as '**amphibolic pathway**'.

UNIT-7 NITROGEN METABOLISM

Content

- 7.1 Objectives
- 7.2 Introduction
- 7.3 Sources of Nitrogen
- 7.4 Nitrogen Fixation
- 7.5 Nitrogen Cycle
- 7.6 Nitrogen Assimilation
- 7.7 Summary
- 7.8 Glossary
- 7.9 Self Assessment Question
- 7.10 References
- 7.11 Suggested Readings
- 7.12 Terminal Questions

7.1 OBJECTIVES

After reading this unit students will be able to-

- Explain and define the nitrogen metabolism.
- Describe various steps of atmospheric nitrogen fixation.
- Write an account of nitrogen cycle
- Describe the process of Nitrogen assimilation

7.2 INTRODUCTION

In this section first we will try to know about the existence of nitrogen in atmosphere. We all know that, Nitrogen in its elemental form is considered to be as the major component of the air constituting about 78% of the gases in the earth atmosphere. In the **atmosphere**, there are also different nitrogen gaseous compounds that exist in form of NH_3 , NO and N_2O . The importance of nitrogen to life is that it constitutes (with carbon, hydrogen and oxygen) the major part of proteins of all living materials. Except few microorganisms (nitrogen fixing bacteria, algae and fungi), living organisms are not able to use N_2 directly as a source of nitrogen and need different forms of fixed nitrogen (NH_4^+ and NO_3^- for plants and organic nitrogen for animals and humans) as a supply for their requirement of **protein synthesis**. Nitrogen is also considered to be an important part of many cells and several processes such as synthesis of amino acids, proteins and even DNA. It is also needed to make chlorophyll in plants, and chlorophyll is used in photosynthesis to make their food.

The continuous process by which nitrogen is exchanged between organisms and the environment known as **nitrogen metabolism**. Few of the atmosphere's free nitrogen combine with other elements to form compounds are deposited into the soil. By means of nitrification these nitrogenous compound are then converted by prokaryotic microorganisms i.e. bacteria, into the nutrients that are absorbed by the roots of green plants. Thereafter, nitrogen is passed into the food chain and returned back to the soil as result of the metabolism and decaying of plants and animals.

On this basis we can say that **Nitrogen metabolism**, an important biochemical process which can also be described as the important part of plant metabolism. Nitrogen is a very important constituent of cellular components. Alkaloids, amides, amino acids, proteins, DNA, RNA, enzymes, vitamins, hormones and many other cellular components contain nitrogen as one of the elements. It is not overstating to say that Nitrogen is the key element for it is the most important constituent of proteins and nucleic acids. Thus N_2 plays a significant role in the formation of proteins and nucleic acids which in turn control cellular activities. Without nitrogen, no living organism can survive. In reality, all the living organisms are virtually submerged in a sea of atmospheric nitrogen (i.e. 78%), but unfortunately not all organisms are capable with the potentiality to utilize this abundantly available molecular N_2 . Only some

microorganisms like certain bacteria, blue green algae and few fungi, have the potentiality to utilize molecular N_2 directly.

Here in the figure given below, we will see that Nitrogen enters roots in form of NO_3^- or NH_4^+ . We can see in figure 1, How NH_4^+ is formed as a result of nitrate reduction or N_2 fixation and NH_4^+ is incorporated into amino acids by the glutamine synthetase (GS) reaction. The NH_4^+ is incorporated into amino acids in roots as well as in leaves and the accumulation of amino acids into proteins.

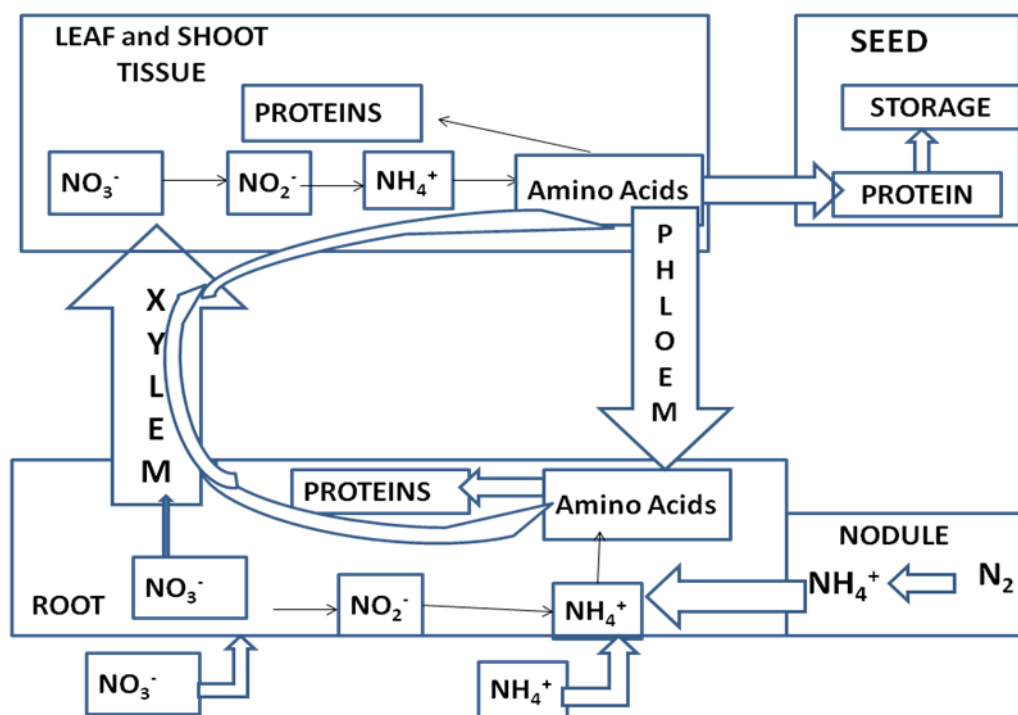


Fig.7.1- Nitrogen Metabolism

7.3 SOURCES OF NITROGEN

Usually the plants get nitrogen in a fixed form from the soil except those plants having the ability to fix the nitrogen. Here we will discuss first various forms of nitrogen available to plants.

7.3.1 Ammonical form of nitrogen: Ammonical form of N_2 in soil in the form of urea or NH_4 in free state is available. Urea is often supplied to the plants as nitrogenous fertilizer either by means of soil or foliar application. Urea, if present in the soil is first split into NH_3 and CO_2 . In many plants foliar application of urea is to be considered as a very effective method of relieving of nitrogen storage. During urea assimilation urea is hydrolysed by the enzyme urease to form ammonia and CO_2 .



Thus the ammonia is absorbed by water to form ammonium ion, which is assimilated later by the process ammonium assimilation.



It is also necessary to know, that free ammonium is the only utilizable form of N_2 that can be directly incorporated into amino acids.

7.3.2 Organic Form of Nitrogen: Organic forms are a very diverse group of nitrogen-containing organic molecules including simple amino acids through to large complex proteins and nucleic acids in living organisms and humic substances in soil and water. As result of decaying of dead plants and animals, releasing of different nitrogenous compounds of which amino acids, amides and other such Nitrate compounds constitute organic form of N_2 . These are absorbed by the root system and utilized directly. Thus the decaying organic matter acts as the rich source of organic nitrogen that can be utilized by not only higher plants but also by micro-organisms.

7.3.2.1. Nitrate / Nitrite Form: N_2 available in the soil is in the form of nitrates and nitrites are also found but in small quantities. These are available ionic forms and easily absorbed by the roots or cellular surfaces from its surrounding soil solution. The physiological mechanism i.e. diffusion process involves in the absorption of NO_3^- or NO_2^- ions and this absorption process is also facilitated by specific carriers. Once the nitrate or nitrite ions enter into cellular environment they have to be converted to NH_4^+ , before the same can be incorporated into cellular components.

The Mechanism of Conversion of NO_3^- and NO_2^- to NH_4^+

Plant roots as well as leaves can utilize nitrates and can be converted to NH_4^+ . But more of nitrate reductive activity is found in leaves than in roots. However, the mechanism of nitrate and nitrite reduction is performed by different enzymes as NO_3^- is reduced by nitrate reductase enzymes and the NO_2^- is reduced by nitrite reductases.

Nitrate Reductase is an Inducible Enzyme

The presence of Nitrate reductase enzyme is very low in the tissues in the absence of NO_3^- . With the addition of NO_3^- as the substrate, the amount of nitrate reductase enzyme increases. Light must be needed for the enzyme induction to the fullest extent. Moreover, phytohormones, particularly cytokinin also induces nitrate reductase synthesis initially even in the absence of light and NO_3^- . Thus we can say that the nitrate reductase is an inducible enzyme.

Nitrate Reduction

Nitrate reduction to NH_4 is not a single step process, but it involves a series of reactions in which the first step is performed by nitrate reductase. The occurrence of this enzyme is well known in various organisms like bacteria, *Chlorella*, blue green algae, alfa alfa, ectomycorrhizal fungi and other higher plants. Nitrate reductase enzyme is associated with 2 cofactors i.e. FAD and two molybdenum ions. Nitrate reductase enzyme also requires reducing power supplied by $\text{NADH}+\text{H}$ or $\text{NADPH}+\text{H}$. The former is available in non chlorophyllous tissues and the latter is found in chloroplast containing leaves.

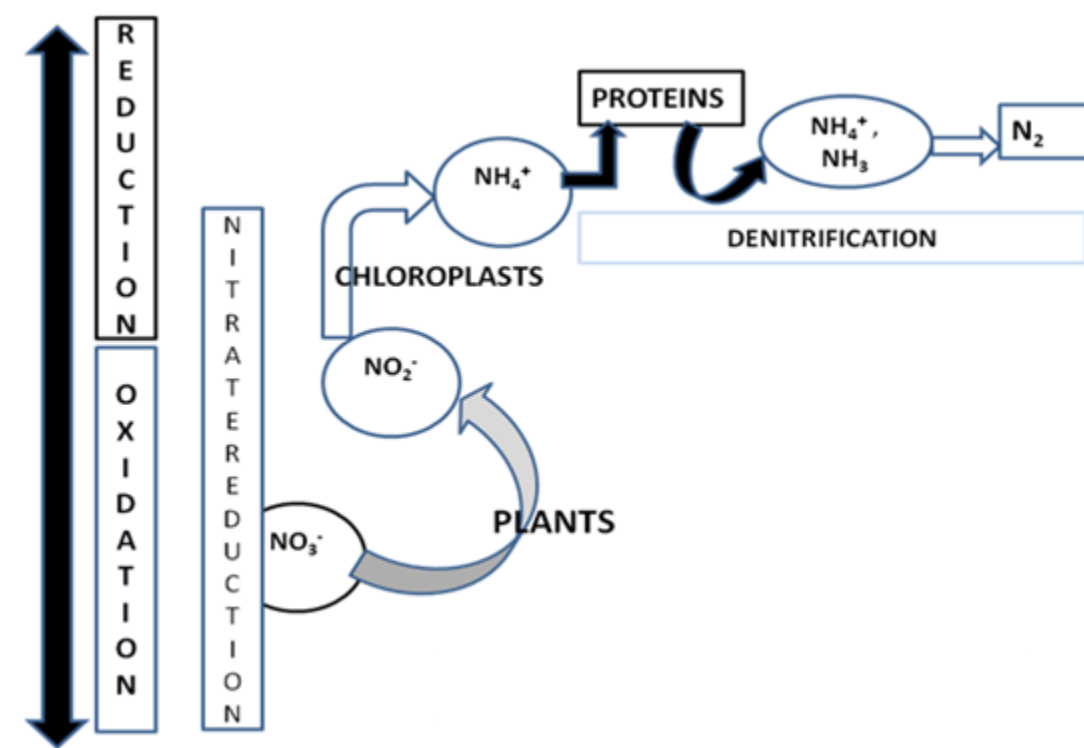
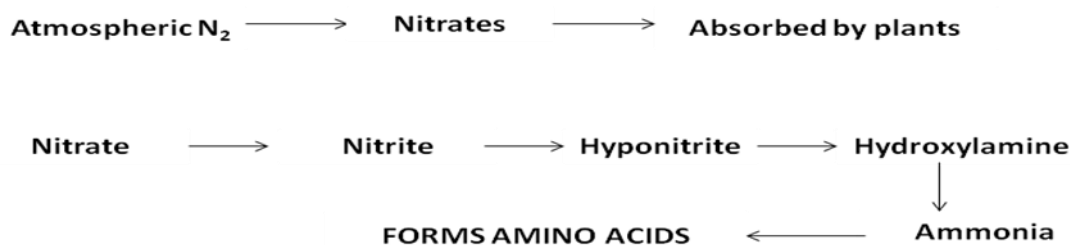


Fig.7.2 Nitrate Reduction

The reduction of nitrate to ammonia is a multistep reaction in which nitrates are reduced to nitrites, which are then converted to hyponitrites then to hydroxylamines and finally to ammonia.

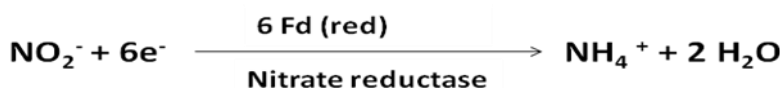


Depending upon the tissues involved nitrate reductase accepts NADH_2 (roots) or $\text{NADPH}+\text{H}$ (leaves), where hydrogen is transferred to the coenzyme FAD to form FADH_2 . In the next step, protons (H^+) and electrons are transferred to NO_3 simultaneously. However, electrons are transferred to NO_3 through molybdenum ions.

For the maximal activity of nitrate reductase, it requires an optimal concentration of MO_2^+ , Fe_3^+ and Ca_2^+ ions. Though calcium has no catalytic activity in this enzymatic reaction, unlike iron and molybdenum which are involved in electron transport and it facilitates the transport of nitrite across the chloroplast membranes. Thus the nitrite synthesized in this reductive step in the cytoplasm is transported into chloroplasts. But in roots, lower fungi and bacteria, the entire process takes place in the cytoplasm.

Nitrite Reduction

The production of nitrite is carried out by the activity of nitrate reductase to be converted to ammonia by the presence of nitrite reductase enzyme in the plant tissues in maximum quantity as compared to nitrate reductase.



In most of the higher plants so far studied, the nitrites synthesized in cytoplasm or transported into plastids, where the nitrites are reduced to hyponitrite by an enzyme called nitrite reductase. The enzyme has a mol. Wt. of 60-70KD and it has a special heme component called siroheme.

Actually there are two forms of nitrite reductases, of which one form uses $\text{NADPH}+\text{H}$ as the proton/electron donor in photosynthetic tissues, but root tissues and others including bacteria and fungi use $\text{NADH}+\text{H}$ as the hydrogen donors. The enzyme nitrite reductase possesses flavin and iron groups. Surprisingly, these enzymes are induced by nitrates than nitrites. However, nitrite reductase leads to the reduction of nitrite to NH_4 in a multistep reaction, where the intermediary products remain attached to the surface of enzyme and only the final product is release from the surface. In this process, we will see that, a total of six electrons and six protons are transferred to nitrite to produce ammonia.

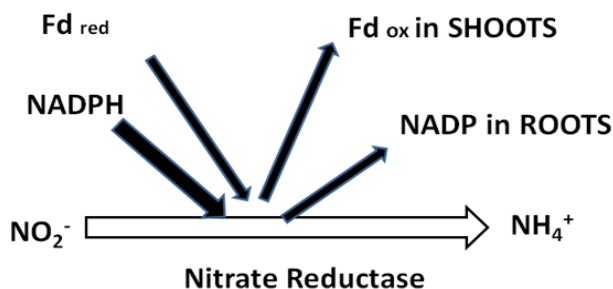
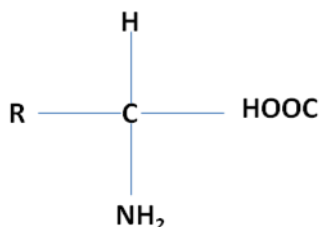


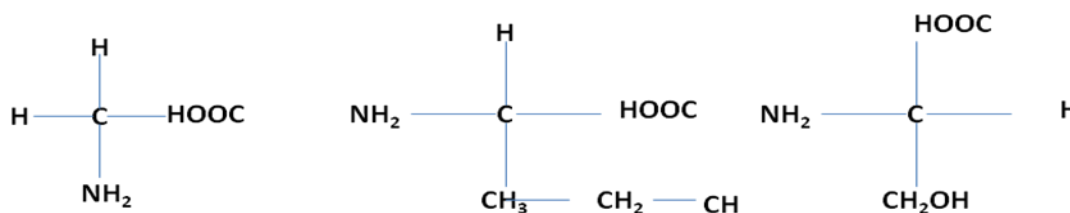
Fig. 7.3 Nitrite Reduction

Nonetheless in some cases one of the intermediate products like hydroxylamine has been found to be converted to NH_4 by the activity of hydroxylamine reductase. Such reactions have been observed in mesophyll tissues of higher plants, *Neurospora*, *Aspergillus* and some bacteria. The overall pathway from NO_3 or NO_2 to NH_4 is catalyzed by a group of enzymes or multi enzyme complexes, but the synthesis of NH_4 is very essential for amino acid synthesis.

Here we will discuss little bit about the Amino acid synthesis. Basically amino acid synthesis showing the conversion of ammonia nitrogen to amine nitrogen. Amino acids are the organic compounds having carboxyl group (COOH) and amino groups (NH_2). The general formula of amino acid can be written as:



R is a variable grouping of atoms, fundamentally a carbon chain or ring. As we can say that in case of glycine R is H, in Valanine R is expressed by $\text{CH}_3\text{-CH}_2\text{-CH}$ etc.



On this basis we can say that amino group is usually incorporated into amino acid. For the biosynthesis of amino acids point of view we will discuss two possible ways-

Reductive amination: During reductive amination the ammonia formed by the reduction of nitrate combines with a ketoglutaric acid of Kreb's cycle to form glutamic acid. This is a reversible reaction. During the synthesis of glutamic acid, Ammonia is first incorporated into a ketoglutaric acid to produce the aminoglutaric acid. As result of this aminoglutaric acid is converted to glutamic acid with the help of enzyme glutamate dehydrogenase and coenzyme $\text{NADH} + \text{H}^+$. Glutamate dehydrogenase (GLDH) is an enzyme present in most microbes and the mitochondria of eukaryotes, as are some of the other enzymes required for urea synthesis, that converts glutamate to α - ketoglutarate, and vice versa.



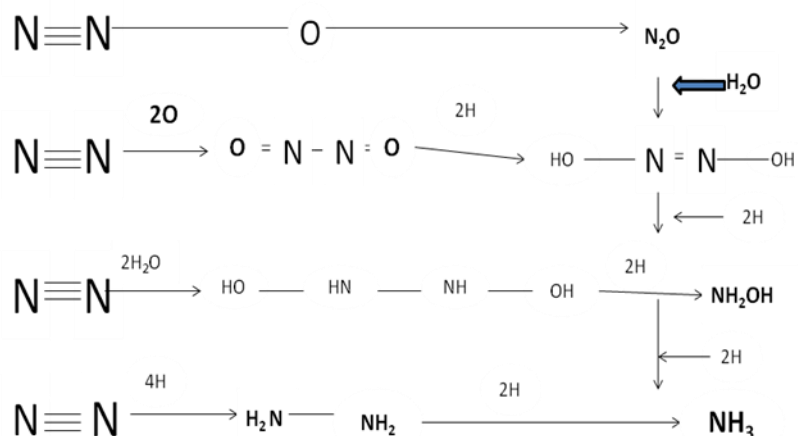
Transamination: Transamination is a chemical reaction that transfers an amino group to a keto acid to form new amino acids. This is one of the major degradation pathways which convert essential amino acids to nonessential amino acids. The most usual and major keto acid involved with transamination reactions is alpha-ketoglutaric acid, an intermediate in the citric acid cycle. A specific example is the transamination of alanine to make pyruvic acid and glutamic acid. Here it involves the transfer of an amino acid to carboxy keto group as keto acid. These reactions catalyzed by the enzyme transaminases and coenzyme pyridoxal phosphate.



In these **transamination** reactions, the α -amino group is transferred to the α -carbon atom of α -ketoglutarate, leaving behind the corresponding α -keto acid analog of the amino acid. The effect of transamination reactions is to collect the amino groups from many different amino acids in the form of only one, namely, L-glutamate. The glutamate channels amino groups either into biosynthetic pathways or into a final sequence of reactions by which nitrogenous waste products are formed and then excreted.

7.3.2.2. Molecular Nitrogen

Abundantly available molecular N_2 is more or less inert. Molecular Nitrogen or diatomic nitrogen (N_2) is better known as highly stable because of its triple bonded ($\text{N}\equiv\text{N}$) nature. Because of this, molecular nitrogen as such is not very reactive in the atmosphere under normal conditions. In the atmosphere, molecular nitrogen is about 78.03% by volume and it has a very low boiling point (-195.8°C) which is even lower than oxygen. Proteins present in living organisms contain about 16% nitrogen.



With the exception of some bacteria, fungi and blue green algae none of the higher plants are capable of utilizing molecular N_2 directly. However, nature has devised mechanisms to fix this type of N_2 into utilizable form of N_2 i.e. NH_4 by non biological and biological methods.

After getting the knowledge about the existence of nitrogen in the atmosphere and various sources of nitrogen which becomes available to the plant, now we will discuss the process of nitrogen fixation.

7.4 NITROGEN FIXATION

Nitrogen fixation is a process in which Nitrogen in the atmosphere is converted into ammonia NH_3 . Nitrogen fixation, whether natural or synthetic, is essential for all forms of life because nitrogen is required to biosynthesize the basic building blocks of plants, animals and other life forms. For example, nucleotides are required for the synthesis of DNA and RNA and we should be very clear that the coenzyme NAD (Nicotinamide Adenine Dinucleotide) acts its role in metabolism (transferring electrons between molecules) as well as the synthesis of amino acids for proteins. Therefore, as part of the nitrogen cycle we can say that it is essential for agriculture and the manufacture of fertilizer.

7.4.1 Atmospheric Nitrogen Fixation

Nitrogen in its gaseous form (N_2) cannot be used by most of the living organisms. It has to be converted or 'fixed' to a more usable form through a process called fixation. There are two ways by which nitrogen can be fixed to be useful for living things:

Biological Method of Nitrogen Fixation: Nitrogen gas (N_2) diffuses into the soil from the atmosphere, and species of bacteria convert this nitrogen to ammonium ions (NH_4^+), which can be used by plants. Legumes (such as clover and lupins) are often grown by farmers because they have nodules on their roots that contain nitrogen-fixing bacteria. The direct use of molecular nitrogen is known as asymbiotic nitrogen fixation and the indirect use of molecular nitrogen is known as symbiotic nitrogen fixation.

Through lightening: Lightning converts atmospheric nitrogen into ammonia and nitrate (NO_3^-) that enter soil with rainfall.

Industrially: People have learned how to convert nitrogen gas to ammonia (NH_3) and nitrogen-rich fertilizers to supplement the amount of nitrogen fixed naturally.

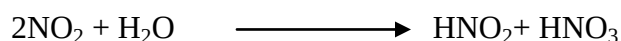
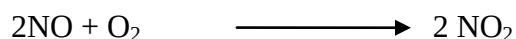
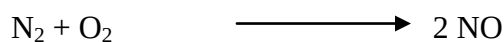
By means of two methods of atmospheric nitrogen fixation is carried out:-

(A) Non Biological or Physico-chemical Fixation

(B) Biological Nitrogen fixation

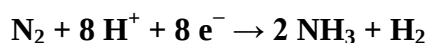
(A) Non Biological or Physico-chemical Fixation (By Electric Discharge and Rainfall)

Air, in which 79% nitrogen gas (N₂), is the major reservoir of nitrogen. The enormous energy of lightning breaks nitrogen molecules and enables their atoms to combine with oxygen in the air forming nitrogen oxides. These dissolve in rain, forming nitrates that are carried to the earth.



These nitrite and nitrates react with many saline substances in soil and forming salts. These salts especially in form of calcium nitrite, calcium nitrate, potassium nitrite and potassium nitrate are ionized in the presence of water. These ionized salts are absorbed by the plant roots. It should be importantly noticed by us that generally main absorbed ionized nitrogen radicals are nitrates (NO₃⁻) because nitrites are to be converted into nitrates by many processes. It is to be understood that atmospheric nitrogen fixation probably contributes some 5– 8% of the total nitrogen fixed with this process.

(B) Biological Nitrogen Fixation: Biological nitrogen fixation was discovered by the German agronomist Hermann Hellriegel and Dutch microbiologist Martinus Beijerinck. Biological nitrogen fixation (**BNF**) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called a nitrogenase. The overall reaction for BNF can be given as:



The process is coupled to the hydrolysis of 16 equivalents of ATP and is accompanied by the co-formation of one molecule of H₂. The conversion of N₂ into ammonia occurs at a cluster called FeMoco, an abbreviation for the iron-molybdenum cofactor. The mechanism proceeds via a series of protonation and reduction steps wherein the FeMoco active site hydrogenates the N₂ substrate.

Biological nitrogen fixation is the process whereby atmospheric nitrogen is reduced to ammonia in the presence of nitrogenase. Nitrogenase is a biological catalyst found naturally only in certain microorganisms such as the symbiotic *Rhizobium* and *Frankia* or the free-living *Azospirillum* and *Azotobacter* and Cyanobacteria (BGA). Biological nitrogen fixation can be categorized into two types as Asymbiotic and Symbiotic.

7.4.2 Asymbiotic Nitrogen Fixation

Asymbiotic nitrogen fixation is carried out by means of free-living microbes (non-symbiotic microbes) including the cyanobacteria or blue-green algae (*Anabaena* and *Nostoc*) and genera such as *Azotobacter*, *Beijerinckia*, and *Clostridium*. In free-living microbes the nitrogenase-generated ammonium is assimilated into glutamate through the glutamine synthetase / glutamate synthase pathway. The microbial genes required for nitrogen fixation are widely

distributed in diverse environments. Enzymes responsible for nitrogenase action are very susceptible to destruction by oxygen. For this reason, many bacteria cease production of the enzyme in the presence of oxygen. Many nitrogen-fixing microorganisms exist only in anaerobic conditions, respiring to draw down oxygen levels, or binding the oxygen with a protein such as leghemoglobin.

Among the living plant world, some free living bacteria, fungi and blue green algae (*Cyanobacteria*) are capable of fixing molecular nitrogen into utilizable form of N_2 i.e. NH_4 . *Azotobacter veinlandi*, *Clostridium pasteurianum*, *Rhodospirillum rubrum*, *Chromatium*, *Nostoc*, *Anabaena*, *Rivularia* etc are the microbes having the ability to fix the molecular nitrogen. In recent years, the above said organisms are made available to farmers as bio-fertilizers. When the cultures of them are spread in the fields and allowed to grow, they enrich the soil with a lot of nitrogen as a natural fertilizer. One important aspect of it is to maintain moisture in the soil. Such living fertilizer renewable and enriches the soil all the time.

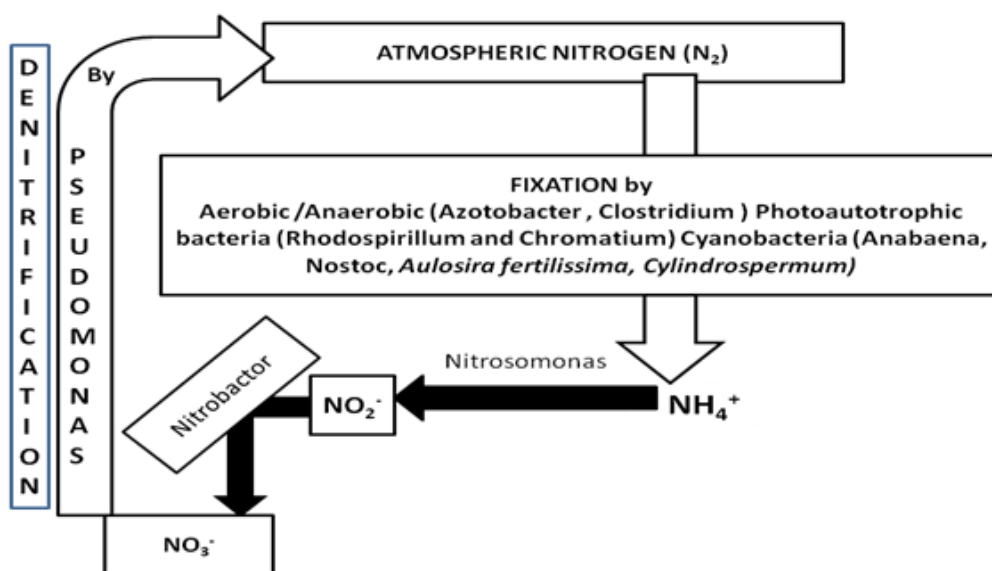


Fig.7.4 Asymbiotic Nitrogen fixation

7.4.3. Symbiotic Nitrogen Fixation

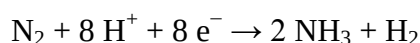
The process which is performed by bacteria found in the nodules of leguminous plants and thereafter forming nitrogenous compounds available to the host plants known as symbiotic nitrogen fixation. Symbiotic nitrogen fixation occurs in plants that harbor nitrogen-fixing bacteria within their tissues. The best-studied example is the association between **legumes** and **bacteria** in the genus *Rhizobium*. On the other hand some other forms of bacteria are also being reported as symbiont in root nodules of many non-leguminous plants (*Frankia* as symbiont in the roots of *Casuarina* and *Alnus*). Each of these is able to survive independently (soil nitrates must then be available to the legume), but life together is clearly beneficial to both. Only together can nitrogen fixation take place. A symbiotic relationship in which both partners get benefits is called mutualism.

Symbiotic nitrogen fixation takes place by the activities of *Rhizobium*, which is pleomorphic bacteria, that enters the root of leguminous plant through the root hair and causes the nodule formation on the roots. There is a symbiotic relationship between *Rhizobium* and legumes.

Before the entrance of *Rhizobium* into the roots of leguminous plants first they sense flavanoids secreted by the root hairs of leguminous plants. These flavanoids activates the secretion of nod factors which are in turn recognized by the host plant and then lead to root hair deformation. Then these bacteria modify themselves in form of infection threads and enter the roots through deformed root hairs.

The *Rhizobium* after entering intracellularly reached the parenchymatous cells of cortex. The infection triggers cell division in the cortex of the root where a new organ, the nodule, appears as a result of successive processes (Fig.7.5).

Infection threads grow to the nodule, infect its central tissue and release the *Rhizobia* in these cells, where they differentiate morphologically into bacteroids and fix nitrogen from the atmosphere into a plant-usable form, ammonium ($\text{NH}_3 + \text{H}^+ \rightarrow \text{NH}_4^+$), using the enzyme nitrogenase. The reaction for all nitrogen-fixing bacteria can be given as:



For the continuation of symbiotic nitrogen fixation, bacterial requirements are fulfilled by means of supplying the carbohydrates, proteins and sufficient oxygen from the plant. Leghaemoglobin, a plant protein helps to provide oxygen for maintaining the free oxygen concentration just to continue the nitrogenase activity.

It is necessary for us to know about **Leghaemoglobin** (also termed as **leghaemoglobin** or **legoglobin**), which is a nitrogen or oxygen carrier and hemoprotein found in the nitrogen fixing root nodules of leguminous plants. It is produced by legumes in response to the roots, which colonized by nitrogen-fixing bacteria, known as *Rhizobia*, a key player of the symbiotic interaction between plant and bacterium.

Thus we can say that those roots, which are not colonized by *Rhizobium* not able to synthesize leghaemoglobin. Any how, the final compounds formed by bacteria during the symbiotic nitrogen fixation are either Ammonia or Hydroxylamine (NH_3 or NH_2OH). Thus leguminous plants or any plants where such kind of association is found with soil increase the fertility of soil.

As the nod genes are activated and nodule formation is continued, on the contrary aspect host cellular factors in turn activate the expression of nitrogen fixing genes found in rhizobial cells. The nif genes remain unexpressed whenever the rhizobial cells are free from host cells. As the bacterial cells are associated with the host cells, then the genes remain unexpressed if the nitrogen sources like nitrate and ammonia are present in the medium.

As in this section we have studied that the nitrogenase and other related enzymes are considered as inducible enzymes. So it is very important for us to know that, the interaction

between the host cellular components and bacterial cellular components is very important in the expression of each other's genomes for N_2 fixation.

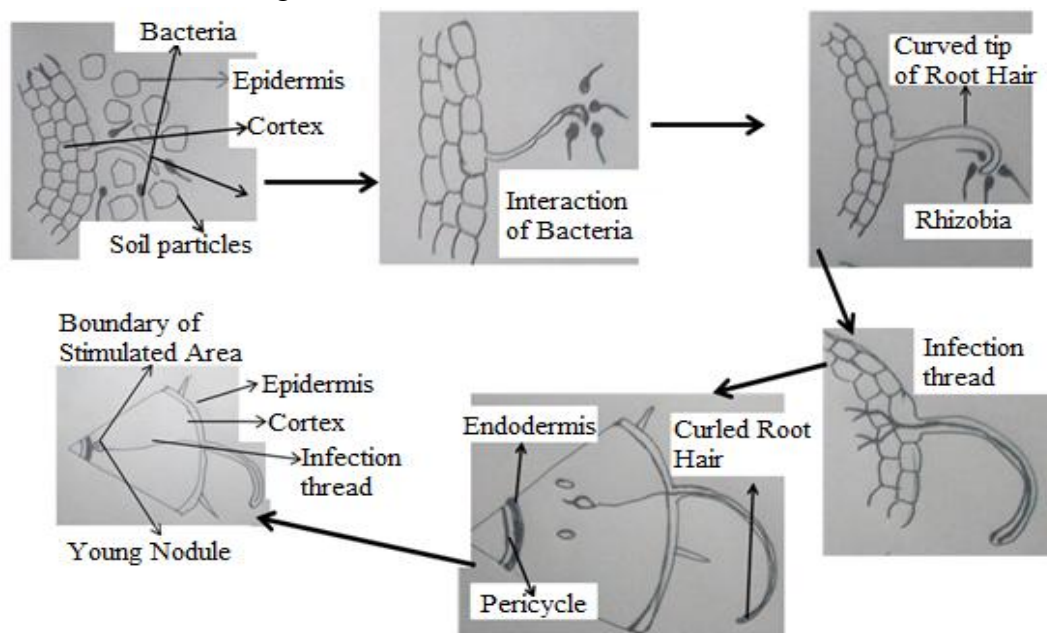


Fig.7.5 Entrance of Rhizobium into the roots of Leguminous plant and as result of infection formation of nodule

The primary enzyme encoded by the *nif* genes is the nitrogenase complex which is involved to convert atmospheric nitrogen (N_2) to other nitrogen forms such as ammonia which the organism can use for various purposes. The *nif* genes also encode a number of regulatory proteins involved in nitrogen fixation. The *nif* genes are also found in both free living nitrogen fixing bacteria and in symbiotic bacteria associated with various plants.

7.5 NITROGEN CYCLE

The significance of nitrogen can be explained in such a way that it is the major builder of proteins and nucleic acids. These proteins and nucleic acids are well known as the essential constituents of living organisms and regulators of biological functions. The nitrogen cycle is the biogeochemical cycle by which nitrogen is converted into various chemical forms as it circulates among the atmosphere, terrestrial and marine ecosystems. The conversion of nitrogen can be carried out through both biological and physical processes.

Important processes in the nitrogen cycle include fixation, ammonification, nitrification and denitrification. Nevertheless plants obtain most of their nitrogen from soil as nitrate or ammonium ions. Globally, atmospheric fixation accounts for around 10 kg per hectare/ year of the biospheric nitrogen flow. Overall, atmospheric fixation represent 2-3% of global nitrogen assimilation and the rest being cycled in nongaseous forms. However, atmospheric nitrogen has limited availability for biological use, leading to a scarcity of usable nitrogen in many types of

ecosystems. Much of the terrestrial fixation is carried out by symbiotic bacteria in plant roots and to a lesser amount by free living soil bacteria. Cyanobacteria (blue green algae) also fix nitrogen and are of much significance in aquatic ecosystems.

During the process of nitrification (oxidation of the ammonium compounds to nitrates) some specialized soil bacteria play their vital role. Some bacteria oxidize ammonium ions to nitrite and others convert nitrite to nitrate. We should also know that during this process energy is also obtained by the chemoautotrophs as result of such transformations.

Table 1: Important organisms of Nitrogen Cycle

S. No	Mechanisms	Microbial Type	Name of Microorganism
1.	Symbiotic Nitrogen Fixation	Bacteria	<i>Rhizobium leuminosarum</i> , <i>Rhizobium phaseolin</i> , <i>Rhizobium trifoli</i>
2.	Asymbiotic Nitrogen fixation	Free living or Saprophytic Bacteria	Chromatium, Chlorobium, Clostridium pasteruianum, Azatobactor, Pseudomonas
		Blue green Algae	Anabaena, Phormidium, Nostoc
3.	Ammonification	Bacteria	Bacillus mycoides, Bacillus ramosus, Bacillus vulgaris, Clostridium
4.	Nitrification	Bacteria	Nitrosomonas, Nitrosococcus, Nitrobacter
5.	Denitrification	Bacteria	Thiobacillus denitrificans, Micrococcus denitrificans

Nitrogen is present in the environment in a wide variety of chemical forms including organic nitrogen, ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O), nitric oxide (NO) or inorganic nitrogen gas (N_2).

The processes of the nitrogen cycle transform nitrogen from one form to another. Many of those processes are carried out by microbes, either in their effort to harvest energy or to accumulate nitrogen in a form needed for their growth. The following diagram below shows how these processes fit together to form the nitrogen cycle.

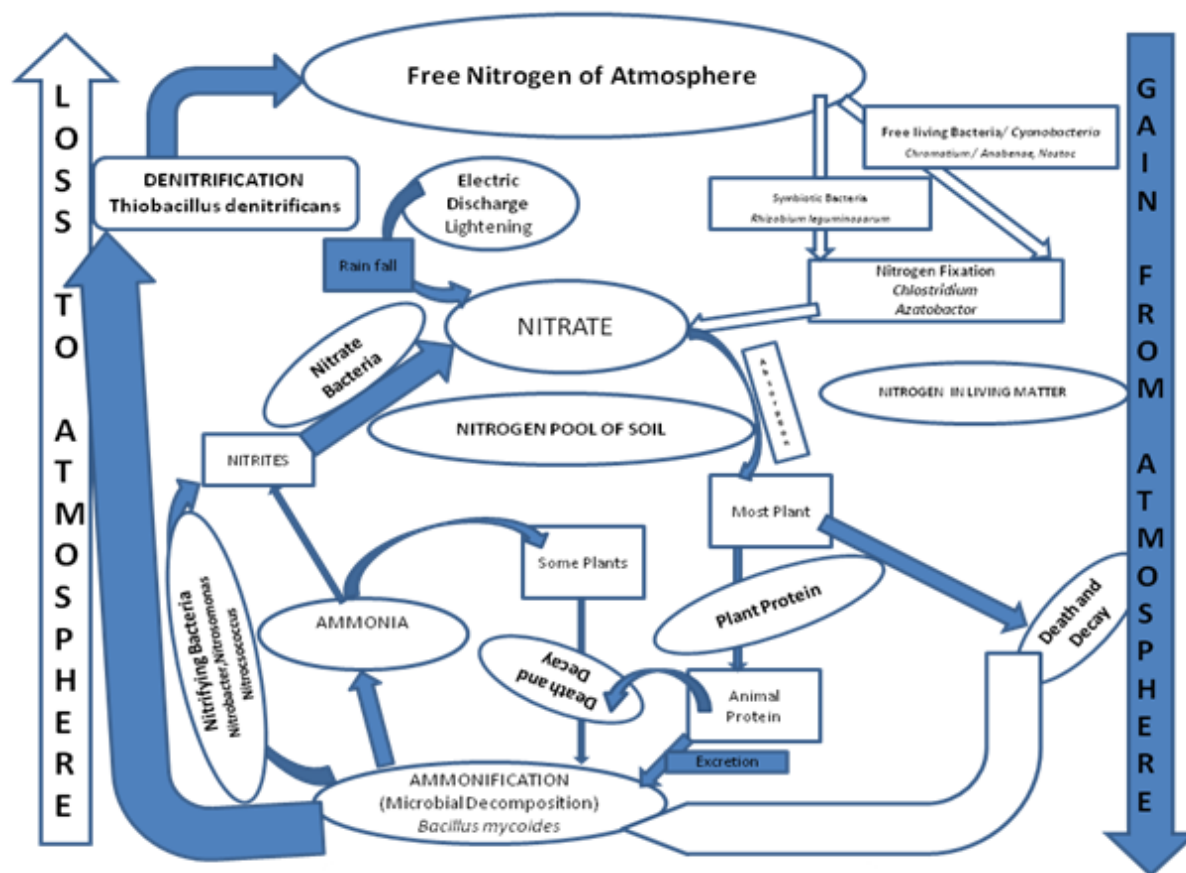


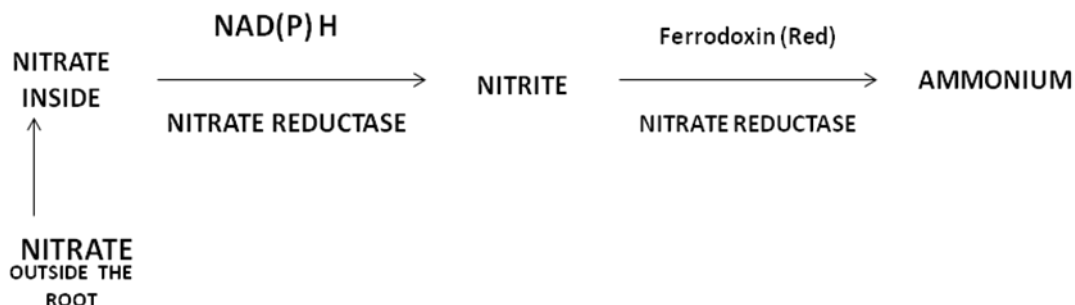
Fig.7.6 Nitrogen cycle in nature

7.6 NITROGEN ASSIMILATION

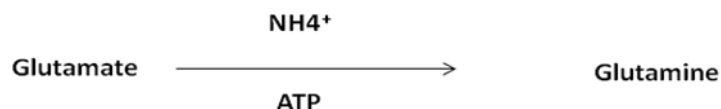
Nitrogen assimilation is the formation of organic nitrogen compounds like amino acids from inorganic nitrogen compounds present in the environment. Organisms like those plants, fungi and certain bacteria which cannot fix nitrogen gas (N_2) but able to assimilate nitrate or ammonia for their needs. Other organisms, like animals, depend entirely on organic nitrogen from their food.

During the growth and development of plants, Nitrogen is moved into and out of proteins in different organs and transported between organs in a limited number of transport compounds. Plants obtain the nitrogen from soil in form of nitrite or ammonium ions. Active processes are mediated for the transportation of nitrate into the root cells. A proton gradient is generated by a H^+ -ATPase across the plasma membrane is to be responsible for uptake of nitrate against the concentration gradient. Assimilation of mineral nitrogen into organic molecules is to be understood as a complex process. Nitrate is first reduced to ammonium ion before it can be assimilated by plants. The reduction mechanism of nitrate to ammonia by higher plants is completed in two steps:

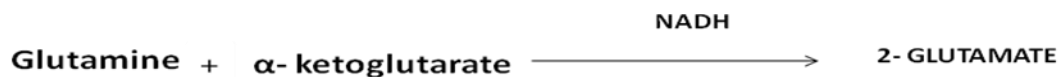
- In first step, nitrate is reduced to nitrite by enzyme nitrate reductase. This reaction takes place in the cytosol. Nitrate reductases are composed of two identical subunits and each containing three prosthetic groups which are FAD, heme (cyt-b₅₅₇) and a Mo containing organic molecule termed as pterin. It requires reducing agent NAD(P)H
- Nitrite is reduced to ammonia by nitrite reductase. Nitrite reductase constituting Fe₄S₄ and siroheme. This reaction is carried out in root's protoplasts and shoot's chloroplasts.



There after the ammonia is converted into amino acids and this conversion mechanism involves the sequential action of two enzymes glutamine synthetase and glutamate synthase. Glutamine synthetase combines ammonium ion with glutamate to form glutamine.



Glutamine is then converted back to glutamate by transfer of the amide group to a molecule of α -ketoglutarate. It is catalyzed by glutamate synthase (GOGAT= **Glutamine-2-oxoglutarate amino-transferase**) and requires reducing potential in form of NADH. Plants constitute two type of GOGAT i.e. one type which accepts electrons from NADH and another type which accepts electron from ferredoxin. Thus we will see that during the reaction two molecules of glutamate are formed:



Ammonium ions can also be assimilated by an alternative pathway i.e. reductive amination. Glutamate dehydrogenase catalyses the reductive amination of α -ketoglutarate which leads to the formation of glutamate. Glutamate dehydrogenase is an unusual enzyme as it is also able to utilize either NAD⁺ or NADP⁺.

Once the assimilation of nitrogen into glutamine or glutamate form, nitrogen is incorporated into other amino acids by transamination reactions. The enzymes which are responsible for catalyzing transamination reactions called aminotransferases.

Plants absorb nitrogen from the soil in the form of nitrate (NO_3^-) and ammonium (NH_4^+). In aerobic soils where nitrification can occur and nitrate is usually the predominant form of available nitrogen which is absorbed by the plants.

We can also sum up the nitrogen assimilation process with the figure.7.7 given below:

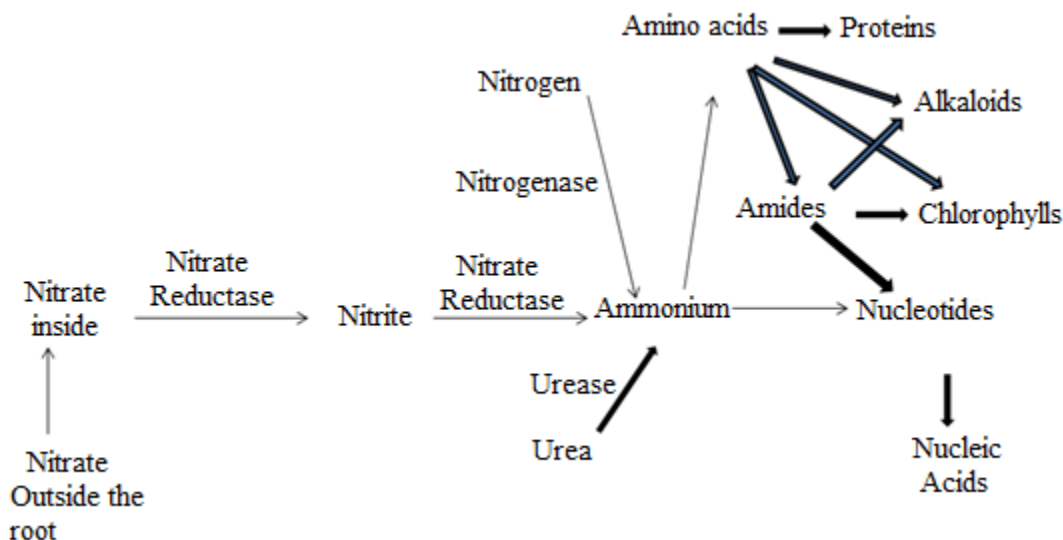


Fig.7.7 An outline of Nitrogen Assimilation

Plants can absorb nitrate or ammonium ions from the soil via their root hairs. If nitrate is absorbed, it is first reduced to nitrite ions and then ammonium ions for incorporation into amino acids, nucleic acids, and chlorophyll. In plants that have a symbiotic relationship with *Rhizobia*, some nitrogen is assimilated in the form of ammonium ions directly from the nodules. Animals, fungi, and other heterotrophic organisms obtain nitrogen by ingestion of amino acids, nucleotides and other small organic molecules.

Several reports have confirmed that cereals utilize nitrate better than ammonium however some plants viz., potato, pine apple and rice prefer ammonium over nitrate. Therefore such preferences for the utilization of different salts might be dependent upon the pH and soil condition.

7.7 SUMMARY

In this unit we have discussed the existence of nitrogen in the atmosphere and how this nitrogen is being utilized by the living organisms and how the nitrogen is metabolized and assimilated. So let us summarize the whole section in form of specific points:

1. Nitrogen is a very important constituent of cellular components.
2. N_2 plays a significant role in the formation of Alkaloids, amides, amino acids, proteins, DNA, RNA, enzymes, vitamins, hormones and many other cellular compounds which in turn control cellular activities. Without nitrogen, no living organism can survive.

3. The nitrogen cycle involves three major steps: nitrogen fixation, nitrification, and denitrification.
4. Nitrogen cycle is a cycle within the biosphere including the atmosphere, hydrosphere, and lithosphere.
5. Nitrogen is found in several locations, or reservoirs. It is most prevalent in sediments and rocks, second in the atmosphere (78%).
6. Nitrogen is considered to be an important for life because it is a major part of amino and nucleic acids. Also, it is well known as important part of Adenosine Tri Phosphate, which is the basic energy molecule for all living things.
7. Neither plants nor animals can obtain nitrogen directly from the atmosphere.
8. Therefore, they depend on a process which is known as nitrogen fixation.
9. Some free living bacteria, fungi and blue green algae (**Cyanobacteria**) are capable of fixing molecular nitrogen into utilizable form of N_2 i.e. NH_4 . *Azotobacter vinelandii*, *Clostridium pasteurianum*, *Rhodospirillum rubrum*, *Chromatium*, *Nostoc*, *Anabaena*, *Rivularia* etc are the microbes having the ability to fix the molecular nitrogen (asymbiotic Nitrogen Fixation).
10. Another biological method of nitrogen fixation is known as symbiotic nitrogen fixation.
11. Key components in this process are legumes and the symbiotic bacteria which are associated with the legume's root nodules. These bacteria are known as nitrogen-fixing bacteria.
12. **Leghemoglobin** (also termed as **leghaemoglobin** or **legoglobin**), which is a nitrogen or oxygen carrier and hemoprotein found in the nitrogen fixing root nodules of leguminous plants.
13. These organisms convert nitrogen in the soil to ammonia, which can then be taken up by plants.
14. This process also occurs in aquatic ecosystems, where Cyanobacteria (Blue green algae i.e. *Nostoc*, *Anabaena*) participate.
15. After nitrogen has been fixed, other bacteria convert it into nitrate, in a process known as **nitrification**.
16. In the first step of this process, **Nitrosomonas** convert ammonia into nitrite, and in the second step, nitrite is converted into nitrate, by **Nitrobacter**. This nitrate is then consumed by plants.
17. The final step of the nitrogen cycle is called **denitrification**. This process is performed by a variety of microscopic bacteria, fungi, and other organisms. Nitrates in the soil are broken down by these organisms, and nitrogen is released into the atmosphere. This step completes the cycle.

7.8 GLOSSARY

Protein synthesis: Combination of inorganic compound of nitrogen and their derivatives with carbohydrate for the formation of proteins

Photosynthesis is the process used by plants, algae and certain bacteria to utilize energy from sunlight and convert into chemical energy.

Alkaloids: The group of naturally occurring chemical compounds that mostly contain basic nitrogen atoms.

An amide: A functional group contains a carbonyl group linked to a nitrogen atom and It is the conjugate base of ammonia (NH_3).

Glutamine synthetase (GS): An enzyme that plays an essential role in the metabolism of nitrogen by catalyzing the condensation of glutamate and ammonia to form glutamate.

Urease: An enzyme that changes urea into ammonium carbonate, occurring in microbes i.e. bacteria, fungi etc.

Humic Substance: An organic residue of decaying organic matter

Nitrite reductase: An enzyme that catalyze the reduction of nitrite.

Nitrate reductases: Molybdoenzymes that reduce nitrate (NO_3^-) to nitrite (NO_2^-).

Diffusion Movement of gaseous molecules from higher concentration region to lower concentration region

Phytohormone: Plant hormone that control or regulate the germination, growth, metabolism or other physiological activities

Ectomycorrhizal fungi: A symbiotic relationship that occurs between a fungal symbionts and the plant roots.

Molybdenum: An essential trace element in plant metabolism.

Siroheme: A heme like prosthetic group used by some enzyme to attain the six electron reduction of sulfur and nitrogen.

Amino acid: An organic compound serves as a building block for proteins and contains an **amino** group and a carboxylic **acid** group.

α - ketoglutarate: As anion, **α -ketoglutarate (α -KG**, also called oxo-glutarate) is an important biological compound.

Transaminase (aminotransferase): An enzyme that catalyzes a type of reaction between an amino acid and an α -keto acid.

Pyridoxal phosphate: A major coenzyme which is involved in amino acid metabolism.

ATP (Adenosine Tri Phosphate) An organic compound ($\text{C}_{10}\text{H}_{16}\text{N}_5\text{O}_{13}\text{P}_3$) is composed of adenosine and three phosphate groups and acts as a source of energy for many metabolic processes.

GTP (Guanosine Tri Phosphate): A nucleotide composed of guanine, ribose, and three linked phosphate groups

ADP: An organic compound $\text{C}_{10}\text{H}_{15}\text{N}_5\text{O}_{10}\text{P}_2$ is converted to ATP for the storage of energy during cell metabolism.

TCA (Tri Carboxylic Acid) cycle: It is a series of enzyme-catalyzed chemical reactions that form a key part of aerobic respiration in cells.

NADPH (Nicotinamide adenine dinucleotide phosphate): It leads to the reduction of nitrate into ammonia for plant assimilation in nitrogen cycle.

NADH(Nicotinamide adenine dinucleotide): It exists in reduced form.

Legumes: Any plant of the legume family

FeMoco: The primary cofactor (Containing iron and molybdenum) of nitrogenase acts during nitrogen fixation.

Leghaemoglobin: a haemoglobin like red pigment in the root nodules of leguminous plants is essential for nitrogen fixation

Bio-fertilizers: Any fertilizer of biological origin, which is a substance contains living microorganisms.

Pleomorphic bacteria: Some **bacteria** having the ability of to alter their shape or size in response to environmental conditions.

Flavonoids: Large group of water-soluble plant pigments constitute antioxidant, anti-inflammatory, and antiviral properties that are beneficial to health

Intracellularly: Occurring within a cell or cells

Biogeochemical cycle: The flow of chemical elements and compounds between living organisms and the physical environment.

Ammonification: The formation of ammonia or its compounds by decomposition of organic matter.

Nitrification: Oxidation of an ammonium compound into a nitrite or nitrite into nitrate by the action of nitrifying bacteria

Denitrification: Reduction of nitrates or nitrites commonly by bacteria (as in soil) that usually results in the escape of nitrogen into the air.

GOGAT (Glutamine-2-oxoglutarate amino-transferase) : An enzyme manufactures glutamate from glutamine and α -ketoglutarate and also plays a central role in the regulation of nitrogen assimilation in photosynthetic eukaryotes and prokaryotes.

7.9 SELF ASSESSMENT QUESTIONS

7.9.1 One word answer Questions:

1. In nitrogen cycle, which bacteria change proteins to ammonia?
2. Which organism is participated most actively in nitrogen cycle in nature?
3. Where free living nitrogen fixing bacteria are found?
4. Which pigment is essential for nitrogen fixation by leguminous plants?
5. What is most limiting factor for nitrification in the in the soil?
6. Which enzyme is essential for the reduction of nitrogen into ammonia?
7. What is the name of enzyme, which changes urea into ammonium carbonate?
8. Give an example aerobic symbiotic nitrogen fixing bacterium?
9. Which pigment protects the enzyme nitrogenase from the inhibitory effect of CO_2 ?
10. Which water fern is used as bio fertilizer?
11. Which gymnosperm fixes nitrogen?
12. Which organism is found in *Anthoeceros* to fix the nitrogen?

7.9.2 Multiple Choice Questions:

1. Leghaemoglobin is a pink coloured pigment found in:
(a) Leg of Monkey (b) Brain of cat
(c) Root of leguminous plants (d) Leg of dog
2. Which of the following is a free living nitrogen fixing organism?
(a) Nitrosomonas (b) Azotobactor
(c) Bacillus ramosus (d) Azatobactor
3. Which bacteria convert NO_2 to NO_3 ?
(a) Nitrobactor (b) Bacillus vulgaris
(c) Nitrosomonas (d) Nitrosococcus
4. Which enzyme becomes inert in contact of oxygen?
(a) Nitrogenase (b) Amylase
(c) Nitrate reductase (d) Nitrite reductase
5. Most plants obtain their nitrogen from the soil in form of:
(a) Nitric acid (b) Nitrates
(c) N_2 gas (d) nitrogen oxides
6. Which of the following plants cannot fix atmospheric nitrogen directly?
(a) Pea (b) bean
(c) Castor (d) horse gram
7. Organisms which fix atmospheric nitrogen are:
(a) Mosses (b) bacteriophages
(c) Liverworts (d) blue green algae
8. Plants incorporate nitrogen into:
(a) Carbohydrates (b) cellulose
(c) Fats (d) proteins
9. Which of the following elements is involved in nitrogen metabolism in reduction of nitrates:
(a) Zn (b) Bo
(c) Mn (d) Mo
10. Which of the following does not contribute to the available nitrogen compound in soil:
(a) Electric discharge (b) Animal remains
(c) Plant remains (d) Liming

11. In nitrogen cycle which of the following does not play an important role:

- (a) *Rhizobium* (b) *Mucor*
(c) *Nitrobacter* (d) *Azotobacter*

12. Nitrogen fixing bacteria are associated with the family:

- (a) Leguminaceae (b) Gramineae
(c) Malvaceae (d) Crucifereae

13. Free living nitrogen fixing bacteria are found in:

- (a) Soil (b) root nodule
(c) Air (d) none of these

7.9.3 Fill up the following blanks:-

1. -----plants absorb ammonium ions more rapidly.
2. -----is the simplest amino acid.
3. ----- is the pigment present in root nodules.
4. Utilization of different salts might be dependent upon the ----- and soil condition.
5. ----- is the final step of nitrogen cycle.
6. In ----- thallus *Anabaena* or *Nostoc* are found.
7. ----- enters roots through root hairs of leguminous plants in form of infection thread.
8. -----are a diverse group of prokaryotes that includes Cyanobacteria.
9. Relationship between nitrogen fixing bacteria and a legume plant is described as-----.

7.9.1 Answer key: 1. Decaying bacteria, 2. Bacteria, 3. Soil, 4. Leghaemoglobin, 5. Temperature, 6. Nitrogenase, 7. Urease, 8. *Azotobacter*, 9. Leghaemoglobin, 10. *Azolla*, 11. *Cycas*, 12. Cyanobacteria

7.9.2 Answer key: 1. (c), 2. (c), 3. (a), 4. (a), 5. (b), 6. (c), 7. (d), 8. (d), 9. (d), 10. (d), 11. (b), 12. (a), 13. (a)

7.9.3 Answer key: 1. Rice, 2. Glycine, 3. Leghaemoglobin, 4. pH, 5. Denitrification, 6. Anthoceros, 7. *Rhizobium*, 8. Diazotrophs, 9. Mutualistic

7.10 REFERENCES

- Donald Voet and Judith G. Voet (1995). Biochemistry : Second edition, John Wiley and Sons
- Shah A., Kumar Seth, R. and Shukla, D. N. (2014). Role of Blue Green Algae in Paddy Crop. European Journal of Experimental Biology, 4(5):24-28
- Hortensteiner, S. and Feller, U. (2002). Nitrogen metabolism and remobilization during senescence. 53(370): 927-937.

- Jain, J. L., Jain, S. Jain, N. (2005). Fundamentals of Biochemistry. S.Chand Publishers
- Celine Masclaux-Daubresse, Francoise Daniel-Vedele, Julie Dechorgnat, Fabien Chardon Laure Gaufichon and Akira Suzuki (2010).
- Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Ann Bot., 105 (7): 1141-1157.
- Bernard SM, Habash DZ (2009). The importance of cytosolic glutamine synthetase in nitrogen assimilation and recycling. New Phytologist, 182:608-620.
- Yan Liang & Jeanne M. Harris (2005). Response of root branching to abscisic acid is correlated with nodule formation both in legumes and nonlegumes..*American Journal of Botany*, **92**(10): 1675–1683.
- Gupta, P. K. (2011). Molecular Biology and Genetic Engineering. Rastogi Publications
- Sharma, P. D. (2012). Ecology and Environment. Rastogi Publications
- Fabrice Foucher & Eva Kondorosi (2000). Cell cycle regulation in the course of nodule organogenesis in Medicago. Plant Molecular Biology, 43: 5-6.

7.11 SUGGESTED READINGS

- Fundamentals of Biochemistry: Jain, J. L., Jain, S. Jain, N. (2005)
- Molecular Biology and Genetic Engineering: P. K.Gupta (2011)
- Ecology and Environment: P. D. Sharma (2012)
- Plant Physiology and Biochemistry: S. K. Gupta & D. K. Gupta (2015)
- Biochemistry: Donald Voet and Judith G. Voet (1995)
- plantcellbiology.masters.grkraj.org/.../Plant_Cell_Biochemistry_And_Metabolism4-Nitrogen_Metabolism.htm Nitrogen 1
- https://en.wikipedia.org/wiki/Nitrogen_fixation
- <https://www.britannica.com/science/nitrogen-fixing-bacteria>
- www.peoi.org/Courses/Coursesen/bot/bot10.html nitrogen fixation 4
- https://en.wikipedia.org/wiki/Glutamate_dehydrogenase

7.12 TERMINAL QUESTIONS

7.12.1 Long Answer Type Questions:

- Q.1. Write an account of nitrogen metabolism.
- Q.2. Write an account of nitrogen cycle and nitrogen fixation in plants.
- Q.3. Describe in brief about the reduction of nitrates in plant.
- Q.4. Describe in brief about the nitrogen assimilation.
- Q. 5. Describe about the asymbiotic and symbiotic nitrogen fixation.
- Q. 6. Describe the biosynthesis of amino acids.
- Q. 7. What are the various forms of nitrogen available to plant? Describe in brief.

Q. 8. Describe in brief about the atmospheric nitrogen fixation.

7.12.2 Short Answer Type Questions:

Q.1. Make a list of microbial involvement during biological nitrogen fixation.

Q.2. Describe in short the role of blue green algae in nitrogen fixation.

Q.3. Write in brief about the role of leghaemoglobin.

Q.4. Describe in short about the occurrence of Rhizobium in soil.

Q.5. Write a short note on symbiotic nitrogen fixation

Q.6. Write a short note on ammonia assimilation.

UNIT-8 GROWTH AND PHASES OF DEVELOPMENT

Content

- 8.1 Objectives
- 8.2 Introduction
- 8.3 Definition of Growth and Development
- 8.4 Phases of Growth and Development
- 8.5 Concept of Photoperiodism
- 8.6 Physiology of Flowering
- 8.7 Biological Clocks
- 8.8 Physiology of Senescence
- 8.9 Fruit Ripening
- 8.10 Seed Dormancy
- 8.11 Seed Germination
- 8.12 Summary
- 8.13 Glossary
- 8.14 Self Assessment Question
- 8.15 References
- 8.16 Suggested Readings
- 8.17 Terminal Questions

8.1 OBJECTIVES

After reading this unit students will be able to-

- Explain and define the meaning of growth and development
- Describe the phases of growth and developmental patterns in plant.
- Describe the concept of photoperiodism
- Describe the physiology of flowering, biological clocks and physiology of senescence.
- Describe the fruit ripening process
- Explain the term seed dormancy and describe various methods to break the dormancy in seeds
- Define the term of seed germination and describe the types of seed germination in dicot and monocots

8.2 INTRODUCTION

It is well known fact, whether the plant grows to be as giant plant or not, all plants start their development as unicellular zygotes and by repeated cell divisions and differentiation they grow into fully developed plants. The development of a plant is a highly complex phenomenon. A zygote in the embryo sac utilizing available nutrients starts dividing mitotically into two, and then 4 and so on. During divisions, cellular orientation determines the future developmental pattern in which a group of cells develop, into stem and the other group into root system. This type of polarity is determined and fixed at a stage as early as 4-8 called embryo. Even at this stage, all cells look alike, but as the predetermined cell division continues, one finds that some of the cells remain exclusively for cell divisions and the other cell derivatives develop into different cell types which in turn develop into different but specific structure of different organs. Cells derived from such a single or a group of mother cells either divide further and increase their number and then differentiate or directly differentiate and develop into new cell types.

Thus we can say that the growth involves an irreversible increase in size which is usually, but not necessarily, accompanied by an increase in dry weight. The basic process of growth is to be considered as the production of new protoplasm, which is clearly evident in the regions of active cell division. The next stage in growth is increase in plant size, which is the result of absorption of water and the consequent stretching of the tissues, a process which in the strict sense is not growth at all, since it involves little or no increase in the characteristic material of the plant itself. The third and the last stage in growth constitute the entry of plenty of building materials, chiefly carbohydrates, into the expanded young tissues. This results in an increase in the dry weight but no visible increase in external size of the plant. Growth is, however, more than just an increasing amount of the plant. Differential growth of plant parts results in a characteristic shape. Each plant species has a distinctive form, development by growth patterns.

The growth is followed by the differentiation. Differentiation can be recognized at cell level, tissue level, organ level, and at the level of an organism. It becomes more obvious at the level of organ and organism. For example, if we consider flower as an organ of plant, it bears sepals for photosynthesis and protection of inner floral parts followed by beautiful, coloured petals to attract insects for cross-pollination and stamens for producing male gametes as well as the carpel for bearing the ovules which after fertilization produce seeds.

Considering a flowering plant as an organism, we observe that its roots are used for absorption of water and minerals and fixation in the soil; the trunk and stem branches bear leaves for photosynthesis, flowers and fruits and the fruits for bearing the seeds which on germination form each a new plant.

Development implies a whole sequence of qualitative structural changes that a plant undergoes from the zygote stage to its death. The developmental changes may be gradual (slowly) or abrupt. Abrupt changes might be expressed in form of germination, flowering and senescence (ageing leading to death).

Slow developmental changes include formation and maturation of tissues, formation of vegetative and floral buds and the formation of reproductive organs. Unlike growth, development is a qualitative change. Development includes growth (cell division, enlargement and differentiation), morphogenesis, maturation and senescence.

The growth cycle of annual, monocarpic, flowering plants (angiosperms) begins with the fertilized egg, the zygote. The zygote develops into an embryo following cell divisions and differentiation (embryonal stage). The embryo is enclosed within a seed where it undergoes a period of inactivity (dormancy). The resting embryo resumes growth during the germination of seed and develops into a seedling (seedling stage).

The seedling grows into a vegetative plant (vegetative phase). After some period of vegetative growth, the plant undergoes maturation and enters the reproductive phase. It develops flowers and fruits, the latter containing the seeds. Finally senescence sets in (senescence stage) leading to the death of the plant.

8.3 DEFINITION OF GROWTH AND DEVELOPMENT

Before going towards the definition of growth and development we will try to understand this aspect through a practical aspect. If we sow a seed in our garden or in a pot, after few days we will observe that a tiny seedling coming out from the seed. After this, as days pass, the tiny seedling grows in size, the number of leaves increases, and later, it grows into a mature plant and produces flowers and fruits. This is the process of growth and development. In this chapter, you will learn about growth and phases of development in plants.

You must have noticed that all living organisms grow in size. But have you ever thought how do they grow? Growth takes place due to cell division, which increases the number of cells in the body. This process continues and we observe increase in weight, size and volume of all plants. This is called **growth**.

“Growth in living organisms may be defined as an irreversible increase in the number and size of a cell, organ or whole organism”.

Growth in living organisms is not uniform throughout the life span. Growth takes place at a faster rate till the plants attain maturity. Then it slows down and at a particular time it stops. Later in life death occurs. All these changes that occur in an organism starting from its beginning till its death may collectively be termed as development. Development is associated with morphogenesis and differentiation.

Morphogenesis is the process of development of shape and structure of an organism however the differentiation is the process of change in cells, tissues or organs to carry out different functions.

“Development is the whole series of qualitative and quantitative changes such as growth, differentiation and maturation, which an organism undergoes throughout its life cycle”.

8.4 PHASES OF GROWTH AND DEVELOPMENT

8.4.1 Growth

A vascular plant starts its life from a single celled zygote formed by fertilization of an egg cell by a sperm cell. Then it begins to divide to form a plant embryo through the process of embryogenesis. As this happens, the resulting cells will organize so that one end becomes the first root (underground), while the other end forms the tip of the shoot (above ground). In phanerogams, the embryo will develop one or more "seed leaves". By the end of embryogenesis, the young plant will have all the parts necessary to begin in its life.

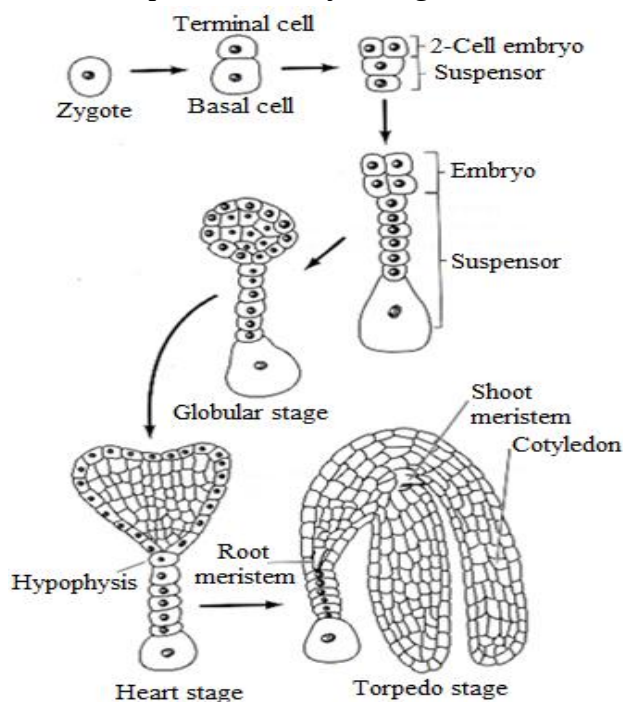


Fig.8.1 Showing Growth phases of plant

Once the embryo germinates from its seed or parent plant, it begins to produce additional organs (leaves, stems, and roots) through the process of organogenesis. New roots grow from root meristems located at the tip of the root, and new stems and leaves grow from shoot meristems located at the tip of the shoot (Fig.8.1).

Here we can understand how primary and secondary growth is carried out? Growth from any such meristem at the tip of a root or shoot is termed primary growth and results in the elongation of that root or shoot. Secondary growth results in widening of a root or shoot from divisions of cells in a cambium

In addition to growth by cell division, a plant may grow through cell elongation. This occurs when individual cells or groups of cells grow longer. The variation occurs in different growth patterns for different plant cells. When cells on one side of a stem grow longer and faster than cells on the other side, as result of this the stem bends to the side of the slower growing cells.

On the basis of these concepts growth can be categorized as primary, secondary, unlimited, limited, vegetative and reproductive growth:-

1-Primary and secondary growth- The mitotic divisions of meristematic cells present at the root and shoot apex increases the length of the plant body. This is called the primary growth. The secondary meristem increases the diameter of the plant body and it is called the secondary growth.

2-Unlimited Growth- The root and the shoot system of plants grow continuously from germination stage to the death or throughout the life span of the plant. It is called 'Unlimited' or 'indeterminate' type of growth.

3- Limited growth - The leaves, fruits and flowers stop growing after attaining certain size. This is called 'limited' or 'determinate' type of growth.

4- Vegetative growth- The earlier growth of plant producing leaves, stem and branches without flowers is called 'vegetative growth'/ Phase.

5-Reproductive growth- After the vegetative growth, plants produce flowers which is the reproductive part of the plant. This is called reproductive growth/phase.

8.4.2 Phases of Plant Growth

As all we know that, a plant is made up of cells and its growth might be considered as the sum total of the growth of its cells (Fig.8.2).

The growth of cells involves three main phases:

- (1) The phase of cell division (formative phase),
- (2) Cell enlargement and cell differentiation.
- (3) Cell Differentiation or Cell Maturation.

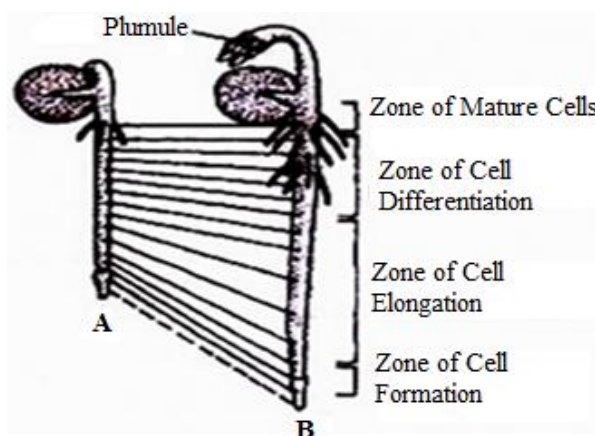


Fig.8.2 Region of Growth phases in root

1- Phase of Cell Division (Formative Phase)

Cell division is the basic event for the growth of multicellular plants. All cells in an organism result from the division of pre-existing cells. The type of cell division that occurs during the growth of an organism is mitosis. It is a quantitative as well as qualitative division that is generally completed in two stages: the division of the nucleus (karyokinesis), followed by the division of the cytoplasm (cytokinesis).

During mitosis, the cell passes through prophase, metaphase, anaphase and telophase, resulting in equal distribution of the genetical material and the cytoplasm in each of the two daughter cells thus formed. Further, the daughter cells are genetically similar to the parent cell. As a result of this process, cells having the same genetic constitution get multiplied. In higher plants, cell divisions continuously occur in the meristematic regions, such as apical meristem. As a result, an increase in the number of cells takes place in the meristematic region. Some of the daughter cells retain the meristematic activity, while others enter the next phase of growth—the phase of cell enlargement.

2 -Phase of Cell Enlargement

The cell enlargement plays an important role in contributing to the size of the tissue and organs. The enlargement occurs by synthesizing protoplasm, absorbing water (hydration), developing vacuoles and adding new cell wall material to the stretched, thin elastic walls to make them slightly thicker and permanent. Cell enlargement may be linear or in all directions.

3- Phase of Cell Differentiation or Cell Maturation

During the last phase, the enlarged cells eventually acquire a specific size and form according to their location and role following biochemical, physiological and morphological changes, i.e., the cells undergo specialization or transformation. As a result, various kinds of cells get differentiated. These differentiated cells form different kinds of simple and complex tissues which perform different functions.

8.4.3 Growth Curve

The rate of growth of a plant or plant part is not always the same during its life span. Sometimes it is slow and at other times rapid. If we plot the increase in cell number (growth rate) against time, a typical S-shaped curve is obtained. This is called growth curve or **sigmoid growth curve**. This curve has three phases of growth.

- (i) **Lag Phase** – This is the initial phase of growth when the rate of growth is very slow.
- (ii) **Log Phase** – It shows rapid growth and is maximum during the entire life span.
- (iii) **Stationary Phase** – Here the rate of growth starts decreasing and finally it stops.

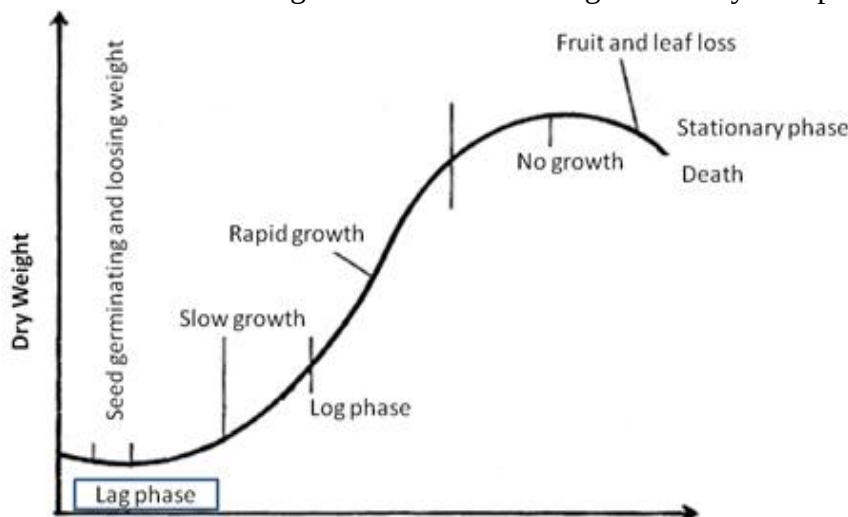


Fig.8.3 Sigmoid Curve

Thus, the total time period during which the fastest growth of the organ or organism occurs is known as **grand period of growth**.

Here we will learn how plant growth is influenced by a number of factors both external and internal.

8.4.4 External growth factors

Simply, external factors are those factors which present in the environment and that affect the growth of the plants directly or indirectly. These factors are light temperature water and mineral nutrients. Let us know about these factors in brief:

1- Light: As all we have already learnt about the necessity of light for the process of photosynthesis. Besides photosynthesis, light is also essential for seed germination, growth of seedling, differentiation of various tissues and organs, and reproduction.

2- Temperature: For growth point of view, we see that some plants grow in cold climate and some in hot climate. The optimum temperature required for growth of plants ranges between 28-30°C, but it may occur in the temperature range of 4-45°C. All metabolic activities of plants are directly affected by variation of temperature. A very low temperature causes injuries to the plant due to chilling and freezing, and very high temperature stops its growth.

3-Water: You have already learnt that a plant absorbs water by its roots, uses it in photosynthesis and other biochemical processes and some of it is lost through transpiration. For proper growth of plants a particular quantity of water is required. Both deficiency and excess of water retards the growth of plants.

4 - Mineral Nutrients: Since our primary level we are always studying about the importance of mineral nutrients for plant growth and development. All metabolic processes require inorganic nutrients. Plant growth is adversely affected by the deficiency of nutrients.

8.4.5 Internal Growth Factors

In addition to the external factors as discussed above, there are some substances produced in the plant body itself, which affects the growth of the plant. These are called plant hormones or phytohormones or growth hormones.

The growth and development of the plant can also be influenced by certain synthetic chemicals resembling plant hormones both in structure and functions. These are called growth regulators. They are not produced by plants naturally. Growth regulators are chemical substances, other than naturally produced hormones, which promote, inhibit or modify growth and development in plants.

The naturally produced growth hormones are broadly grouped under five major classes. They are

(i) Auxins (ii) Gibberellins (iii) Cytokinins (iv) Ethylene (v) Abscissic acid

Let us know details about these hormones.

(i) Auxins: Auxin is a growth promoter, generally produced by the growing apex of stem and root of the plants. It helps in the elongation of shoot and root tips behind apical meristem. The naturally produced auxin is Indole-3-Acetic Acid (IAA). They are also produced by chemical synthesis, which show same physiological responses like Auxin. Some of the synthetic auxin are Indole-3-butyric acid (IBA), 2,4- Dichlorophenoxy Acetic Acid (2,4-D), and Naphthalene acetic acid (NAA).

The Greek word **Auxin** means “to grow”. It was first isolated from human urine. An experiment was performed by Fritz Went on oat seedling to see the effect of auxins. When tip of oat coleoptile (early shoot) is removed, growth stops. Then the removed tip is placed on a block of agar (gelatinous material from sea weeds) for about an hour. This agar block is then placed on the cut end of the seedling.

It was observed that the growth of the seedling started again. It shows that there is something that has passed from the cut tip into the agar block, which helps to restart the growth. This was named **Auxin**, a plant hormone.

Functions of Auxins

(a) It promotes cell elongation;

(b) It suppresses the growth of lateral bud. If the tip of a plant is removed, the lateral branches begin to grow; In most of the plants apical bud suppresses the development of lateral buds. This is called **apical dominance**.

- (c) It delays fall of leaves. (leaf abscission)
- (d) NAA (Naphthalene acetic acid) is used for preventing fruit drop in apples before they are ripe.
- (e) 2, 4-D (2, 4-dichlorophenoxy acetic acid) acts as a dicot weedicide.

(ii) Gibberellins: Gibberellin or Gibberellic Acid (GA) was initially isolated from a fungus *Gibberella fujikuroi*. In plants, it is produced in embryos, roots, and young leaves and it enhances growth.

Functions of Gibberellins

- (a) It helps in elongation of stems in genetically dwarf plants. By using gibberellin the height of the dwarf plants can be increased.
- (b) It breaks dormancy of seeds and buds.
- (c) It induces parthenocarpy. (Formation of seedless fruits without fertilization) or provides stimulus received by pollination.

(iii) Cytokinins: They were extracted from coconut milk. Cytokinins are synthesized in root apex, endosperm of seeds, and young fruits where cell division takes place continuously.

Functions of Cytokinins

- (a) They stimulate cell division, cell enlargement and cell differentiation.
- (b) They prevent aging of plant parts.
- (c) They inhibit apical dominance and help in growth of lateral buds into branches.

(iv) Ethylene

Ethylene is a gaseous hormone. It is found in ripening fruits, young flowers and young leaves.

Functions of Ethylene

- (a) It induces ripening of fruits.
- (b) It promotes senescence and abscission of leaf, and flowers.
- (c) In cells it only increases the width not the length.

(v) Abscissic acid

Abscissic acid also known as Dormin is a naturally occurring growth inhibitor found in wide variety of plants. It is synthesized in leaves.

Functions of Abscissic acid:

- (a) It induces dormancy of buds and seeds as opposed to Gibberellin, which breaks dormancy.
- (b) It promotes the senescence of leaf, i.e., fall of leaves happen due to abscissic acid.
- (c) It inhibits seed germination and development.
- (d) It causes closing of Stomata.

8.4.6 Development

As we know the developmental process is started through embryogenesis, the formation of a multicellular embryo from a single-celled zygote, is one of the most dramatic and best-characterized aspects of plant development. Four key developmental processes take place during embryogenesis (Fig.8.1). First, the zygote expresses apical-basal polarity, meaning that the apical and basal ends of the zygote cell differ structurally and biochemically. When the zygote divides, it typically divides asymmetrically, giving rise to a small apical cell with dense cytoplasm and a large basal cell with watery cytoplasm. Although these two cells have identical nuclei, their fates differ dramatically. The apical cell gives rise to the embryo itself, while the basal cell gives rise to a short-lived structure called a suspensor and the tip of the root system. The progeny of the apical cell grow and divide to form a spherical mass of cells, the globular-stage embryo. Second, differential growth within the globular embryo gives rise to the "heart" stage embryo, the earliest stage when the precursors of cotyledons, root, and stem can be recognized. This key embryogenic process is called organogenesis. Third, distinctive planes of cell divisions bring about histogenesis, the process by which cells within embryonic cotyledons, root, and stem acquire different shapes, forming the precursors of the plant tissue systems. Last, the apical meristems of the shoot and root systems are formed at the apical and basal ends of the embryo.

In broad terms, the stages of development in plants can be divided into the following: vegetative, reproductive, ripening, and senescence. Each stage can be subdivided into various component sub stages or phases.

Although plant development is cyclical, here the seed is considered as the starting point for the sequential events leading to a mature plant, the formation of seed, and finally death. The stages of development are listed hereunder in order of occurrence and should also provide at least a general picture of how plants grow from seeds until they die. Here let we discuss about the major stages of development in plants (Fig.8.4):-

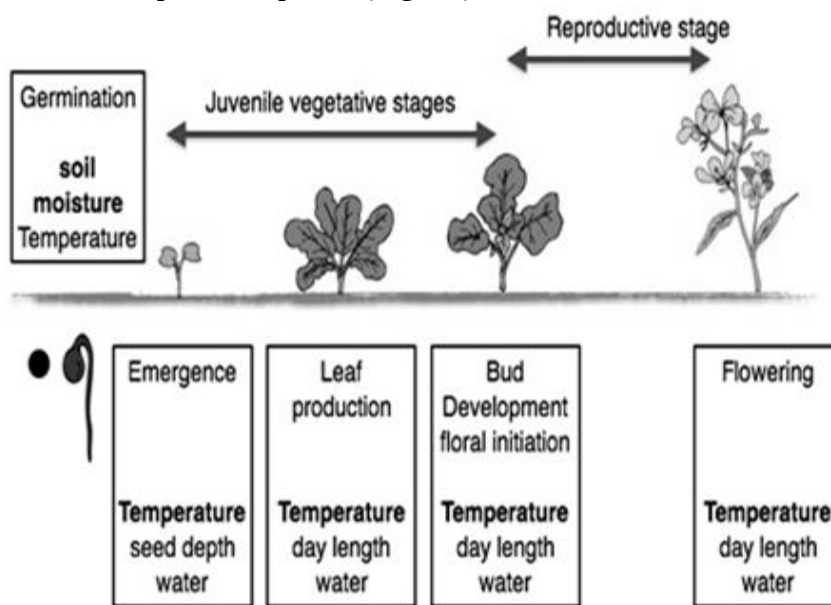


Fig.8.4 Major stages of development in plants

1-Vegetative Stage - This is generally a lengthy period of development in plants, starting from seed germination until prior to reproductive stage. In seed germination the young, quiescent plant (embryo) within the seed initiates active growth and ultimately the embryonic root (radicle) and the embryonic shoot (epicotyl) extend outward from the seed (Fig.8.5).

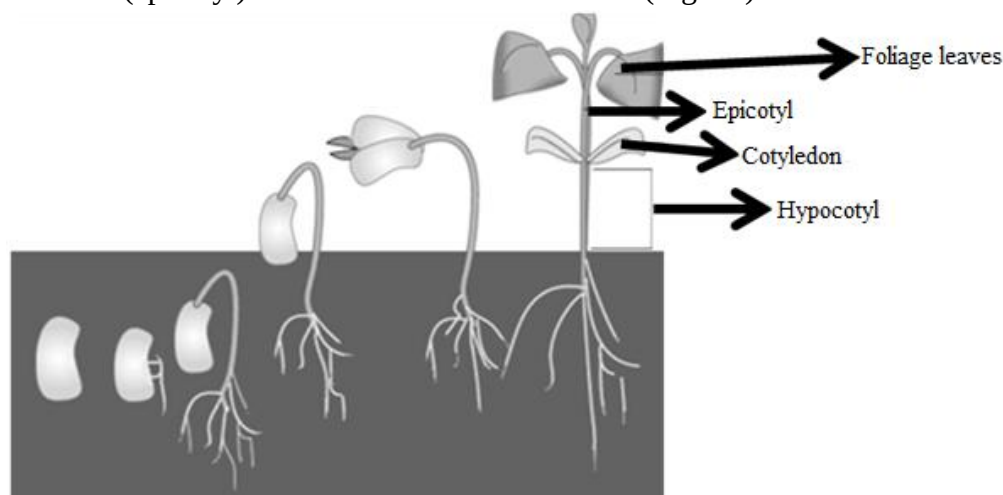


Fig.8.5 Seed germination and formation of vegetative parts of plant

2-Reproductive Stage - This stage of development in plants occurs after the vegetative or juvenile stage is completed. At this stage the plants are considered mature, that is, they are physiologically capable of commencing the production of reproductive parts: the flowers, fruits and seeds.

This stage consists of the period from the time that the plant starts to form inflorescence or flower primordia (called booting in rice) until flowering, pollination, and fertilization. Therefore, in fruit trees the reproductive stage commences with a *transition phase* during which few flowers are produced.

3-Ripening stage - In annual crops, this is the developmental stage during which fruits and seeds are formed. But for horticultural crops, ripening has been defined as “the composite of the processes that occur from the latter stages of growth and development through the early stages of senescence and that results in characteristic food quality, as evidenced by changes in composition, color, texture, or other sensory attributes”.

We can understand the ripening stage in rice, as ripening starts after fertilization (syngamy) and ends when the grains (commonly called seeds) become mature. A seed is physiologically mature when it has achieved maximum accumulation of dry matter (and dry weight). However, at this stage of maturity the seeds would have high moisture content.

4-Senescence - This is the final stage of development in plants during which physical and chemical changes occur leading to the death of the whole plant. In annual plants, senescence may start during the reproductive stage and plant death sets in soon after seed maturity which marks the end of irreversible growth.

Progressive change in color from green to yellowish (chlorosis) is a major indicator of leaf senescence and the start of whole-plant death.

8.5 CONCEPT OF PHOTOPERIODISM

As we always observe that some plants like spinach, wheat, etc. which produce flowers in summer and dahlia, cosmos etc. produce flower in winter. Why is it so? Because the plants that bear flower in summer require longer duration of light per day than those flowering in winter. Thus, we can say that duration of light plays an important role in flowering of plants. This effect of duration of light on the growth of plants is known as photoperiodism (Fig.8.6).

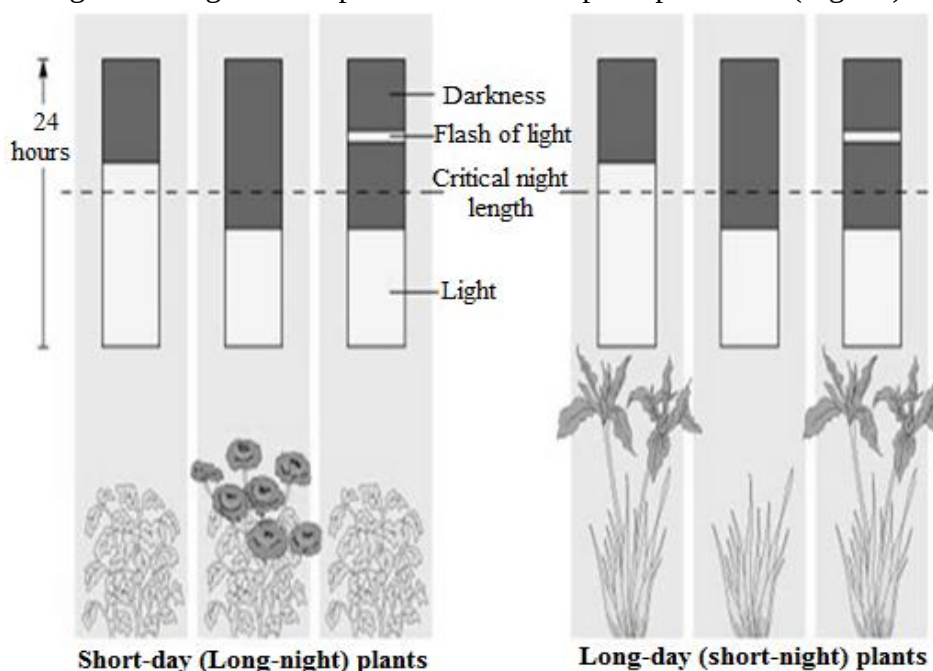


Fig.8.6 Effect of duration of light on the growth of plants

Photoperiodism is the response in growth, transpiration, photosynthesis, and reproduction (flowering) of a plant to the specific duration of light, which falls on it per day. On the basis of day-length required by the plants for flowering, the plants are classified into the following three categories:

1-Short-day Plants (SDP): Some plants produce flowers when exposed to a light period shorter than a required day-length. These are called Short-day Plants. Chrysanthemum, Cosmos, Dahlia, Soyabean, are short-day plants.

2-Long-day Plants (LDP): They produce flowers when exposed to a light period longer than a fixed day-length. Gulmohar, radish, spinach, are long-day plants.

3-Day-neutral Plants (DNP): In these plants flowering is not affected by length of light period i.e. they produce flower in almost all photoperiods. Cucumber, Tomato, and Sunflower, are day-neutral plants.

Though flowering is the best known example of photoperiodism, many other plant processes are also controlled by duration of light. Bud dormancy, bulb formation in onion, and tuber formation in potato are affected by period of light.

8.6 PHYSIOLOGY OF FLOWERING

We know that plants start their life through a period of vegetative growth. The extent of vegetative growth is well furnished with its genetic potentiality. Accordingly, they may grow into herbs or shrubs and some may develop into trees or climbers. Generally, every plant after going through a period of vegetative growth, responds towards environmental aspects, start producing floral structures, which may be in the form of characteristic single flowers or inflorescences. All plants have to acquire ripeness to flowering. Annuals complete their vegetative growth and flowering in one season and then they die. Biennials produce vegetative growth in one season and flower in the next season. But perennials remain for many years and flower seasonally.

Plants growing in different regions of the globe are exposed to different climatic conditions and different day length periods. In fact they are adapted to environs in such a way, they exhibit alternate vegetative and flowering cycles. It means that plants with their inherent genetic potentiality interact with environmental conditions according to their response and behaviour. Photoperiodism and vernalization are two most important mechanisms underlying flowering response. Phytochromes being omnipresent in the plant body, they are always subjected to both red and far red radiations in the day conditions. Accumulation of P_R forms and P_{FR} forms of phytochrome in sufficient amounts in plants is critical and important. The effective concentration of any of these forms over a threshold values in the perceptive organs like leaves is absolutely essential to bring about certain biochemical functions which may ultimately lead to the induction of flowers.

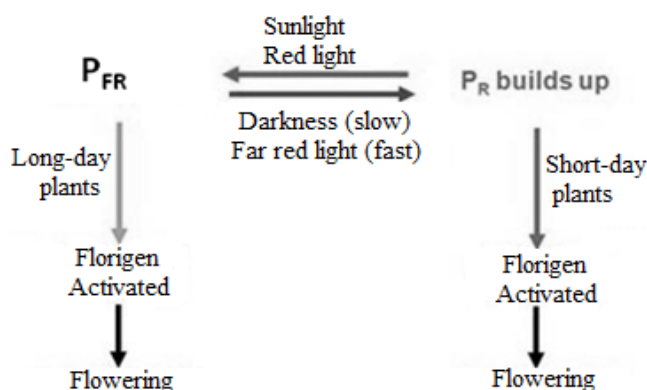


Fig.8.7 Accumulation of P_R forms and P_{FR} forms of phytochrome for Induction of Flowering

8.6.1 Discovery of flowering response

Though it is a common knowledge that different kinds of plants respond to different seasons of the year and produce flowers, it was left to G. Gassner & W.W. Garner to explain the

phenomenon by their pioneering scientific studies. Gassner observed that winter variety of petkus rye plants called *Secale cereale*, responded favorably to cold treatments. Almost at the same period of time, **Garner** and **Allard** demonstrated how plants produce flower in response to different lengths of the day and night in a 24 hours day cycle. The above two phenomenon are popularly called as Vernalization and Photoperiodism respectively. The above studies have lead to the discovery of how plants rhythmically respond and behave to the day and night duration or to temperature fluctuation in different seasons of the year and they also observed rhythmical behavior of the plants which is referred to as 'biological rhythm' or circadian rhythm. And the operational time measuring system found within the plant structures is called 'Biological Clock'.

8.7 BIOLOGICAL CLOCKS

In the 1920s, when two scientists in Germany, Erwin Bunning and Kurt Stem, were studying the movement of bean plant leaves, they saw that the plants were moving their leaves towards the sun throughout the day, and that at night they were gathering their leaves vertically upwards and assuming a sleeping position. The ability to measure time is an ability that one does not usually expect to see in other living things other than man. It may be thought that this is limited to man, but both plants and animals possess a time-measuring mechanism, or "biological clock."

The existence of this biological clocks points to a single reality, the fact of Creation. Under natural conditions, plants select certain times for certain activities. They do this in line with certain changes in the sunlight. Because their internal clocks are tuned to sunlight, they complete their rhythmic activities in 24 hours. In other cases, there are some rhythms which are much longer than 24 hours. For example, in most plants flowers open at a particular time of year, i.e. at the best possible time. Plants' clocks, which regulate this time, also calculate the duration of sunlight falling on the leaves. Every plant's biological clock calculates this period in accordance with the plant's particular features.

Plants use this perfect sense of timing in many of their functions, not just opening flowers. For example, it causes the time the poppy flower disperses its pollen to coincide with the days and hours when pollinators are most prevalent. *Thus, on the basis of this fact we can say that* the plant must have accurate knowledge of the time when the creatures which will fertilize it emerge, the length of the journey they will undertake, and the times they feed. The mechanisms of biological clocks inside every cell of the plant and are controlled by a series of components. To describe these components to you, let we know more about the secret life of plants. Plants, just like us, are very diverse and each plant is totally different. If you look at various people you will see that each one of us is unique. Some people have blue eyes and some have brown eyes, some people are tall and others are shorter and such kind of differences is endless.

Let we try to understand the mechanism of biological clocks in the plants. This mechanism is tiny and is found inside each of the cells that make up your body. In scientific terms it would be called your "DNA" and the instructions inside mechanism are your "genes".

Each plant also has its own mechanism (DNA) complete with instructions (genes) and some of these genes work like a clock.

The clock genes work on a central feedback loop. This central feedback loop means that three genes work together in a seesaw-like action. These genes have the curious names of **CCA1** (Circadian Clock Associated 1 is a gene that is central to the circadian oscillator of angiosperm). It was first identified in *Arabidopsis thaliana* in 1993. Another one is **LHY** (Late Elongated Hypocotyl) and last one is **TOC1** (TOC1 is named after the sound a clock makes). **TOC1** (Timing of CAB expression 1) is a protein that in *Arabidopsis thaliana* is encoded by the **TOC1** gene. **TOC1** is also known as two-component response regulator-like **APRR1**. **TOC1** was the first plant gene that, when mutated, yielded a circadian phenotype. Each gene tells the plant to make a protein bearing the same name as the gene. These proteins are like little engines that drive certain processes inside the plant.

The whole mechanism starts with when the sun rises, the relevant instructions tell the plant to make the **CCA1** and **LHY** proteins. When these are being made the plant knows that it is daytime. These two proteins prevent the plant from making **TOC1**. In the afternoon the plant destroys **CCA1** and **LHY**, which allows **TOC1** to be made. When there is lots of **TOC1**, the plant knows it is night time. **TOC1** is destroyed just before dawn, but the last job it does is to tell the plant to make **CCA1** and **LHY**, starting the whole process off again. This complicated feedback loop is actually only the central part of a network of interacting genes but it acts like the tiny cogs on the inside of a watch that move the hands around.

Of course all this activity is well hidden inside the plant and it's not easy to get physical evidence of it. However we can play smart by coupling the activity of **CCA1** with a sort of "glow-in-the-dark" luminous marker. Such a marker is found in fireflies. The pulse of light generated by a firefly is the result of the activity of a gene inside the fly called Luciferase. We have replicated this gene, and inside the plant have linked it to the **CCA1** gene. So under certain conditions, we can see light coming from the plant as a consequence of **CCA1** activity. The intensity of this light equals the activity of the gene: high intensity equals high gene activity.

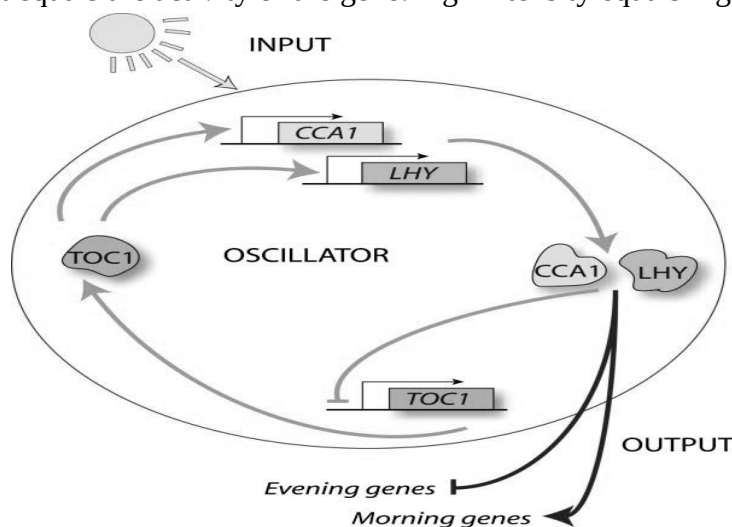


Fig.8.8 Circadian Rhythms

Throughout each day the daisy's leaves flap, the flower petals open and close, the stem grows in spurts, and small pores on the leaf surface called stomata open and close. As these events take place every day, they are known as "Circadian Rhythms". Circadian rhythms are controlled by the biological clock that is found in each cell of the daisy. The central part of this clock is made up of three instructions (genes): **CCA1**, **LHY** and **TOC1** which run together in a loop. **CCA1** and **LHY** activity is highest in the morning while **TOC1** is highest in the evening. Light and temperature are the inputs from the environment the clock uses to maintain this loop at 24 hours.

8.8 PHYSIOLOGY OF SENESCENCE

As the young plant grows, it undergoes ageing and develops into mature plant in an orderly fashion. The later part of the developmental process which ultimately leads to death is called senescence. Senescence may be defined as the period between reproductive maturity and death of a plant or a part of it. It is characterized by a collective, progressive and deteriorative developmental process which ultimately leads to complete loss of organization and function of the plant or parts of it. The study of plant senescence is called phyto gerontology.

By means of three ways plants can senescence:

- a. They die simply because of aging *i.e.* accumulated entropy causes increased sensitivity to environmental stress, bringing on death.
- b. Simple nutrient withdrawal by the seeds from the leaves eventually brings on starvation or some other nutrient deficiency, causing death *i.e.* the seeds take what nutrients they need by diffusion from the adjacent cells and this sets up a source sink diffusion gradient where the nutrients pass from the leaves to the seeds, with no active breakdown of the leaves.
- c. A hormonal or other kind of signal is released by the developing flower or seed, or the photo-induced leaves. This causes the plant to begin degrading itself and transporting the resulting nutrients out of the cells into the developing seed.

8.8.1 Types of Senescence

Leopold (1961) has recognized 4 types of senescence patterns, which are as follows:-

1. Whole plant senescence
2. Shoot Senescence
3. Sequential senescence of Organ senescence
4. Simultaneous senescence

1-Whole plant senescence: It is found in monocarpic plants which produce flower and fruit only once in their life cycle. The plants may be annual (e.g. rice, wheat, gram, mustard etc.), biennials (e.g. cabbage, henbane) or perennials (e.g. certain bamboos). The plant dies soon after ripening of seeds.

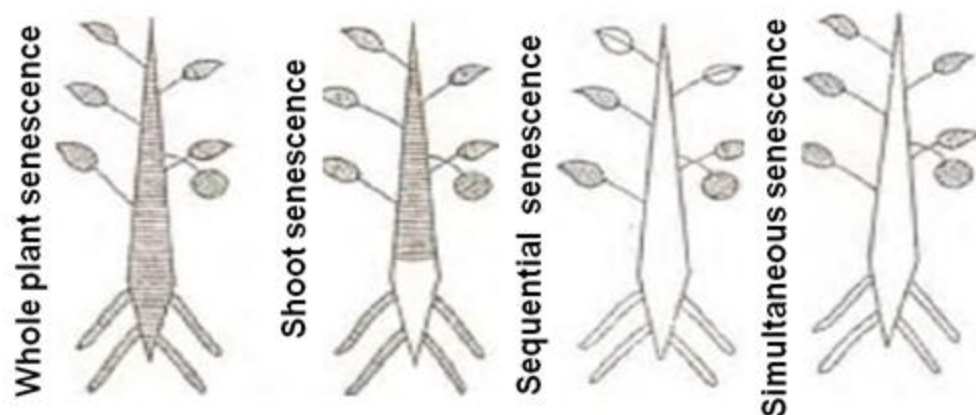


Fig. 8.9 Types of Senescence

2-Shoot senescence: This type of senescence is found in certain perennial plants which possess underground perennating structures like rhizomes, bulbs, corm etc. The above ground part of the shoot dies each year after flowering and fruiting, but the underground part (stem and root) survives and puts out new shoots again next year. For example; banana, gladiolus, ginger etc.

3-Sequential Senescence: This is found in many perennial plants in which the tips of main shoot and branches remaining a meristematic state and continue to produce new buds and leaves. The older leaves and lateral organs like branches show senescence and die. Sequential senescence is apparent in evergreen plants. e.g. *Eucalyptus*, *Pinus* etc.

4-Simultaneous or Synchronous senescence: It is found in temperate deciduous trees such as elm and maple. These plants shed all their leaves in autumn and develop new leaves in spring. Because of this shedding of leaves, autumn season is also called fall. Such a senescence of leaves or plant organs is called synchronous.

8.8.2 Physiology of senescence

The process of senescence involves a number of structural and physiological changes in the senescing organs. Some of the important changes are:

1. Cells undergo reduction in their size.
2. The membrane bound sub-cellular inclusions are disrupted.
3. Photosynthesis is reduced and starch content decreases in the cells.
4. Breakdown of chlorophyll is accompanied by synthesis and accumulation of anthocyanin pigments.
5. Protein synthesis is decreased and protein break down enhances.
6. Amino acids are withdrawn from senescing leaves and transported to the growing regions.
7. RNA content is decreased.
8. Chromatin material changes its property and DNA molecules degenerate.

8.9 FRUIT RIPENING

Ripening is a process in fruits that causes them to become more palatable. In general, fruit becomes sweeter, less green, and softer as it ripens. Even though the acidity of fruit increases as it ripens, the higher acidity level does not make the fruit seem tarter. This is attributed to the Brix-acid ratio. The ripening process makes the fruit more appealing – the color of the skin changes as chlorophyll (the green stuff in plants) is broken down and in some cases new pigments are made, the acids that make the fruit sour are broken down, the mealy starches are converted into sugar, hard pectin is softened, and larger molecules are made into smaller ones that then evaporate as aroma. Suddenly, we have a soft, juicy, sweet, fragrant, colorful animal-attractor.

This produce makeover is accomplished by a group of enzymes that are made on cue. They take their cue from a ripening signal – a burst of a gas called ethylene. Ethylene is a simple hydrocarbon gas produced when a fruit ripens. Ethylene flips the switch to trigger the genes that in turn make the enzymes that cause ripening. Plants send signals all the time using hormones. This ripening signal is unique, though, because it involves an airborne hormone (the ethylene). Ethylene is produced by rapidly growing tissue (the tips of roots, flowers, ripening fruit, damaged tissue). Thus, a wound can activate ethylene production; just the act of picking green fruit can cause the ripening process to begin.

Colour change is the result of pigments, which were always present in the fruit, becoming visible when chlorophyll is degraded. However, additional pigments are also produced by the fruit as it ripens. In fruit, the cell walls are mainly composed of polysaccharides including pectin. During ripening, a lot of the pectin is converted from a water-insoluble form to a soluble one by certain degrading enzymes. These enzymes include polygalacturonase. This means that the fruit will become less firm as the structure of the fruit is degraded.

Enzymatic breakdown and hydrolysis of storage polysaccharides occurs during ripening. The main storage polysaccharides include starch. These are broken down into shorter, water-soluble molecules such as fructose, glucose and sucrose. During fruit ripening, gluconeogenesis also increases. Acids are broken down in ripening fruits and this contributes to the sweeter rather than sharp tastes associated with unripe fruits. In some fruits such as guava, there is a steady decrease in vitamin C as the fruit ripens. This is mainly as a result of the general decrease in acid content that occurs when a fruit ripens.

Ethylene: The Ripening Hormone

Ethylene is a small hydrocarbon gas. It is naturally occurring, but it can also occur as a result of combustion and other processes. You can't see or smell it. Some fruit will produce ethylene as ripening begins. Apples and pears are examples of fruit that produce ethylene with ripening. Ethylene is responsible for the changes in texture, softening, colour, and other processes involved in ripening. Fruits such as cherries and blueberries do not produce much ethylene and it doesn't influence their ripening.

Ethylene is thought of as the aging hormone in plants. In addition to causing fruit to ripen, it can cause plants to die. It can be produced when plants are injured, either mechanically or by disease. Ethylene will cause a wide range of effects in plants, depending on the age of the plant and how sensitive the plant is to ethylene. Ethylene effects include fruit ripening, loss of chlorophyll, abortion of plant parts, stem shortening, abscission of plant parts, and epinasty (bending of stems).

8.10 SEED DORMANCY

A dormant seed is one that is unable to germinate in a specified period of time under a combination of environmental factors that are normally suitable for the germination of the non-dormant seed. Dormancy is a mechanism to prevent germination during unsuitable ecological conditions, when the probability of seedling survival is low.

Therefore dormancy can be defined as the share of inhibited growth of seeds or other plant organs as result of internal reasons. One important function of most seeds is delayed germination, which allows time for dispersal and prevents germination of all the seeds at the same time. Another form of delayed seed germination is seed quiescence, which is different from true seed dormancy and occurs when a seed fails to germinate because the external environmental conditions are too dry or warm or cold for germination. Many species of plants have seeds showing delay germination for many months or years, and some seeds can remain in the soil seed bank for more than 50 years before germination. Some seeds have a very long viability period.

True dormancy or innate dormancy is due to conditions within the seed that prevent germination under normally ideal conditions. Often seed dormancy can be categorized into two major types based on what part of the seed showing dormancy *i.e.*, exogenous and endogenous. There are three types of dormancy based on their mode of action: physical, physiological and morphological.

8.10.1 Exogenous Dormancy

Exogenous dormancy is caused by conditions outside the embryo and of three types such as physical, mechanical and chemical.

1-Physical dormancy: Dormancy that is caused by an impermeable seed coat is known as physical dormancy. Physical dormancy is the result of impermeable layer 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. In natural systems, physical dormancy is broken by several factors including high temperatures, fluctuating temperatures, fire, freezing/thawing, drying or passage through the digestive tracts of animals.

Generally, physical dormancy might also be due to one or more palisade layers in the fruit or seed coat. In few families such as Anacardiaceae and Nelumbonaceae the seed coat is not well developed. Therefore, palisade layers in the fruit perform the functional role of preventing water uptake. Specialized structures in ***Ipomoea lacunosa*** (Convolvulaceae) which function as a "water-gap" are associated with the impermeable layers of the seed to prevent the uptake of water. The water-gap is closed at seed maturity and is opened in response to the appropriate environmental signal. For example, legume (Fabaceae) seeds become permeable after the thin-walled cells of lens (water-gap structure). Following disrupted pulls apart to allow water entry into the seed.

2- Mechanical dormancy: Mechanical dormancy occurs when seed coats or other coverings are too hard to allow the embryo to expand during germination. These endogenous factors include low embryo growth potential.

3-Chemical dormancy: Chemical dormancy is associated with growth regulators that are present in the coverings around the embryo. They may be leached out of the tissues by washing or soaking the seed, or deactivated by other means. Other chemicals that prevent germination are washed out of the seeds by rainwater or snow melt.

8.10.2 Endogenous dormancy

Endogenous dormancy is caused by conditions within the embryo itself, and it is categorized into three subgroups such as physiological dormancy, morphological dormancy and combined dormancy.

1-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. Mostly Seeds with physiological dormancy do not germinate even after the seed coat or other structures that interfere with embryo growth are removed. We should be also aware with the fact that some conditions also affect physiological dormancy of seeds including drying, photo dormancy and thermo dormancy.

(a) Drying: Some plants including a number of grasses and those from seasonally arid regions need a period of drying before germination. If the seeds remain moist after dispersal, germination can be delayed for many months or even years. Many herbaceous plants from temperate climate zones have physiological dormancy that disappears with drying of the seeds. Other species will germinate after dispersal only under very narrow temperature ranges, but as the seeds dry they are able to germinate over a wider temperature range.

(b) Photo dormancy 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.

(c) Thermo dormancy is seed sensitivity to heat or cold. Some seeds including cocklebur (*Xanthium*) and amaranth germinate only at high temperatures. Many plants that have seeds that

germinate in early to mid summer have thermo dormancy and germinate only when the soil temperature is warm. Often thermo dormancy requirements disappear as the seed ages or dries.

2-Morphological dormancy: In morphological dormancy, the embryo is underdeveloped or undifferentiated stage. Some seeds have fully differentiated embryos that need to grow more before seed germination, or the embryos are not differentiated into different tissues at the time of fruit ripening.

3-Combined dormancy: Seeds have both morphological and physiological dormancy. Combinational dormancy occurs in some seeds, where dormancy is caused by both exogenous (physical) and endogenous (physiological) conditions. Some Iris species have hard impermeable seed coats and showing physiological dormancy.

8.10.3 Methods of Breaking Seed Dormancy

Here we will discuss about various methods used to break the dormancy of seed. Simple and widely used methods are as following:-

1-Scarification: Any treatment i.e. physical or chemical that weakness the seed coat, is known as scarification. Scarification method is applied, when dormancy is imposed by hard seen coat e. g. in legumes- *Cajanus cajan* (tur), gram etc.

In this method there are various ways to break hard seed coat such as:

- (a) Seeds are either rubbed on a sand paper manually. At the time of rubbing care should be taken that not to damage the axis of the seed e.g. Green gram & subabool.
- (b) When seed coat is too hard i.e. of woody nature, the seed coat has to be removing completely by breaking it. E.g. Rubber (*Havea app*) seed India teak wood seed.
- (c) Soaking treatment: Soaking hard seed coat in concentrated or diluted solution of sulphuric acid for 1 to 60 minutes, it removes seed coat impermeability.

2-Temperature Treatments:

- (a) When the dormancy is due to embryo factor i.e. the seed is incubating at low temp. (0- 50°C) over a substratum for 3 to 10 days placing it at optimum temp. Required for germination. E.g. mustard. – (*Brassica campestris*)
- (b) Some seeds required a brief period of incubation (from a few hours to one to five days) at 40 to 50°C before germinating at required temp. (in this method care should be taken that moisture content of the seed is not more than 15% e.g. paddy (*Oryza Sativa*)
- (c) Hot water treatment is also an effective method of breaking hard- seed ness in legumes. In this method the seeds are soaked in water at 80°C temp. For 1 – 5 minutes (depending up on the type of seed) before putting for germination.

3- Light Treatments: Same seeds do not germinate in dark thus it provides continuous or periodic exposure of light is essential e. g. Lettuce (*Lactuca sativa*) required red light (660nm) or white light is essential for germination to occur.

4-Exposure to light: As we know that light has the effect on hastening the germinability of seeds of some species while in other it has retarding effect. The seeds of *Vernonica longifolia* show better germination in the presence of light and at low temperature.

8.11 SEED GERMINATION

Germination may be defined as the activation of dormant embryo so as to initiate growth with the result that the embryo comes out of seeds in form of seedling.

Several factors i.e. water availability, air, temperature and sunlight and food material are necessary for the seed germination. **Water** is crucial to seed germination. The seed must go through imbibition to activate root growth. However, too much water can be a bad thing, as most gardeners know. When a plant is still growing underground, during root formation, it cannot use the sun to make food, like most grown plants do. It must rely on the stored food inside the seed, and oxygen from the environment to make energy. If the soil is too soggy, there will not be enough oxygen and the plant will not thrive.

1-Water: Water or moisture is considered as to be most essential for the germination of seeds. This is due to no vital activity is possible in high concentration of protoplasm of reserve food material. The seeds when kept in moist soil, they absorb water through micropyle, swell up and bring about the activation of dormant protoplasm. Due to the moisture the seed coat get softens and through softened seed coat embryo core comes out easily.

2- Temperature: Temperature is also an important factor. Some seeds germinate when it is cold, such as plants in northern environments. Other seeds only germinate when the weather reaches spring temperatures, which is why we see so much plant growth in the spring in temperate climates. Other seeds only germinate after extreme temperatures, such as after a fire in the grasslands. For the proper germination of seeds a suitable temperature is required (25⁰C to 30⁰C temperature is required for most of seeds and some time above 45⁰C to 50⁰C).

3-Oxygen (Air): It is essential during germination for respiration and other physiological activities. For proper germination of seeds the much amount of oxygen is essential. This is why the seeds which are deeply sown and fail to germinate, the soil is ploughed so that air may penetrate in between the soil particles.

The following three types of seed germination are being described here as:

- (a) Hypogeal Germination
- (b) Epigeal Germination

(c) Vivipary (Viviparous Germination).

Here Fig.8.10 showing the glimpse of two modes of seed germination. All monocotyledons show hypogeal germination. Among dicotyledons such as gram, pea and groundnut are some common examples of hypogeal germination.

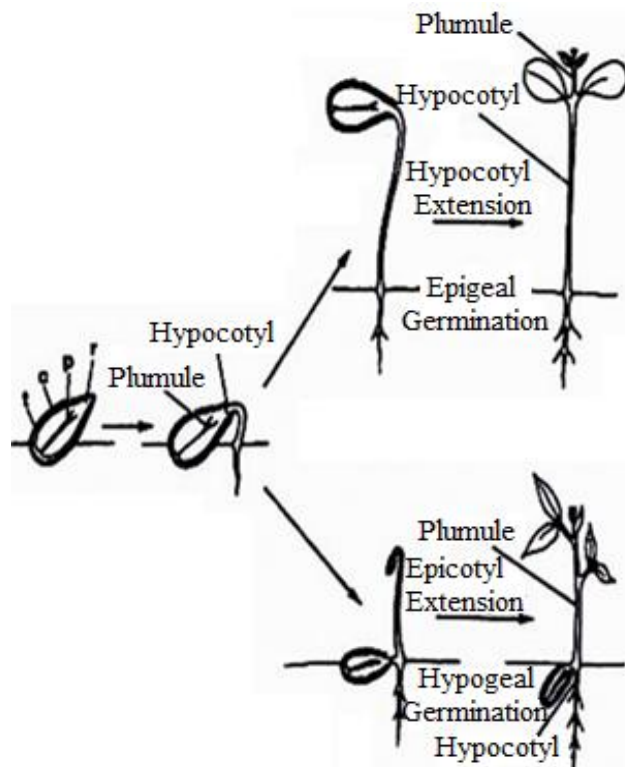


Fig.8.10 Epigeal and Hypogeal Germination

(a) Hypogeal Germination: In this kind of germination, the cotyledons do not come out of the soil surface. In such seeds the epicotyl (i.e., part of embryonic axis between plumule and cotyledons) elongates pushing the plumule out of the soil. Among dicotyledons groundnut, pea (Fig.8.11) are some common examples of hypogeal germination. All monocotyledons show hypogeal germination (Fig.8.12).

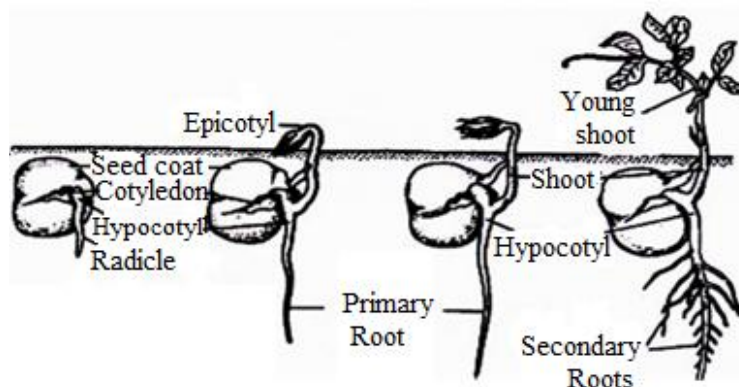


Fig.8.11 Hypogeal germination in Pea (Dicot) Seed

(i) Germination of Maize Grain: The grain imbibes water from moist soil. The coleorhiza pierces the base of caryopsis (fruit) and appears as a shining knob. After sometimes, the coleorhiza gets ruptured due to growth of radicle. After sometime coleoptile comes out. Three seminal roots develop from above the radicle (but variation in number). The radicle and seminal roots with two branches persist throughout the life of the plant. Adventitious roots are formed from the lowermost nodes above the mesocotyl (Fig.8.12).

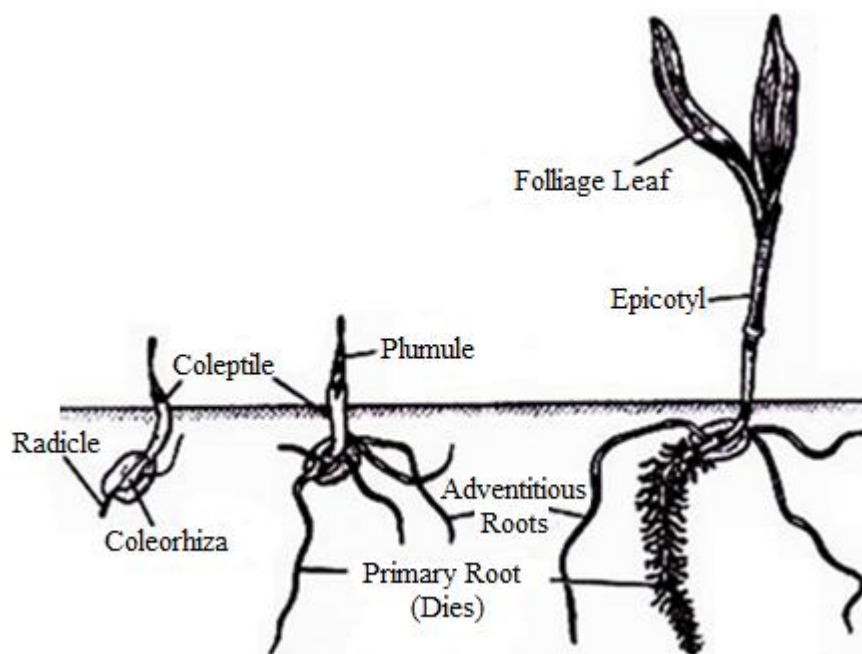


Fig.8.12 Hypogeal germination in Maize (Monocot) seed

(b) Epigeal Germination: In seeds with epigeal germination, the cotyledons are brought above the soil due to elongation of the hypocotyl. Here the cotyledons, besides food storage, also perform photosynthesis till the seedling becomes independent. In some other plants like bean, the cotyledons being thick, do not become leaf-like; they shrivel and fall off after their food reserves are consumed by the seedling.

(i) Germination of Gourd (*Cucurbita maxima*): The straight radicle comes out of the seed and fixes the seed to the soil with the secondary roots developing from the radicle. Next, the hypocotyl grows so quickly that it forms a loop which comes out of the soil and pulls out the rest of the seed. The seed coat is cast off and the cotyledons open out like two leaves, become green, large and thin so that they look and behave like ordinary leaves. The plumule within the cotyledons becomes exposed and soon grows into the aerial shoot (Fig.8.13).

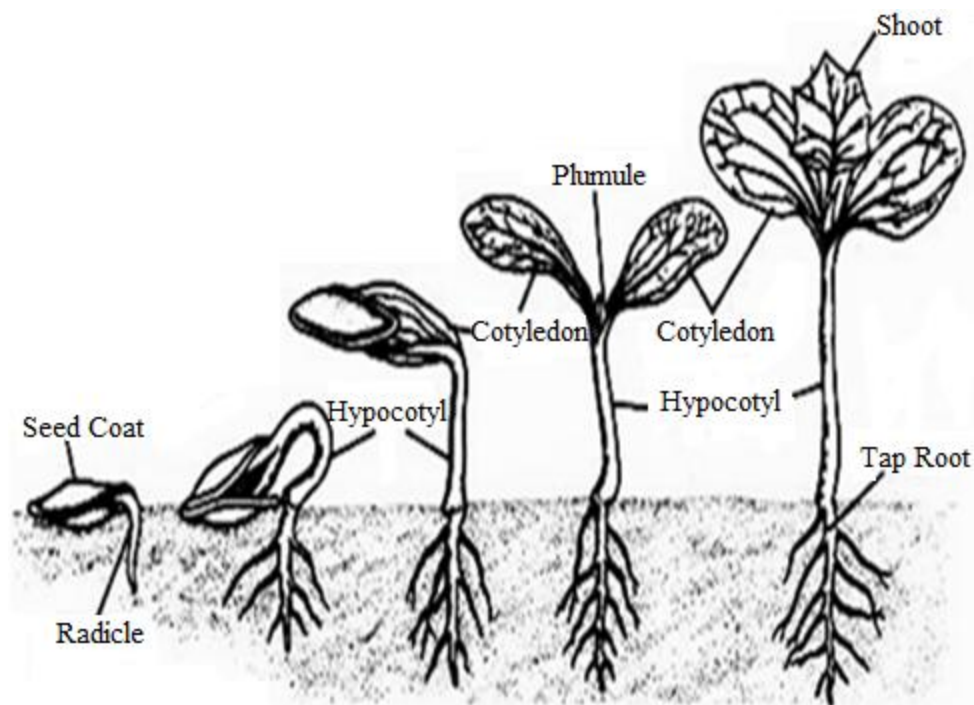


Fig.8.13 Epigeal germination in Gourd (Dicot) seed

(c) Vivipary (Viviparous Germination): Vivipary can be defined as the giving birth to young ones in advanced stage of development. It might be occurred in mammals (among animals) and mangrove plants. In mangrove plants (e.g., *Rhizophora*, *Sonneratia*, *Heritiera*) the seeds cannot germinate on the ground because of the excessive salt content and lack of oxygen in marshy habitat. In such plants seed dormancy is absent.

Let us see in the Fig.8.14, the embryo of the seed (present inside the fruit) continues growth while the latter is attached to the parent plant. Hypocotyl elongates and pushes the radicle out of the seed and the fruit. Growth continues till the hypocotyl and radicle become several centimetres long (more than 70 cm in *Rhizophora*). The seedling becomes heavy. As a result it breaks its connection with the fruit and falls down in the salt rich muddy water in such a position that the plumule remains outside the saltiest water while the tip of the radicle gets fixed in the mud. This protects the plumule. The radicle quickly forms new roots and establishes the seedling as a new plant.

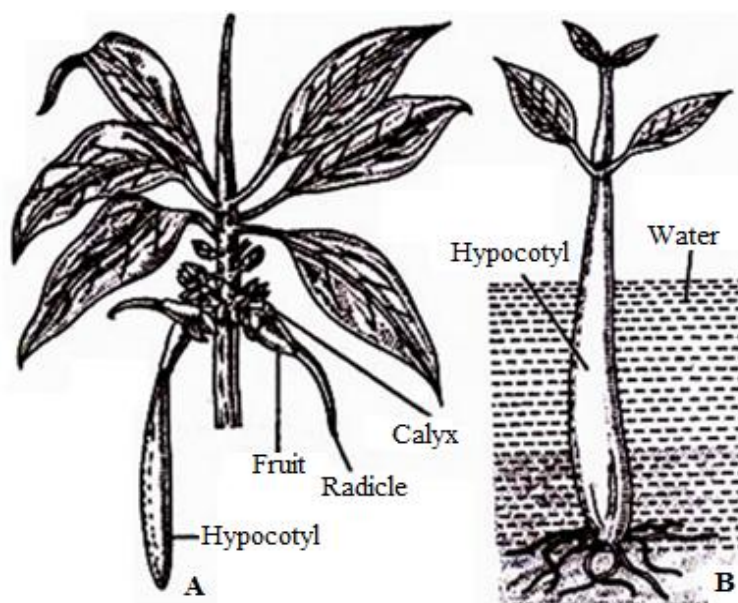


Fig.8.14 Viviparous germination in *Rhizophora* twig

8.12 SUMMARY

In this unit we have discussed the meaning of growth and development in plants and their unique features related to growth phases and developmental patterns. We also learnt how various changes are carried out since the development of seedling to the death of plant. So, let us try to summarize the whole process during the growth and development of plant in key points:

1. Plant growth and development are controlled by several environmental and genetic factors. Growth is any irreversible increase in size of an organism or its parts.
2. Development and differentiation involve the progressive specialization of cells into tissues and then plant organs.
3. The growth involves an irreversible increase in size which is usually, but not necessarily, accompanied by an increase in dry weight.
4. The basic process of growth is to be considered as the production of new protoplasm, which is clearly evident in the regions of active cell division.
5. The next stage in growth is increase in plant size, which is the result of absorption of water and the consequent stretching of the tissues, a process which in the strict sense is not growth at all, since it involves little or no increase in the characteristic material of the plant itself.
6. The third and the last stage in growth constitute the entry of plenty of building materials, chiefly carbohydrates, into the expanded young tissues. This results in an increase in the dry weight but no visible increase in external size of the plant.
7. Growth is, however, more than just an increasing amount of the plant. Differential growth of plant parts results in a characteristic shape. Each plant species has a distinctive form, development by growth patterns.

8. The growth cycle of annual, monocarpic, flowering plants (angiosperms) begins with the fertilized egg, the zygote. The zygote develops into an embryo following cell divisions and differentiation (embryonal stage).
9. The embryo is enclosed within a seed where it undergoes a period of inactivity (dormancy). The resting embryo resumes growth during the germination of seed and develops into a seedling (seedling stage).
10. The growth is followed by the differentiation. Differentiation can be recognized at cell level, tissue level, organ level, and at the level of an organism.
11. It becomes more obvious at the level of organ and organism. For example, if we consider flower as an organ of plant, it bears sepals for photosynthesis and protection of inner floral parts followed by beautiful, coloured petals to attract insects for cross-pollination and stamens for producing male gametes as well as the carpels for bearing the ovules which after fertilization produce seeds.
12. Considering a flowering plant as an organism, we observe that its roots are used for absorption of water and minerals and fixation in the soil; the trunk and stem branches bear leaves for photosynthesis, flowers and fruits and the fruits for bearing the seeds which on germination form each a new plant.
13. The seedling grows into a vegetative plant (vegetative phase). After some period of vegetative growth, the plant undergoes maturation and enters the reproductive phase.
14. Flowering is an example of environmental responses during plant development. Phytochrome, a photo reversible protein pigment, is involved in light-sensing responses in plants and is associated with several responses including flowering, photoperiodism and vernalization.
15. As result of flowering fruits are developed and latter containing the seeds. Finally senescence sets in (senescence stage) leading to the death of the plant.

8.13 GLOSSARY

Zygote: After fertilization, the fertilized egg is termed as zygote

Embryo Sac: The megaspore of a seed-bearing plant, situated within the ovule, giving rise to the endosperm and forming the egg cell or nucleus from which the embryo plant develops after fertilization.

Differentiation: It involves a series of qualitative changes occurring in plants

Pollination: The transfer of pollen from the male part (anther) of a flower to the female part (pistil).

Cross-Pollination: The transfer of pollen from an anther of a flower of one plant to a stigma of a flower of another plant of the same species.

Stamen: Typically consists of a stalk called the filament and an anther which contains microsporangia

Fertilization: The action or process of fertilizing an egg or a female animal or plant, involving the fusion of male and female gametes to form a zygote.

Seed: a structure containing a tiny embryo plant and stored food which can give rise to a new plant under suitable conditions.

Germination: Resumption of active growth in an embryo which results in its emergence from the seed and development of those structures essential to plant development.

Epicotyl: Portion of the axis of a plant embryo or seedling stem between the cotyledons and the primary leaves

Hypocotyl: That part of the embryonic axis which is between the cotyledons and the radicle.

Ageing: The process of becoming older.

Tissue: Group or layer of similarly specialized cells that together perform certain special functions.

Floral Buds: A plant bud that produces only a flower.

Morphogenesis: Formation of the structure of an organism or part

Irreversible Increase: Increase in cell numbers by cell division and an increase in cell size.

Dry Weight: Weight of any plant (or other organism) part after all its water content has been removed by drying.

Protoplasm: The semi fluid, translucent substance that forms the living matter in all plant

Carbohydrate: Neutral compound of carbon, hydrogen and oxygen. Sugar, starch and cellulose are carbohydrates.

Plant Development: It is an overall term which refers to the various changes that occur in a plant during its life cycle.

Plant Growth: It is the irreversible, quantitative increase in size, mass, and/or volume of a plant or its parts.

Annual: Refers to a plant that completes its entire life cycle in growing season. Marigolds are examples of an annual plant.

Biennial: Those plants that sprout from seed and grow through one season, overwinter then flower, go to seed and die in the second growing season.

Perennial: A plant which completes its life cycle over several to several hundred years.

Cotyledon: Energy storage components of a seed that feed the plant before the emergence of its first true leaves.

Dormant: To remain inactive when conditions are not suitable. Seeds are often dormant until conditions are right for germination.

Dormancy: This term is most often used when referring to a seed's inability to sprout for a given time after it is produced.

Hormone: Chemical substance that modifies the growth and development of a plant.

Inflorescence: The name for the structure on the plant that carries the flower.

Meristem: Tip of a plant's growth.

Nutrient: An Element Fundamental to Plant Life.

Oxygen: Tasteless, colourless element, necessary in soil to sustain plant life

Photoperiod: The relationship between the length of light and dark in a 24 hour period

Photosynthesis: The building of chemical compounds (carbohydrates) within a plant through the use of light energy, water and carbon dioxide.

Phototropism: The specific movement (often termed bending) of an entire plant or just a plant part towards a light source.

Prune: To purposely alter the shape and growth pattern of a plant by cutting stems, shoots or roots

Stratification: The process by which seed dormancy is broken and hence germination percentages improved.

Flower Induction (floral induction, flower initiation) : The process where the leaf generates a flowering stimulus in response to appropriate photoperiods.

Long-Day Plants: The plants which flower under dark period shorter than the critical night length (light period longer than the critical day length).

Long-Short-Day Plants: The plants which flower when placed under long-day conditions and then under short-day conditions.

Photoperiodic Cycle: The cycling of day and night (light and darkness).

Photoperiodic Flowering: The flowering which occurs in response to the photoperiodic cycle.

Short-Day Plants: Plants which flower when exposed to a dark period longer than the critical length (light period shorter than the critical length).

Vernalization: The flowering induced by low temperature (0 to 5°C). Also the promotive effects of low temperatures on flowering. Sometimes spelled "vernalization".

CCA1: Circadian Clock Associated 1 is a gene that is central to the circadian oscillator of angiosperm.

LHY: Late Elongated Hypocotyl gene

TOC1 Gene: Responsible for encoding TOC1 (Timing of CAB expression 1) a protein that in *Arabidopsis thaliana*

Ethylene: The Ripening Hormone

8.14 SELF ASSESSMENT QUESTION

8.14.1 Very Short Answer type Question:

1. What is the basic process of growth is to be considered as?
2. By which process the growth is followed?
3. Which part of plant is used for absorption of water and minerals and fixation of plant in the soil?
4. Which process is responsible for the development of shape and structure of an organism?
5. What term is used for growth from any such meristem at the tip of a root or shoot?
6. Which growth of plant responsible for producing leaves, stem and branches without flowers?
7. Name the phase of the growth when the rate of growth is very slow?
8. Which stage of development in plants occurs after the completion of vegetative or juvenile stage?

9. At what stage of development in plants physical and chemical changes occur leading to the death of the whole plant?
10. What phenomenon is used for effect of duration of light on the growth of plants?
11. What was the first plant gene that, when mutated, yielded a circadian phenotype?
12. The central part of biological clock is made up of which three instructions (genes)?
13. What is the name of Ripening Hormone?
14. Which dormancy is caused by conditions outside the embryo?
15. Which factor is regarded to be as crucial to seed germination?

8.14.2 Multiple choice questions

1. Which one of the followings is a gaseous plant hormone?
(a) Ethylene (b) Gibberellin
(c) IAA (d) Absciscic acid
2. Importance of day length in flowering of plants was first shown in:
(a) Petunia (b) Letting
(c) Tobacco (d) Cotton
3. Seed dormancy is due to the:
(a) Ethylene (b) Absciscic acid
(c) IAA (d) Starch
4. Flowering of plants depend on duration and timing of light and dark periods, is termed as
(a) photoperiodism (b) Differentiation
(c) Both (d) None
5. Which are of the following the sites of vernalisation.
(a) Apical buds (b) Roots
(c) Laeves (d) None
6. Miniature plants contains one or two seed leaves called
(a) Radicle (b) Plomule
(c) Cotyledon (d) Zygote
7. When seed is sown into soil, first thing it does is
(a) take up water (b) take up oxygen
(c) burst apart (d) split in two parts
8. A photoreceptor molecule which mediates several developmental and morphogenetic responses of plants to light
(a) Phytochrome (b) Gibberellin

- (c) auxins (d) ethylene

9. Which of the following hormones can replace vernalisation?

- (a) auxin (b) Cytokinins
(c) Gibberellin (d) ethylene

10. What will be the effect on phytochrome in a plant subjected to continuous red light?

- (a) phytochrome synthesis will increase (b) level of phytochrome will decrease
(c) phytochrome will be destroyed (d) first (b) then (a).

8.14.3 Fill up the following blanks:

- _____ involves a series of qualitative changes occurring in plants
- _____ is the specific movement (often termed bending) of an entire plant or just a plant part towards a light source
- _____ is the process by which seed dormancy is broken and hence germination percentages improved.
- _____ is the energy storage components of a seed that feed the plant before the emergence of its first true leaves
- _____ are the plants which flower under dark period shorter than the critical night length
- The seedling grows into a _____
- _____ involves the formation of the structure of an organism or part
- _____ is referred to be as the Ripening Hormone
- In _____ plants the seeds cannot germinate on the ground because of the excessive salt content and lack of oxygen in marshy habitat.
- In _____ germination the cotyledons are brought above the soil due to elongation of the hypocotyl.

8.14.1 Answer key: 1. The production of new protoplasm, 2. The differentiation, 3. Roots, 4. Morphogenesis, 5. Primary growth, 6. Vegetative growth, 7. Lag phase, 8. Reproductive stage, 9. Senescence, 10. Photoperiodism, 11. TOC1 gene, 12. CCA1, LHY and TOC1, 13. Ethylene, 14. Exogenous dormancy, 15. Water

8.14.2 Answers Key: 1. (a); 2. (c); 3. (b); 4. (a); 5. (a); 6. (c); 7. (a); 8. (a); 9. (c); 10. (d)

8.14.3 Answers Key: 1. Differentiation; 2. Phototropism; 3. Stratification; 4. Cotyledon; 5. Long-day plants; 6. vegetative plant (vegetative phase); 7. Morphogenesis; 8. Ethylene; 9. Mangrove plants; 10. Epigeal germination

8.15 REFERENCES

- Baurle, I; Laux, T. "Apical meristems: The plant's fountain of youth".Bio Essays, (2003); 25(10): 961–70. doi:10.1002/bies.10341. PMID 14505363. Review.

- Abhijit Kantankar*, P. Latha, L. Sandeep, D. Rashmi and J. Venkatesh (2016) Impact of ASA (Acetyl Salicylic Acid) and APAP (Acetyl Para Amino Phenol) on Morphological Characteristics and yield formation of Tomato Plant (*Solanum lycopersicum* L.) *ejpmr*, 2016,3(11), 541-547.
- William D., Teale Ivan A. , Paponov & Klaus Palme (2006). Auxin in action: signalling, transport and the control of plant growth and development. *Nature Reviews Molecular Cell Biology* 7, 847-859 (November 2006) | doi:10.1038/nrm2020.
- Isabelle Debeaujon, Karen M. Leon-Kloosterziel and Maarten Koornneef (2000). Influence of the Testa on Seed Dormancy, Germination, and Longevity in *Arabidopsis*. *Plant Physiology*, vol. 122 (2) 403-414.
- H. Nohl (1993). *Involvement of free radicals in ageing: a consequence or cause of senescence*. *Br Med Bull* 49 (3): 653-667.
- Andrew Watkinson (1992). Plant senescence. *Trends in Ecology and Evolution*, 7(12): 417-420. Elsevier
- Irwin A. Ungar (1962) Influence of Salinity on Seed Germination in Succulent Halophytes. *Ecology* (Ecological Society of America) 43:763–764.
- David Alabadi, Tokitaka Oyama, Marcelo J. Yanovsky, Franklin G. Harmon, Paloma Mas and Steve A. Kay (2001). Reciprocal Regulation Between **TOC1** and **LHY/CCA1** within the *Arabidopsis* Circadian Clock. *Science* 293, (5531): 880-883
- Todd Mockler, Hongyun Yang, Xu Hong Yu, Dhavan Parikh, Ying-chia Cheng, Sarah Dolan and Chentao Lin (2002). Regulation of photoperiodic flowering by *Arabidopsis* photoreceptors. *Proceedings of National Academy of Sciences America* 100(4): 2140–2145.

8.16 SUGGESTED READINGS

- Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Carol C. Baskin and Jerry M. Baskin. 1998
- The Rhythms Of Life: The Biological Clocks That Control the Daily Lives of Every Living Thing. Leon Kreitzman and Russell Foster. 2011
- Photoperiodism in Plants. Brian Thomas and Daphne Vince- Prue. 1996
- Plant Physiology. Frank B. Salisbury. 1992
- Plant Physiology. Lincoln Taiz & Eduardo Zeiger. 2010
- Plant Physiology. Frank B Salisbury & Cleon W. Ross. 1985
- Plant Physiology and Development. Lincoln Taiz & Eduardo Zeiger. 2014
- Plant Growth & Development :A Molecular Approach. D.E. Fosket, Academic Press, San Diego, 1994.
- Plant Development and Reproductive Biology. S. K. Gupta and D. K. Gupta. 2014
- The Embryology of Angiosperms. 6th ed. S. S. Bhojwani, S. P. Bhatnagar and P. K. Dantu Vikas publishing house private limited, New Delhi. 2014

- www.cropsreview.com/plant-growth.html
- https://en.wikipedia.org/wiki/Plant_development

8.17 TERMINAL QUESTIONS

8.17.1 Long answer type questions:

1. Describe the different stages of cellular growth.
2. Define the terms growth and development. Differentiate the growth and development.
3. Define the term Photoperiodism. Describe the physiology of flowering.
4. Explain the role of Cytokinins and Ethylene in growth and development of plants.
5. What is meant by seed germination? Describe the various factors responsible for seed germination.
6. What is senescence? Write an essay on types of senescence.
7. What do you mean by seed dormancy? Describe the exogenous and endogenous dormancy.
8. What do you understand by biological clocks? Describe the mechanism of biological clocks in the plants.
9. Write a short essay on fruit ripening process.
10. What is auxin? What is its role in the growth of plants?

8.17.2 Short answer type questions:

1. Distinguish between growth and development.
2. What is differentiation?
3. What role does differentiation play in plant growth and development?
4. What is a sigmoid growth curve? State the different phases of sigmoid curve.
5. State any two functions of Gibberellin?
6. Distinguish between epigeal germination and hypogeal germination.
7. What is senescence?

UNIT- 9 PLANT GROWTH REGULATORS

Content

- 9.1 Objectives
- 9.2 Introduction
- 9.3 Auxins
- 9.4 Gibberellins
- 9.5 Cytokinins
- 9.6 Abscissic acid
- 9.7 Ethylene
- 9.8 Summary
- 9.9 Glossary
- 9.10 Self Assessment Question
- 9.11 References
- 9.12 Suggested Readings
- 9.13 Terminal Questions

9.1 OBJECTIVES

After reading this unit, student will be able to know about:

- General account of plant hormones
- Diagrammatic representation of different experiments regarding the discovery of Auxin
- Discovery of Auxin, Gibberellin, Cytokinin and Abscissic acid
- Physiological effect of Auxin, Gibberellin, Cytokinin, Abscissic acid and Ethylene

9.2 INTRODUCTION

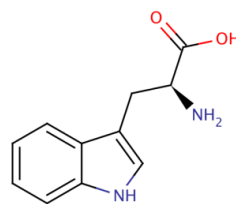
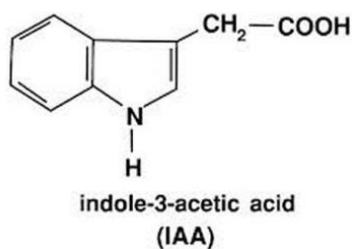
The plant growth regulators are small, simple molecules of diverse chemical composition. They are organic substances synthesized in one part of the plant body and transported to another part where they are active. PGRs (Plant Growth Regulators) are also known as plant hormones or phytohormones.

They are the regulators produced by the plants which in low concentrations regulate the metabolic process. The PGRs can be broadly classified into two categories:

(a) Growth promoters (b) Growth inhibitors, depending on their growth promoting or inhibiting activities respectively.

Generally five types of chemical growth regulating systems have been identified by plant physiologists namely (i) Auxins (ii) Gibberellins or GAs (iii) Cytokinins (iv) Abscissic acid or ABA (v) Ethylene. In this chapter PGRs (Auxin, Gibberellins, Cytokinins, Abscissic acid and Ethylene) of major importance are being described.

9.3 AUXIN



Tryptophan

The term auxin is derived from the Greek word auxein which means to grow. Compounds are generally considered auxins if they can be characterized by their ability to induce cell elongation in stems and otherwise resemble Indole Acetic Acid (IAA, the first auxin isolated) in physiological activity.

Auxins usually affect other processes in addition to cell elongation of stem cells but this characteristic is considered critical of all auxins and thus "helps" define the hormone. Although there is only one naturally occurring auxin: indole-3-acetic acid (IAA: chemically related to the amino acid tryptophan), there are many synthetic auxins as we shall see in laboratory.

9.3.1 Discovery of Auxin

In the late 1870s, **Charles Darwin and his son Francis** were one of the earliest scientists who studied phototropism (the growth of stems and leaves toward light).

The Darwin studied coleoptiles (the protective sheath around the embryonic shoot in grass seeds) of canary grass and oats. They discovered that both plants grow toward the light source.

The Darwin followed up this discovery with these experiments:

- (i) The tips of coleoptiles were covered with a metal foil. This blocked the incoming light and the coleoptiles did not grow toward light. When the foil was removed they grew toward the light.
- (ii) The growing region of the coleoptiles rather than their tips was covered and they discovered that the coleoptiles grew toward the light. The conclusion was made that the growth of coleoptiles toward light was controlled by the tip of the coleoptiles (Fig. 9.1).

The Darwin suggested that, "phototropism was due to an 'influence' produced in the tip of a coleoptile that moved to the growing region, where it caused the coleoptile to grow toward light." Their discovery helped later scientists discover plant hormones.

Peter Boysen-Jensen (1913) further developed on the Darwin's experiments:

- (i) He cut off the tip of a coleoptile and noticed that it stopped its growth, which showed that something within the tip of the coleoptile controlled growth.
- (ii) He then separated the tip from the coleoptile with a tiny piece of agar. He observed that the coleoptiles grew and curved toward the light. He concluded that the tips of the coleoptiles did not have to be in their normal position to affect growth, and the chemical that controlled phototropism moved through agar therefore it was a water-soluble chemical.
- (iii) He replaced the agar block with butter. Since water is insoluble in butter, any water-soluble chemical from the tip could not move through the butter into the growing region. He observed that there was no growth, and from this concluded that the chemical was water-soluble.
- (iv) To test if the signal was electrical he replaced the agar blocks with pieces of foil, and there was no growth. Therefore, the signal was chemical rather than electrical

Discovery of Auxin: Phototropic Response of Grass Seedlings

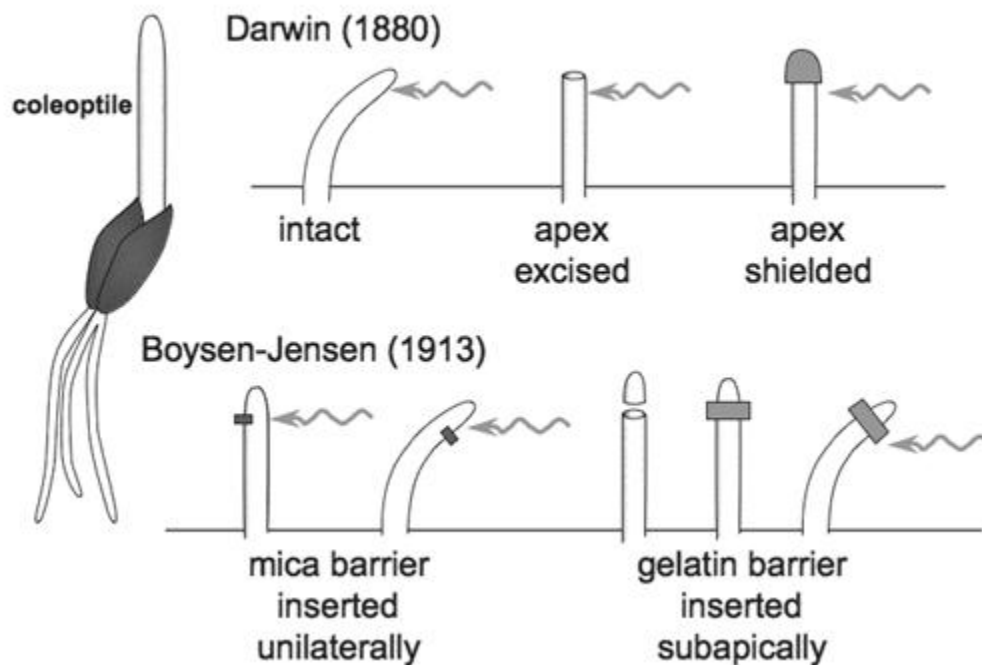


Fig. 9.1: Showing effect of light on coleoptile

In 1918, **Arpad Paal** continued on with Boysen-Jensen's experiments to identify the chemical. He studied coleoptiles grown in the dark:

- (i) He cut off the tips of coleoptiles grown in the dark, and placed them on one side of the cut surface. These curved away from the side onto which the tips were placed, despite them being in the dark. The curvature was identical to that of the plants growing toward light (Fig. 9.2).

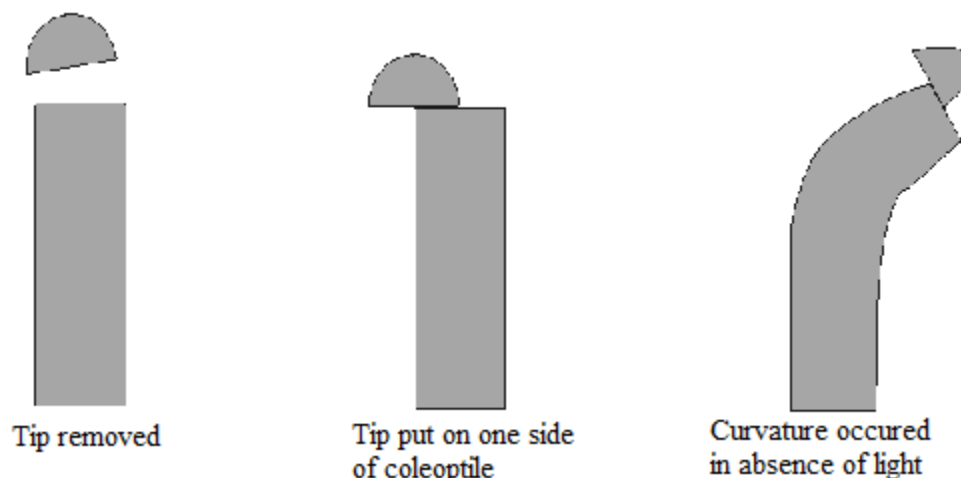


Fig. 9.2: Paal's experiment

Paal concluded that the coleoptile's tip produces something that travels down and stimulates growth, and that light causes the accumulation of the chemical on the shaded side of the coleoptile.

Frits Went (1926) finalized all these experiments: He cut off the tips and placed the cut surfaces onto agar. The tips were removed after an hour and the agar was placed on the cut tips of the coleoptiles grown in the dark (Fig. 9.3). Went's different experiments and results:

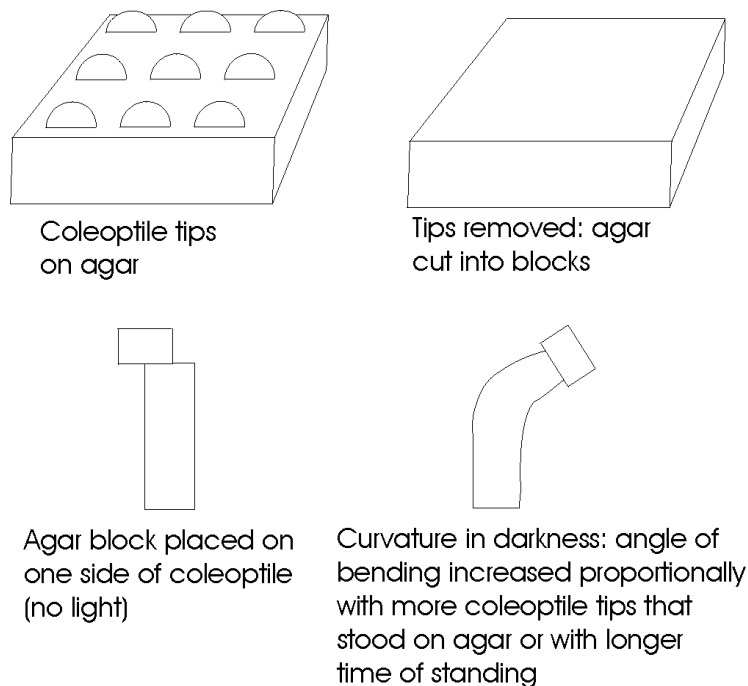


Fig. 9.3: Went's experiment

- (i) Cut off coleoptiles & without agar blocks, did not grow. This confirmed that the tips produced something essential for growth.
- (ii) Agar blocks that contacted cut tips were placed on the center of the cut off coleoptiles and they grew straight up. Therefore, the chemical diffused into the agar from the coleoptile tips, and stimulated their growth.
- (iii) Agar blocks that did not contact the cut tips of coleoptiles did not show any response. Therefore, nothing in the agar caused growth of the coleoptile.
- (iv) Agar blocks that had contacted the cut tips when placed on one side of the cut off coleoptiles, curved away from the agar blocks. This confirmed that the agar blocks had a chemical that stimulated growth of coleoptiles.

Went came to the conclusion that the phototropic response was due to a chemical coming from the coleoptile's tip. He named this chemical auxin which comes from a Greek word meaning "to grow."

9.3.2: Synthesis

The most active auxin in plants is indole-3-acetic acid (IAA) and its most active areas of synthesis are in young leaves, fruits, flowers, shoot tips, embryos, and pollens.

Some synthetic compounds have auxin like effects; such as 2, 4-D and NAA. 2, 4-D is used as a herbicide because it is relatively cheap and non-toxic (?) to humans (some question here relative to potential carcinogenicity). Other uses of synthetic auxins are that they are used to produce roots on cuttings, prevent pre-harvest dropping of fruits and prevent lateral buds from growing.

9.3.3: Effects of Auxin

Apical dominance: In higher plants the apical bud is far more active than the lateral buds. For certain period the growth of the lateral buds is suppressed. This phenomenon is called as apical dominance. According to Thimann and co-workers auxin is responsible for the dominance of apical bud.

Apical dominance seems to result from the downward transport of auxin produced in the apical meristem. In fact, if the apical meristem is removed and IAA applied to the stumps, inhibition of lateral buds is maintained (Fig. 9.4).

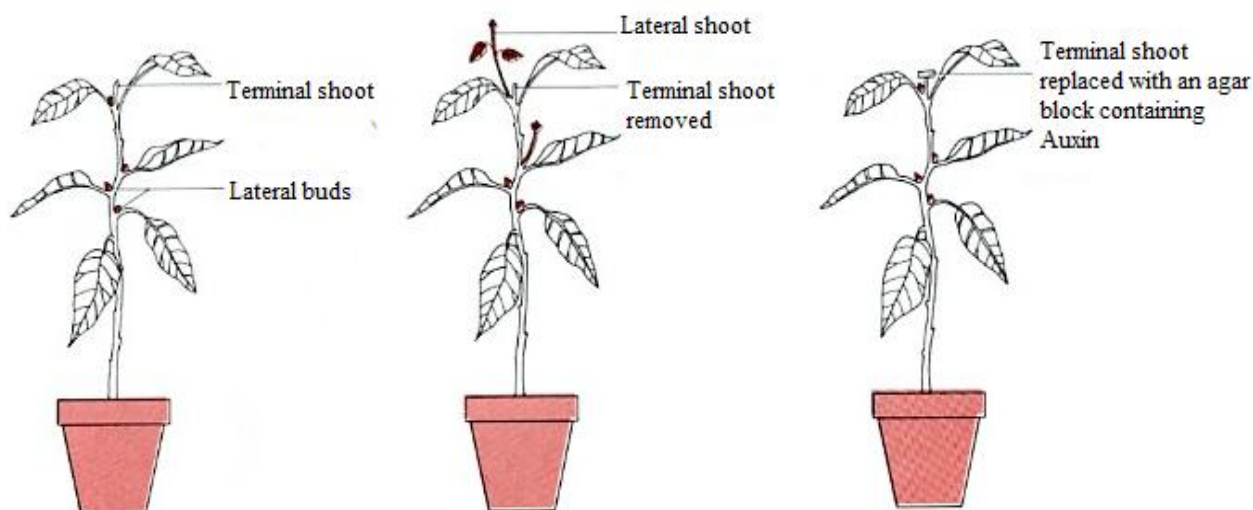


Fig. 9.4: Experiment showing effect of Auxin

The common white potato is really a portion of the underground stem of the potato plant. It has a terminal bud or "eye" and several lateral buds. After a long period of storage, the terminal bud usually sprouts but the other buds do not. However, if the potato is sliced into sections, one bud to a section, the lateral buds develop just as quickly as the terminal bud (Fig. 9.5)

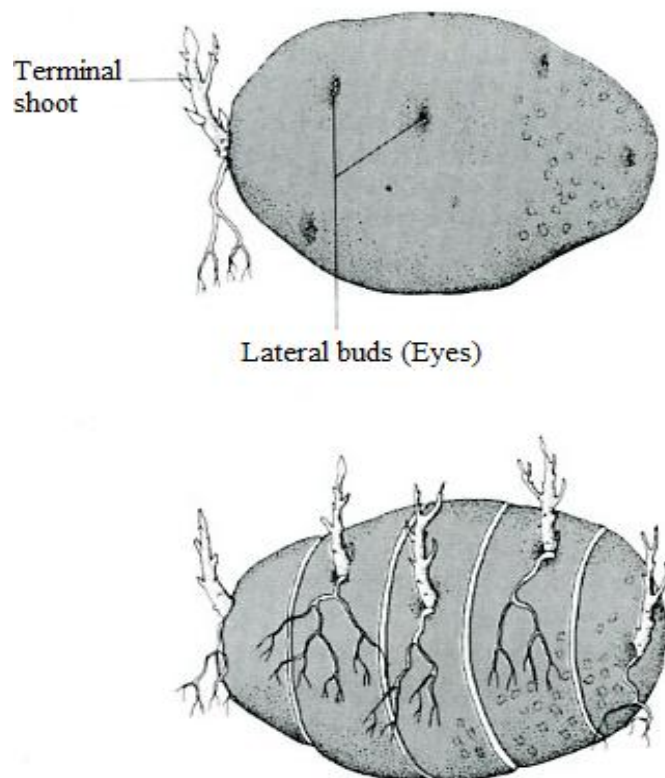


Fig. 9.5: Showing emergence of lateral buds

Parthenocarpy: As a result of pollination the auxin level of ovary is raised resulting in fruit formation, when the ovary is converted into fruit without occurrence of fertilization, the phenomenon is called as parthenocarpy. The auxins (IAA; IBA; NAA; NOAA; 2, 4-D; 2, 4, 5-T) are applied in low concentration (10^{-7} - 10^{-6} M) in a lanolin paste to the stigma of the flower and, as a result parthenocarpy is induced.

Root initiation: The auxins induce rooting in stem cuttings. Application of IAA in low concentration at the cut end of stem induces formation of adventitious roots. Besides, IBA, NAA, and 2, 4-D are also successfully used for this purpose. This property of auxins is of great economic importance for multiplying plants by cutting in nurseries.

Prevention from abscission: Auxin also plays a role in the abscission of leaves and fruits. Young leaves and fruits produce auxin and so long as they do so, they remain attached to the stem. When the level of auxin declines, a special layer of cells - the abscission layer - forms at the base of the petiole or fruit stalk. Soon the petiole or fruit stalk breaks free at this point and the leaf or fruit falls to the ground.

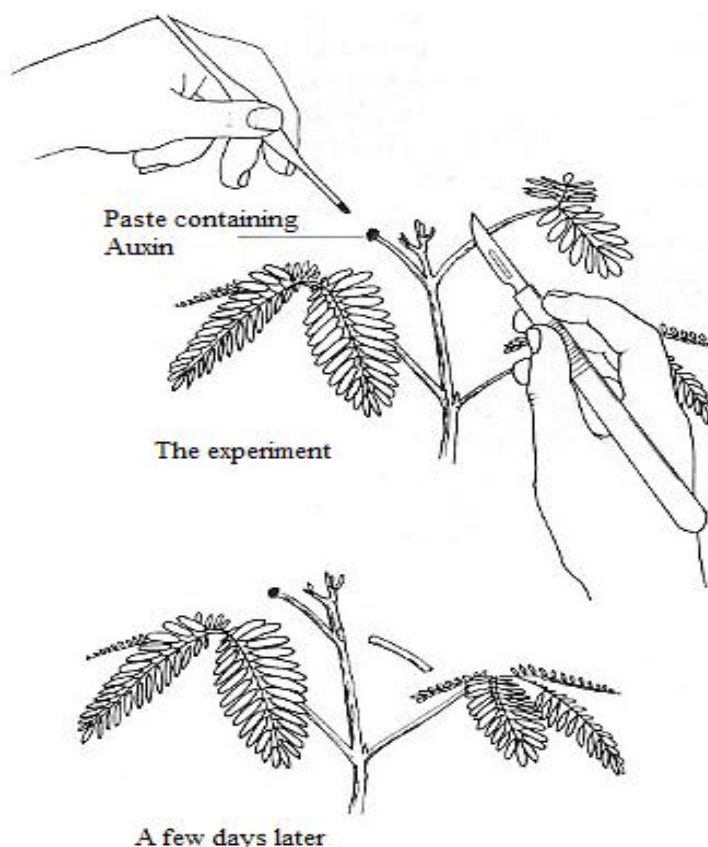


Fig. 9.6: Showing prevention from senescence

The Fig.9.6 shows a nice demonstration of the role of auxin in abscission. If the blade of the leaf is removed, as shown in the figure, the petiole remains attached to the stem for a few more days. The removal of the blade seems to be the trigger as an undamaged leaf at the same node of the stem remains on the plant much longer, in fact, the normal length of time. If, however, auxin is applied to the cut end of the petiole, abscission of the petiole is greatly delayed. Fruit growers often apply auxin sprays to cut down the loss of fruit from premature dropping (Fig. 9.7)

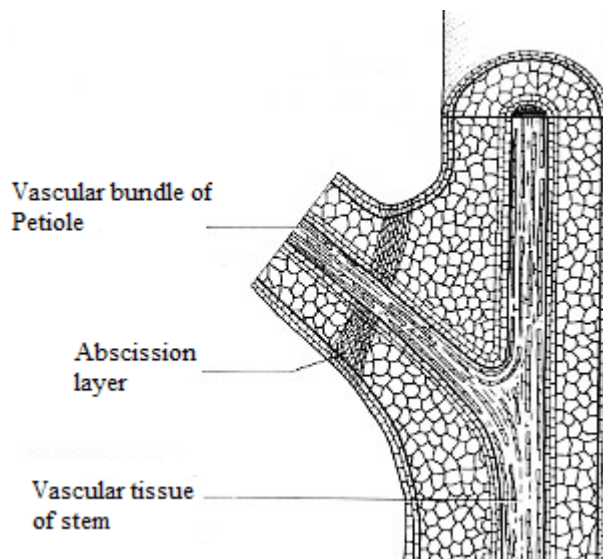


Fig. 9.7: Petiole showing abscission layer

Removal of weeds: A concentration of auxins like 2, 4-D and 2, 4, 5-T destroys dicot weeds (not the monocot weeds). The roots are sensitive to auxins. They block their sieve elements and disturb mitosis. The plant is ultimately destroyed.

Stimulation of respiration: The auxins induce respiration, perhaps by providing more ADP to be converted to ATP.

The auxins also bring about shortening of internodes. This property of auxins is useful in apple where flowers and fruits are borne on dwarf shoots. By auxin sprays more dwarf shoots are formed.

9.4 GIBBERELLINS

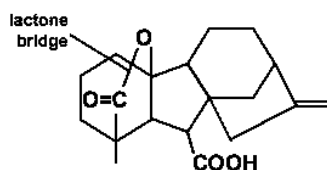
Some Japanese farmers observed a particular disease in rice seedlings on account of which they become thin, tall and pale. They called the disease as bakanae or foolish seedling. It was later on found that these seedlings were infected with the fungus *Gibberella fujikuroi* (*Fusarium moniliforme*). Sawada (1912) thought that the disease was caused due to some substance secreted by the fungus. This was experimentally confirmed by Kurosawa (1926). Yabuta and Sumiki, (1938) were first to extract a crystalline substance from the fungus which they named as gibberellin.

All gibberellins are acidic in nature whose carboxyl group is marked by esterification. Hence they are chemically called as gibberellic acid and abbreviated as G.A. Cross and co-workers (1961) isolated six gibberellins from the fungus *Gibberella* which are identified as GA.1, GA.2, GA.3, GA.4, GA.7 and GA.9.

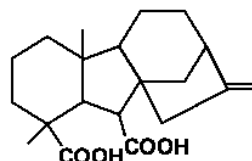
In same year Mac Millan and co-worker isolated three gibberellins from bean seeds which they identified as GA.5, GA.6, and GA.8. So far about sixty gibberellins have been isolated, of which some 15 have been obtained from the fungus *Gibberella*. The seed plants contain about 51 gibberellins. All the gibberellins contain gibbane ring skeleton.

The gibberellins are present in all groups of plants i.e., from algae to Angiosperms but rarely in fungi and bacteria. They may be synthesized at places where they are needed or they may be transported from other regions. The transport is done by diffusion through xylem as also through phloem. Their transport is non-polar. The young leaves are the main sites of gibberellin synthesis. In contrast, the older leaves have only a little ability to do so. The gibberellin synthesis in young leaves renews the activity of the vascular cambium. The roots synthesize gibberellins in sufficient amount. If the roots are repeatedly excised, a marked decrease in the concentration of the gibberellins is observed in the shoot. This suggests that in the shoot's gibberellin is derived from the roots via xylem elements. The immature seeds contain a higher percentage of gibberellin in comparison to mature seeds. This increased concentration is due to synthesis and not due to transport. In grass seeds the gibberellin synthesis mainly occurs in the scutellum and probably in other parts too.

There are two fundamentally different forms of Gibberellins

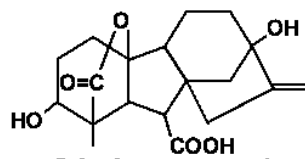


GA₉ is a C-19 gibberellin

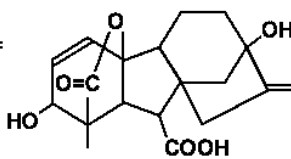


GA₁₂ is a C-20 gibberellin

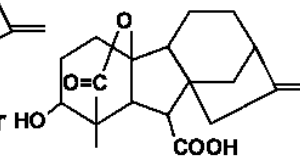
These are three active Gibberellins



GA₁ is more-polar



GA₃ is more-polar



GA₄ is less-polar

9.4.1 Mechanism of action

It stimulates cell division in shoot tip. The cell growth is promoted by the increase in the hydrolysis of starch, fructose, and sucrose. It further well plasticity. Most of the workers are of the opinion that the effect of gibberellin is indirect since they act by altering the auxin status. In the aleurone layer of barley the gibberellin increases the transcription of genes that code for protease and amylase enzymes. The protease activity produces tryptophan which is translocated to coleoptile tip where it is converted to IAA. The IAA shows polar movement.

9.4.2 Physiological effects

1. **Stem elongation:** The gibberellin induces internodal elongation. When treated with gibberellins, the lettuce plant became vine like.
2. **Light induced stem growth:** Dark grown etiolated plants are lean, tall, and yellow. Perhaps light has inhibiting effect on stem growth. It is thought that light induced inhibition of stem growth is overcome by exogenous application of gibberellins.
3. **Genetic dwarfism:** By the application of gibberellins, Lang (1956) observed a rapid growth or 'bolting' on some dwarf plant. Similar behavior has been observed in dwarf rice, maize, watermelon, squash, and cucumber.
4. **Promotion of flowering in long-day plants:** The long day plants generally possess a basal rosette of leaves. Before flowering they show significant internodal growth. After receiving the minimum hours of day light requirement, they bolt and flower. The continuation of rosette form or bolt and flower is linked with the amount of native gibberellins present in the plant.
5. **Increase in flower and fruit size:** By the exogenous application of gibberellin the size of flower of *Geranium* and *Camellia* increased. Similar application of gibberellin also increased the size of the fruit of *Vitis*.
6. **Parthenocarpy:** Like auxins, the exogenous application of gibberellins also induced the production of parthenocarpic fruits. They are also applied to the stigma in a lanolin paste.
7. **Substituting cold treatment:** By exogenous application of gibberellins, many biennials can be induced to behave as annuals and they no more require the natural chilling treatment for their flowering.
8. **Breaking of dormancy:** The exogenous application of gibberellins have been shown to be capable of breaking the dormancy of potato tubers and buds of trees in winter.
In addition to above, the gibberellins promote hypocotyl growth, increase in the number and size of leaves, expression of apical dominance, delay the senescence of leaves and *Citrus* fruits, sexual development of flowers particularly the maleness as well as the enzyme activity.

Commercial uses of Gibberellins

Commercially the gibberellins are employed in the following:

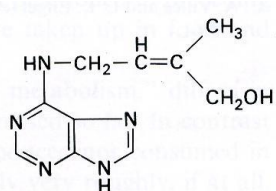
1. Increase in the size of Thompson seedless grape fruits as also the distance in between them.
2. Increase in the height of sugar cane plant and more sugar yield.
3. Increase in the fresh weight of pastures and hay crops.
4. In storage of oranges, gibberellin prevents rind disorder by delaying senescence.

9.5 CYTOKININ

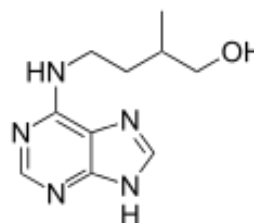
Haberlandt (1913) observed that the vascular sap is capable of including cell division activity in wounded potato tubers. Similar capability was observed by Van Overbeek *et al.* (1941) in

coconut milk (liquid endosperm). Miller (1954) isolated an active substance from autoclaved DNA from Herring sperms which stimulated cell division. He named this substance as Kinetin. It was identified as 6-furfurylamino purine. Several names were given to this substance such as Kinetenoid by Busstorm (1961); Phytokinin by Osborne (1963); Cytokinin by Letham (1963) and so on. However, the name cytokinin was preferred over other names and abbreviated as CK. Skoog *et al.* (1965) defined cytokinin as 'chemicals' which regardless of their activities promote cytokinesis in various plant organs'

The most common and physiologically active cytokinins are **zeatin**, **dihydrozeatin**, and **isopentanyl adenine**. Benzyladenine was regarded as a synthetic cytokinin but Ernst (1983) found 6-benzyladenine riboside in the seeds of *Pimpinella anisum*.



Structure of zeatin



Structure of dihydrozeatin

9.5.1 Zeatin

It was obtained by Letham (1963) in crystalline form from immature maize grain who named it as zeatin. Later, Letham *et al.* (1964) identified as 6 (4-hydroxy 3-methyl trans -2 butenyl) aminopurine. Its synthesis was done by Wilson (1964). According to Letham (1974) the zeatin and zeatin ribose (ribosylzeatin) are most active compound. The higher activity of zeatin is perhaps due to a very reactive allylic-OH group in its side chain.

Distribution

Beside higher plants, the cytokinins occur in diatoms, red and brown algae as well as mosses. In these plants they promote growth. A few pathogenic bacteria and fungi also contain cytokinins. In non-pathogenic bacteria they influence nodulation and formation of mycorrhiza. In higher plants cytokinin occurs in the root extract of sunflower, pea seedling, flower and fruits of apple, pear, plum, and tomato, cambial tissue of eucalyptus and tobacco, young fruits of maize, banana and walnut, fruit and embryo of peach, liquid endosperm of coconut and tumor tissue of tobacco.

9.5.2 Biological activities

1. **Cell division:** The cytokinins induce cell division activity. It is the most characteristic property of cytokinin. By its application a normal cell of *Vinca* was converted to a tumor cell. They also induce cell division activity in bacteria such as *E. coli*.
2. **Cell elongation:** They induced cell enlargement. It was observed in disc etiolated leaves as well as tobacco pith and cortical cells. This property is enhanced in combination with auxins.

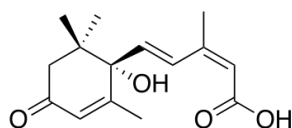
3. **Morphogenesis:** By applying cytokinin and IAA in balanced combination, tobacco pith cells produced callus. If the amount of cytokinin is increased, differentiation of buds was observed in the callus.
4. **Breaking dormancy:** The dormancy of seeds of *Lactuca sativa* is broken by a spray of cytokinin. It is thought that the site of cytokinin action on seed germination is in the cotyledon.
5. **Suppression of apical dominance:** The cytokinin counteracts the phenomenon of apical dominance. In fact this phenomenon is controlled by a balance of concentration between endogenous IAA and cytokinins.
6. **Delay in senescence:** Richmond and Lang (1967) observed that the degradation of proteins and chlorophyll was delayed in the detached leaves of *Xanthium*, if there was cytokinin in the medium. The phenomenon of delay in senescence by cytokinin treatment is also caused as **Richmond-Lang effect**.

9.6 ABSCISSIC ACID

When a fruit ripens or before a leaf falls, a special zone of cells is formed at the base of the pedicel or petiole. This zone is called as **abscission zone**. It is delimited by a protection layer on the stem side and a **separation layer** on the organ side. The fruit or leaf is ultimately separated and the phenomenon is called as **abscission**.

A Swedish botanist, Hemberg (1949) observed that the extract of dormant potato tubers and the buds of *Fraxinus excelsior* (ash) inhibit the growth of *Avena* coleoptiles. Osborn (1955) also observed that the extract of old leaves causes premature fall of young leaves in bean plants. Addicott and co-workers (1963) isolated two compounds, **Abscissin I and Abscissin II**, responsible for the abscission of cotton fruits. Robinson and Warming (1964) isolated a substance responsible for dormancy in *Acer pseudoplatanus* and named it as dormin. It was later found that abscissin II and **dormin** were identical compounds. Addicott (1968) suggested the name Abscissic acid (ABA) which was widely accepted. It is a naturally occurring hormone which appears to be present in all vascular plants and some mosses but not in bacteria, algae, most of fungi and liverworts.

9.6.1: Structure



Structure of Abscissic acid

The ABA is a terpenoid having an asymmetric carbon. It is a C-15 sesquiterpene (with 3 isoprene units). Most of the hormone is synthesized in leaves and fruits. Some of ABA in chloroplast can arise from xanthophyll, **violaxanthin**.

9.6.2 Physiological Effects of Abscissic Acid

1. **Seed and bud dormancy:** Abscissic acid induces dormancy of buds towards the approach of winter. Abscissic acid accumulates in many seeds during maturation and apparently contributes to seed dormancy.
2. **Senescence:** ABA acts as a general inducer of senescence. The onset of senescence is correlated with stomatal closure. The ABA content of aging leaves increases markedly as senescence is initiated.
3. **Flowering:** In long-day plants, the effect of gibberellins on flowering is counteracted by ABA, which accumulated in the leaves during the short winter days. This ABA acts as inhibitor of flowering in long-day plants. On the other hand ABA induces flowering in short-day plants.
4. **Starch hydrolysis:** The GA-induced synthesis of α -amylase and other hydrolytic enzymes in barley aleurone cells is inhibited by abscissic acid. This inhibition can be reversed by increasing the amount of GA supplied.

9.7 ETHYLENE

It was observed in 1864 that the gas illuminating the streets in German cities, due to leakage to pipes caused leaf fall in road side shade trees. This gas was thought to be responsible for causing senescence and abscission of leaves. Later, in 1879 it was observed that an illuminating gas promoted ripening of oranges. The gas was identified as ethylene ($\text{CH}_2=\text{CH}_2$) in both the cases. Galston and Davis (1970) recognized it as a growth regulator.

9.7.1 Physiological Effects of Ethylene

1. Unripe fruits can be made to ripe before proper time if they are kept in ethylene atmosphere.
2. In some plants, it stimulates germination of seed.
3. It inhibits root and stem elongation but induces root hair formation.
4. It also induces cellulose activity leading to promotion of leaf abscission.
5. It induces petal discoloration.

9.8 SUMMARY

1. The plant growth regulators are small, simple molecules of diverse chemical composition. They are organic substances synthesized in one part of the plant body and transported to another part where they are active.
2. The term auxin is derived from the Greek word auxein which means to grow.
3. Compounds are generally considered auxins if they can be characterized by their ability to induce cell elongation in stems.

4. The Darwin suggested that, "phototropism was due to an 'influence' produced in the tip of a coleoptile that moved to the growing region, where it caused the coleoptile to grow toward light." Their discovery helped later scientists discover plant hormones.
5. In 1913, Peter Boysen-Jensen further developed on the Darwin's experiments.
6. He cut off the tip of a coleoptile and noticed that it stopped its growth, which showed that something within the tip of the coleoptile controlled growth.
7. In 1918, Arpad Paal continued on with Peter Boysen-Jensen's experiments.
8. He cut off the tips of coleoptiles grown in the dark, and placed them on one side of the cut surface. These curved away from the side onto which the tips were placed, despite them being in the dark. The curvature was identical to that of the plants growing toward light.
9. Paal concluded that the coleoptile's tip produces something that travels down and stimulates growth, and that light causes the accumulation of the chemical on the shaded side of the coleoptile.
10. Frits Went finalized all these experiments. Went came to the conclusion that the phototropic response was due to a chemical coming from the coleoptile's tip. He named this chemical auxin which comes from a Greek word meaning "to grow."
11. According to Thimann and co-workers auxin is responsible for the dominance of apical bud.
12. As a result of pollination the auxin level of ovary is raised resulting in fruit formation.
13. The auxins induce rooting in stem cuttings. Application of IAA in low concentration at the cut end of stem induces formation of adventitious roots.
14. Auxin also plays a role in the abscission of leaves and fruits.
15. A concentration of auxins like 2, 4-D and 2, 4, 5-T destroys dicot weeds (not the monocot weeds).
16. Some Japanese farmers observed a particular disease in rice seedlings on account of which they become thin, tall and pale. They called the disease as bakanae or foolish seedling.
17. Yabuta and Sumiki (1938) were first to extract a crystalline substance from the fungus which they named as gibberellin.
18. In 1961, Cross and co-workers isolated six gibberellins from the fungus *Gibberella* which are identified as GA.1, GA.2, GA.3, GA.4, GA.7 and GA.9.
19. In same year Mac Millan and co-worker isolated three gibberellins from bean seeds which they identified as GA.5, GA.6, and GA.8. So far about sixty gibberellins have been isolated, of which some 15 have been obtained from the fungus *Gibberella*. The seed plants contain about 51 gibberellins.
20. The immature seed contain a higher percentage of gibberellin in comparison to mature seeds. This increased concentration is due to synthesis and not due to transport. In grass seeds the gibberellin synthesis mainly occurs in the scutellum and probably in others parts too.
21. The gibberellin induces internodal elongation.
22. By the application of gibberellins, Lang (1956) observed a rapid growth or 'bolting' on some dwarf plant. Similar behavior has been observed in dwarf rice, maize, watermelon, squash, and cucumber

23. It is also effective in genetic dwarfism, promotion of flowering in long day plants, Increase in flower and fruit size, parthenocarpy and breaking of dormancy.
24. Skoog *et al.* (1965) defined cytokinin as 'chemicals' which regardless of their activities promote cytokinesis in various plant organs'.
25. The most common and physiologically active cytokinins are zeatin, dihydrozeatin, and isopentanyl adenine.
26. Beside higher plants, the cytokinins occur in diatoms, red and brown algae as well as mosses. In these plants they promote growth.
27. The cytokinins induce cell division activity. It is the most characteristic property of CK. They induced cell enlargement morphogenesis.
28. They also effective in breaking dormancy, suppression of apical dominance and delay in senescence. The phenomenon of delay is senescence by CK treatment is also caused as Richmond-Lang effect.
29. Robinson and Warming (1964) isolated a substance responsible for dormancy in *Acer pseudoplatanus* and named it as dormin. It was later on found that abscissin II and dormin were identical compounds. Addicott (1968) suggested the name Abscissic acid (ABA) which was widely accepted.
30. Abscissic acid induces dormancy of buds towards the approach of winter. Abscissic acid accumulates in many seeds during maturation and apparently contributes to seed dormancy.
31. ABA acts as a general inducer of senescence. It is also effective in flowering and starch hydrolysis.
32. Ethylene gas are helpful for unripe fruits which can be made to ripe before proper time if they are kept in ethylene atmosphere.

9.9 GLOSSARY

Auxin: A plant hormone which causes the elongation of cells in shoots and is involved in regulating plant growth

Gibberellin: Any of a group of plant hormones that stimulate stem elongation, germination, and flowering

Coleoptile: A sheath protecting a young shoot tip in a grass or cereals

Phototropism: The orientation of a plant or other organism in response to light

Agar: A jelly like substance, obtained from algae

Stimulus: A thing or event that evokes a special functional reaction in an organ or tissue

IAA: Most common naturally occurring plant hormone of the auxin class

Apical dominance: Inhibition of the growth of lateral buds by the terminal bud of a plant shoot

Apical meristem: Meristem at the apex of a root or shoot

Stumps: the bottom part of a tree left projecting from the ground after most of the trunk has fallen or been cut down

Lateral buds: A bud located on the side of the stem

Terminal buds: A bud occurs on the end of a stem

Parthenocarpy: The development of a fruit without prior fertilization

Abscission: The natural detachment of parts of a plant, typically dead leaves and ripe fruit

Weed: A wild plant growing where it is not wanted

9.10 SELF ASSESSMENT QUESTIONS

9.10.1 Very short answer type questions:

1. Name natural auxin found in plants.
2. Suggest the most characteristic effect of gibberellins in plants.
3. Name the most common cytokinin reported in plants.
4. Name a hormone that is related with xylem differentiation.
5. Name the two synthetic auxins used for inducing the rooting in woody plants.
6. Richmond and Lang effect related to cytokinins refers to which phenomenon?

9.10.2 Objective type question:

1. Auxins are:
(a) Growth hormones (b) Proteins
(c) Porphyrins (d) Cells osmophores
2. Substances which originate in the stem tip and control growth are:
(a) Vitamins (b) Enzymes
(c) Phenols (d) Auxins
3. The bending of stems towards light is due to uneven growth of cells caused by:
(a) Enzymes (b) Auxins
(c) Gibberellic acid (d) Stored proteins
4. Which of the following is not a naturally occurring hormone?
(a) 2, 4-D (b) Gibberellin
(c) Kinin (d) IAA
5. Auxins are produced by:
(a) Apical root meristem (b) Root cambium
(c) Apical shoots meristem (d) All of the above
6. Auxins inhibit the growth of:
(a) Apical buds (b) Lateral axillary buds
(c) Roots on stem- cuttings (d) Parthenocarpic development of fruits

7. Gibberellin were first extracted from:

- (a) Bacteria
- (b) Virus
- (c) Fungi
- (d) Algae

8. The fungus causing foolish seedling disease was discovered by:

- (a) A Japanese farmer
- (b) An Indian botanist
- (c) A Formosan botanist
- (d) An American botanist

9. Internodal elongation is stimulated by:

- (a) Auxin
- (b) Cytokinin
- (c) Gibberellin
- (d) Phenol

10. Gibberellins cause:

- (a) Curvature of coleoptile
- (b) Elongation of internodes
- (c) Initiation of lateral roots
- (d) Cell division

11. Cytokinins:

- (a) Induces cell division
- (b) induces dormancy
- (c) Causes cell plate formation
- (d) Is meant for spindle fiber formation

12. Which of the following delays senescence:

- (a) Cytokinin
- (b) Gibberellin
- (c) Indole acetic acid
- (d) ABA (Abscissic acid)

13. Which of the following hormone is concerned mainly with root initiation?

- (a) IBA
- (b) ABA
- (c) G.A.-3
- (d) CK

14. The name “auxin” was coined by:

- (a) Paal
- (b) Stark
- (c) Thimann
- (d) Went

15. Which of the following is not a natural auxin :

- (a) Indole 3-acetonitrile
- (b) Indole 3-acetaldehyde
- (c) Indole 3-ethanol
- (d) Indole 3-butyric acid

16. The first cytokinin was isolated from:

- (a) Maize
- (b) Cocos
- (c) Herring sperms
- (d) Tomato fruit sap

17. Zeatin was first isolated in crystalline form by:

- | | |
|--------------|-------------|
| (a) Skoog | (b) Osborne |
| (c) Burstrom | (d) Letham |

9.10.3 Fill in the blanks

1. The *Avena* curvature test for auxins was discovered by.....
2. On unilateral illumination the dark side of the coleoptile tip possesses..... % auxins.
3. The leaves are main sites of gibberellin synthesis.
4. The gibberellins induce flowering in.....day plants.
5. induce cell division and cell elongation activities in plants.

9.10.4 True/False

1. Paal placed coleoptile tip eccentrically on the stump and transferred the experiment in dark which produced no curvature.
2. Generally five types of auxins have been recognized.
3. Immature seeds contain more gibberellins than mature seeds.
4. The first cytokinin isolated was dihydrozeatin.
5. Zeatin and dihydrozeatin are most common and physiologically active cytokinins.

9.10.1 Answer key: 1. IAA, 2. Internodal elongation, 3. Isopentenyl adenine, 4. Auxin, 5. Indole Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA), 6. Delay of senescence

9.10.2 Answer key: 1(a), 2(d), 3(b), 4(a), 5(d), 6(b), 7(a), 8(c), 9(a), 10(c), 11(b), 12(a), 13(a), 14(d), 15(d), 16(c), 17(d)

9.10.3 Answer key: 1. Went, 2. 57%, 3. Young, 4. Long, 5. Cytokinins

9.10.4 Answer key: 1. False, 2. True, 3. True, 4. False, 5. True

9.11 REFERENCES

- Simon, S, Petrášek, P (2011). "Why plants need more than one type of auxin". *Plant Science*. **180** (3): 454–460.
- Ludwig-Müller J (2011). "Auxin conjugates: their role for plant development and in the evolution of land plants". *J. Exp. Bot.* **62** (6): 1757–1773
- Taiz, L, Zeiger, E (1998). *Plant Physiology* (2nd ed.). Massachusetts: Sinauer Associates.
- Friml, J (2003). "Auxin transport-shaping the plant". *Current Opinion in Plant Biology* **6** (1): 7–12.
- Camara, M *Cet al* (2015) General Aspects and Applications of Gibberellins and Gibberellic Acid in Plants. In: *Hardy, J. (Org.). Gibberellins and Gibberellic Acid: Biosynthesis, Regulation and Physiological Effects*. 1st ed. Hauppauge: Nova Science Publishers,v., p. 1-21.

- Riley, JM (2012) "Gibberellic Acid for Fruit Set and Seed Germination". Retrieved 26 Oct.
- Kieber, JJ (March 2002). "Tribute to Folke Skoog: Recent Advances in our Understanding of Cytokinin Biology". *J. Plant Growth Regul.* **21**(1): 1-2.
- Mok DW, Mok MC (2001). "Cytokinin Metabolism and Action". *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52** (1): 89–118.
- Bassaganya-Riera, J. Skoneczka, J. Kingston, DG.Krishnan, A. Misyak, SA.Guri, AJ. Pereira, A. Carter, AB. Minorsky, P.Tumarkin, R.Hontecillas, R (2010)."Mechanisms of action and medicinal applications of abscissic Acid". *Current Medicinal Chemistry.* **17** (5): 467–78
- "Abscissic Acid - Compound Summary". Pub Chem Compound. USA: *National Center for Biotechnology Information.* 16 September 2004.

9.12 SUGGESTED READINGS

- *Fundamental of plant physiology* by V.K. Jain (2015).
- *Plant Physiology* by Edwin Oxlade (2010)
- *Plant Physiology* by S.N. Pandey (2009)
- *Advances in Plant Physiology* by A. Hemantaranjan (2004)
- *Advances in Plant Physiology* by P.C. Trivedi (2006)
- *Text Book of Plant Physiology* by V. Verma (2009)
- *Plant Physiology and Development* by Lincoln Taiz (2015)
- *A Text Book of Plant Physiology, Biochemistry and Biotechnology* by S.K. Verma and Mohit Verma (2008)

9.13 TERMINAL QUESTIONS

9.13.1 Long answer type

1. Write down the physiological effects of Auxins.
2. Describe the discovery of Auxin with relative experiments.
3. Give a brief description of gibberellins.
4. Write down the discovery and types of gibberellins.
5. What are the mechanism underlying the phenomenon by which the terminal/apical bud suppresses the growth of lateral buds? Suggest measures to overcome this phenomenon.

9.13.2 Short answer type

1. Name a hormone isolated from fungus and the workers who isolated it?
2. List the hormones used as weedicides or herbicides.
3. The phenomenon of apical dominance is mainly due to which hormone?

4. Explain apical dominance. Name the hormone that controls it.
5. Why auxins and cytokinins are primarily used in tissue culture?

9.13.3 Diagrammatic Type

1. Show the diagrammatic representation of Boysen-Jensen's experiments.
2. Explain Went's experiments only through the diagram.

BLOCK-3 : BIOCHEMISTRY

UNIT-10 CARBOHYDRATES AND LIPIDS

Content

- 10.1 Objectives
- 10.2 Introduction
- 10.3 Carbohydrates
 - 10.3.1-Classification
 - 10.3.2-Properties
 - 10.3.3-Biological role
- 10.4 Lipids
 - 10.4.1-Classification
 - 10.4.2-Properties
 - 10.4.3-Biological role
- 10.5 Summary
- 10.6 Glossary
- 10.7 Self Assessment Question
- 10.8 References
- 10.9 Suggested Readings
- 10.10 Terminal Questions

10.1 OBJECTIVES

After reading this unit students will be able-

- To understand the structure and classification of carbohydrates.
- To study physical and chemical properties of carbohydrates.
- To study biological role of carbohydrates.
- To understand the structure and classification of lipids.
- To study physical and chemical properties of lipids.
- To study biological role of lipids.

10.2 INTRODUCTION

Carbohydrates are organic and biological molecules. They are an instant energy source for our body. They are very important in the form of dietary fibers like cellulose, pectin, mucilage etc. Dietary fibers like pectin and mucilage are important for proper intestinal health. Consumption of fiber makes waste elimination easier. The excess pressure during defecation may cause hemorrhoids and diverticulitis. Fiber intake reduces the risk of obesity and diabetics also benefit from fiber intake. It also decreases absorption of cholesterol. Carbohydrates also are important in the cellular recognition process. Example: Immunoglobulin's or antibodies and peptide hormone contain glycoprotein sequences. The liver can recognize the glycoprotein sequence and this way carbohydrates mark proteins passages.

The lipids are a large and diverse group of naturally occurring organic compounds that are related by their solubility in nonpolar organic solvents (e.g. ether, chloroform, acetone & benzene) and general insolubility in water. There is great structural variety among the lipid Fatty Acids, Soaps, and Detergents, Fats and oils, Waxes, Phospholipids, Eicosonoids, Terpenes, Steroids, Lipid Soluble Vitamins, etc.

This chapter deals with structure properties of carbohydrates and lipids.

10.3 CARBOHYDRATES

Carbohydrates are the most abundant class of organic compounds which are found in living organisms. They originate as products of photosynthesis, an endothermic reductive condensation of carbon dioxide requiring light energy and the pigment chlorophyll.



Carbohydrates can be defined as chemically as neutral compounds of carbon, hydrogen and oxygen. Carbohydrates form the basic constituent of diet of all living beings exist widely in

animal and vegetable tissue. The composition of carbon, hydrogen and oxygen is in the ratio of 1:2:1. The empirical formula of carbohydrates is $C_n(H_2O)_n$ where n usually ranges from 3–7. Carbohydrates are present in sugars and starches. They make up parts of nucleotides (the energy currency of a cell, and the building blocks for genetic information). They are also present in some components of all cell membranes. They are the central components of energy producing pathways in biology. Carbohydrates may be defined as polyhydroxyl aldehydes or ketones or compounds which produce them on hydrolysis. The term sugar is applied to carbohydrates soluble in water to taste.

Or

Carbohydrates are that substance which on hydrolysis yields either ketones or aldehyde group.

The carbohydrates are sometimes known as *saccharides*. Saccharides comes from a Greek word Sacharon meaning Sugar, Glucose, Fructose, Sucrose, Starch, Cellulose, Glycogen are some of the common carbohydrates.

10.3.1 Classification of Carbohydrates

The name carbohydrate literally means Hydrates of carbon. They are often known as saccharides which mean sugar.

There are a variety of interrelated classification schemes.

Based on functional groups:

1-Aldoses. They contain the aldehyde group - Monosaccharides in this group are glucose, galactose, ribose, and glyceraldehyde.

2-Ketoses . They contain the ketone group - The major sugar in this group is fructose.

3-Reducing: They contain a hemiacetal or hemiketal group. Sugars include glucose, galactose, fructose, maltose, lactose

4-Non-reducing: They contain no hemiacetal groups. Sucrose and all polysaccharides are in this group.

Classification based on number of Carbons:

Monosaccharides can be further classified by the number of carbons present.

Hexoses (6-carbons) are by far the most prevalent.

	Number of Carbons	
Three = Triose	Five = Pentose	Six = Hexose
Glyceraldehyde	Ribose	Glucose
		Galactose
		Fructose

They are broadly classified into 3 groups based on sugar unit:-

1. Monosaccharides

2. Oligosaccharides

3. Polysaccharides

Mono and oligosaccharides are sweet to taste, crystalline in character and soluble in water, hence they are commonly known as sugar.

1-Monosaccharides

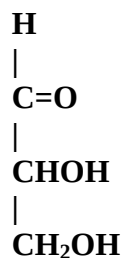
Mono means one. Monosaccharides are a simple group of carbohydrates. The general formula is $C_n(H_2O)_n$. These contain a single carbon chain. Monosaccharides are divided into two categories based on the functional group and number of a carbon atom.

Sugars which have aldehyde ($\begin{array}{c} \text{H} \\ \diagdown \\ \text{C}=\text{O} \\ \diagup \end{array}$) group are called aldoses (e.g.: glyceraldehydes, glucose.) and sugars with keto group are called ketoses (e.g: dihydroxy acetone, fructose). Depending on the number of carbon atoms monosaccharides are named as

1. Triose (C3)
2. Tetrose (C4)
3. Pentose (C5)
4. Hexose (C6)

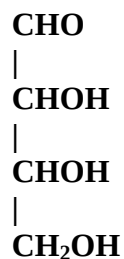
1. Triose (C3): These have three carbon atom.

Glyceraldehyde and dihydroxy acetone are the simplest carbon hydrates with three carbon compounds. Glyceraldehyde has an aldehyde group and dihydroxy acetone has a ketone group.



Glyceraldehyde

2. Tetrose (C4): These have four carbon atom. Erythrose ($C_4H_8O_4$) is an important carbohydrate, whose structure is as follows:



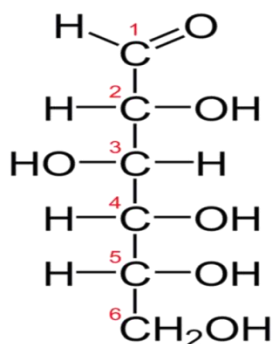
Erythrose

3. Pentose (C5):-These have 5 carbon atoms. Ribulose and Xylose are common pentoses. Riboses have aldehyde while xylose has ketone group.

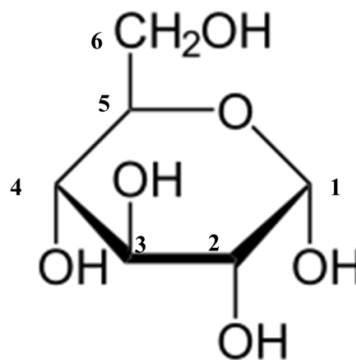


Ribose

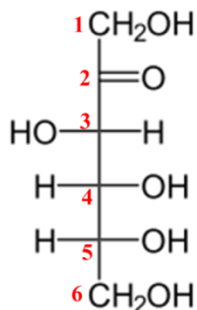
4. Hexoses (C6):-Hexoses have common formula $\text{C}_6\text{H}_{12}\text{O}_6$ and commonly meet with in plants in free conditions. Glucose and fructose are more common carbohydrates. **Glucose** is aldohexose while **fructose** in a keto hexose.



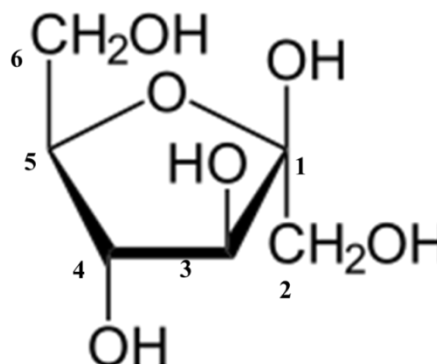
Glucose (Fischer projections)



Glucose (Haworth projections)



Fructose (Fischer projections)



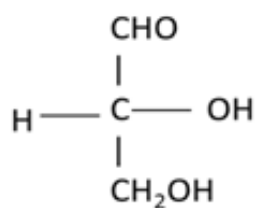
Fructose (Haworth projections)

Note - Fischer projections and Haworth projections of structure

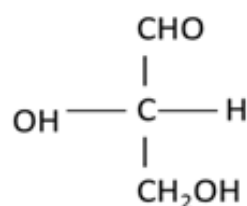
Fisher projections show sugars in their open chain form. In this type, the carbon atoms of a sugar molecule are connected vertically by solid lines, while carbon-oxygen and carbon-hydrogen bonds are shown horizontally.

Haworth projections are often used to depict sugars in their cyclic forms. It is a common way to draw a structural formula which represents the cyclic structure of monosaccharides with a simple three-dimensional perspective

Stereoisomers



D(+)-Glyceraldehyde



L(-)-Glyceraldehyde

Stereoisomers compounds have a same structural formula but differ in the spatial configuration. While writing the molecular formula of monosaccharides, the spatial arrangements of H and OH groups are important, since they contain asymmetric carbon atoms. Asymmetric carbon means four different groups are attached to the same carbon. The reference molecule is glyceraldehyde which has a single asymmetric carbon.

The number of possible stereoisomers depends on the number of asymmetric carbon atoms by the formula 2^n where n is the number of asymmetric carbon atoms.

Reference carbon atom of sugars

The configuration of H and OH at the second carbon of glyceraldehyde makes two mirror images denoted as D and L form. All monosaccharides are derived from glyceraldehyde by successive addition of carbon atoms. Therefore the penultimate carbon atom is the reference carbon atom for naming the mirror images. This is also referred to as absolute configuration.

Optical activity

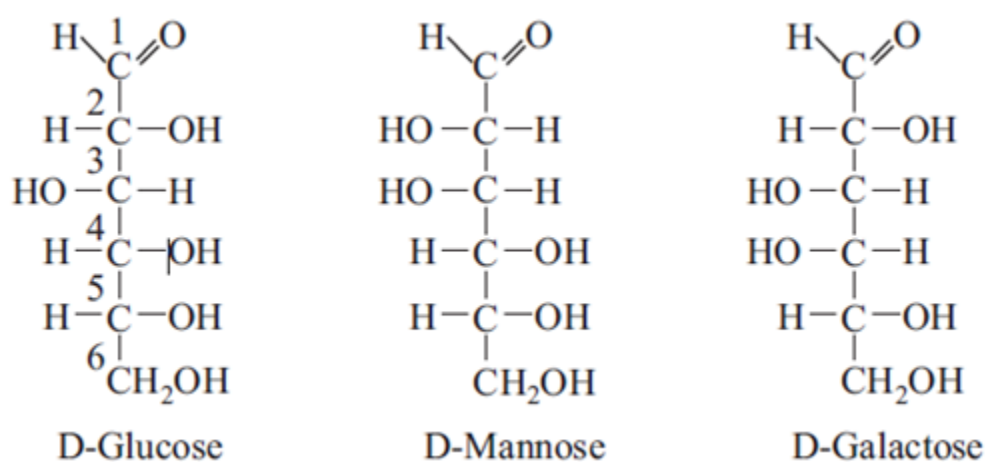
Optical activity is caused by due to the presence of asymmetrical carbon atom. When a beam of plane polarized light is passed through a solution of carbohydrates, it will rotate the light either to right or to left. Depending on the rotation, molecules are called dextrorotatory (+) (d) or levorotatory (-).

Racemic mixture

If D and L- isomers of a carbohydrate are present in unequal concentration, it is known as racemic mixture or DL mixture. The racemic mixture does not exhibit any optical activity because dextro- and levorotatory activities cancel each other.

Epimers

Epimer refers to one of a pair of stereoisomers. In this, sugars are different from one another, only in configuration with regard to the single carbon atom, other than the reference carbon atom, they are called Epimers. Example Glucose and Mannose are an epimeric pair because they differ only with respect to C2. Similarly, galactose is epimer of glucose which differs at C4.

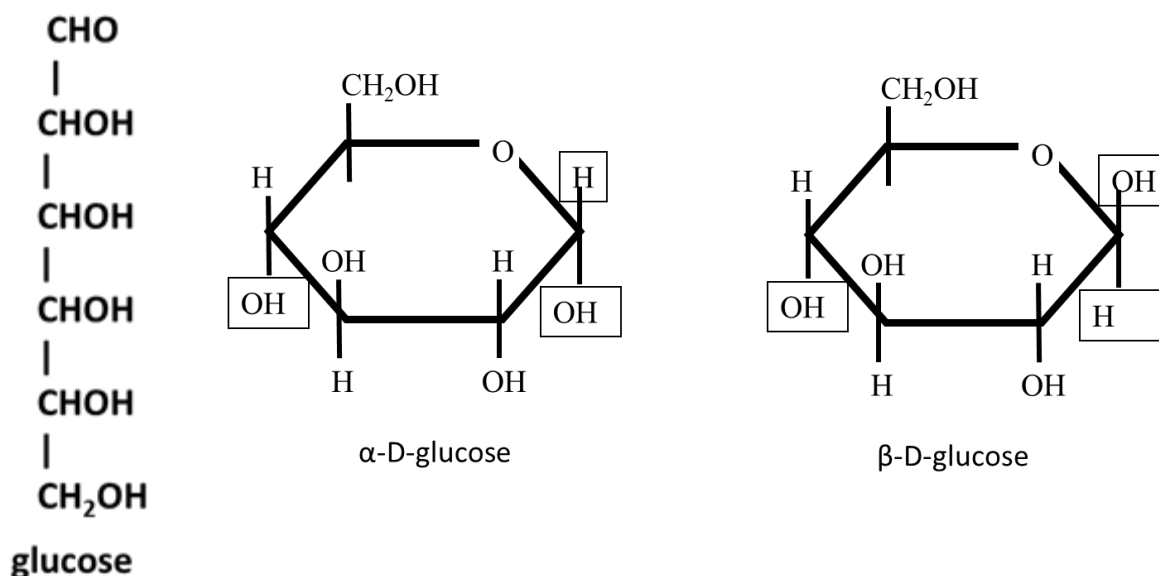


Anomers

An anomer is a type of stereoisomer and epimer found in carbohydrate chemistry. When D-glucose is crystallized at room temperature and a fresh solution is prepared, its specific rotation of polarized light is $+112^\circ$; but it changes to $+52.5^\circ$ after 12-18 hours. If initial crystallization is taking place at 98°C and then solubilized, the specific rotation is found to be $+19^\circ$, which also changes to $+52.5^\circ$ within few hours. This change in rotation with time is called

Mutarotation

D-glucose has two anomers, alpha and beta form. The alpha and beta form are produced by the spatial configuration with reference to the first carbon atom in aldoses and second carbon in ketoses. Therefore these carbon atoms are called anomeric carbon atoms. α -D-glucose has specific rotation of $+112^\circ$ and β -D-glucose has specific rotation of $+19^\circ$. Both undergo mutarotation and at equilibrium one-third molecules are alpha type and two-thirds are beta variety to get the specific rotation $+52.5^\circ$.



Disaccharides

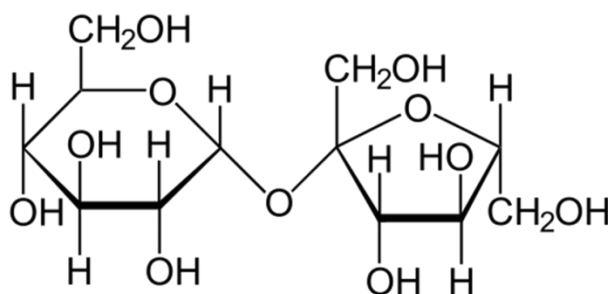
When two monosaccharides are combined together with the elimination of a water molecule it is called disaccharide. Monosaccharides are combined by a glycosidic bond. They are crystalline, water- soluble and sweet to taste. The disaccharides are two types:

1. **Reducing disaccharides** with free aldehyde or keto group e.g. maltose lactose
2. **Non –reducing disaccharides** with no free aldehyde or keto group. e.g. sucrose, trehalose

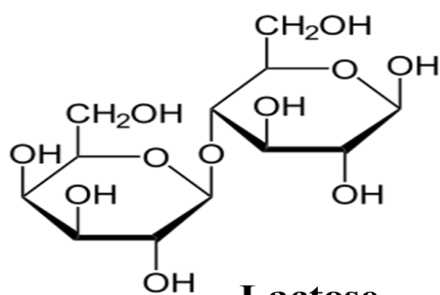
Disaccharide	Description	Component monosaccharides
Sucrose	Common table sugar	glucose α 1 $\rightarrow$ 2 fructose
Maltose	Production of starch hydrolysis	glucose α 1 $\rightarrow$ 4 glucose
Trehalose	Found in fungi	glucose α 1 $\rightarrow$ 1 glucose
Lactose	Main sugar in milk	galactose β 1 $\rightarrow$ 4 glucose
Melibiose	Found in legumes	galactose β 1 $\rightarrow$ 6 glucose

Sucrose

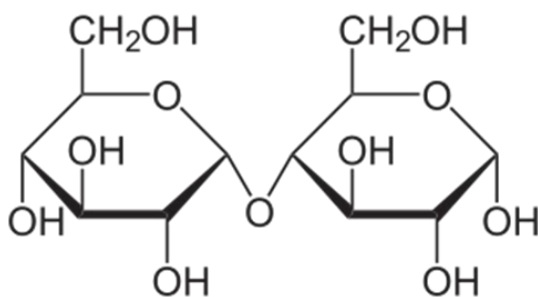
Sucrose also called saccharose, is ordinary table sugar refined from sugar cane or sugar beets. Sucrose is not a reducing sugar. This is because of the glycosidic linkage involves first carbon of glucose and second carbon of fructose, and hence there are no free reducing groups. When sucrose is hydrolyzed the resulting products have reducing property. Hydrolysis of sucrose (optical rotation $+66.5^\circ$) will produce one molecule of glucose ($+52.5^\circ$) and one molecule of fructose (92°). Therefore the products will change the dextrorotation to levorotation, or the plane of rotation is inverted. An equimolecular mixture of glucose and fructose thus formed is called invert sugar.

**Sucrose****Lactose**

Lactose is the sugar present in milk. It is reducing disaccharide.

**Lactose****Maltose**

Maltose consists of two α -D-glucose molecules and joined by $\alpha(1\rightarrow4)$ bond. It is a reducing disaccharide.

**Maltose**

Maltose is common example of oligosaccharides. It is produced when amylase breaks down starch. It is found in germinating seeds as they break down their starch stores to use for food.

Polysaccharides

Polysaccharides are polymerized products of many monosaccharide units. They may be homo or hetero polysaccharides. Many polysaccharides, unlike sugars, are insoluble in water. Dietary

fiber includes polysaccharides and oligosaccharides that are resistant to digestion and absorption in the human small intestine but which are completely or partially fermented by microorganisms in the large intestine.

Homopolysaccharides

Which on hydrolysis yield only a single type of monosaccharide. They have only one type of monosaccharide units. Thus **glucans** are polymer of glucose where as **fructosans** are polymer of fructose.

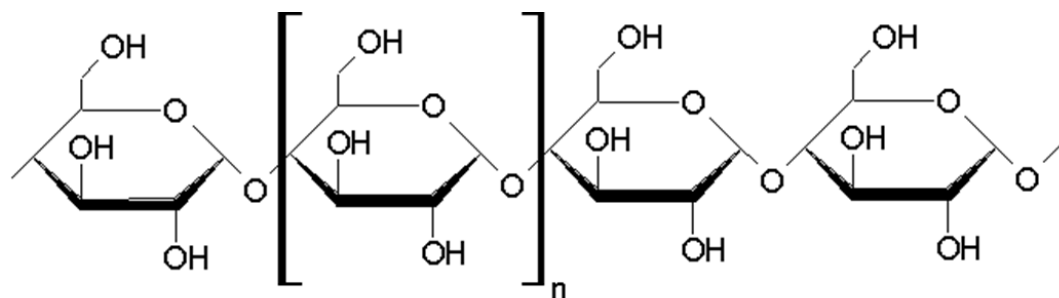
Heteropolysaccharides

They yield a mixture of a few monosaccharides or their derivatives on hydrolysis. Example- glycoproteins.

Starch

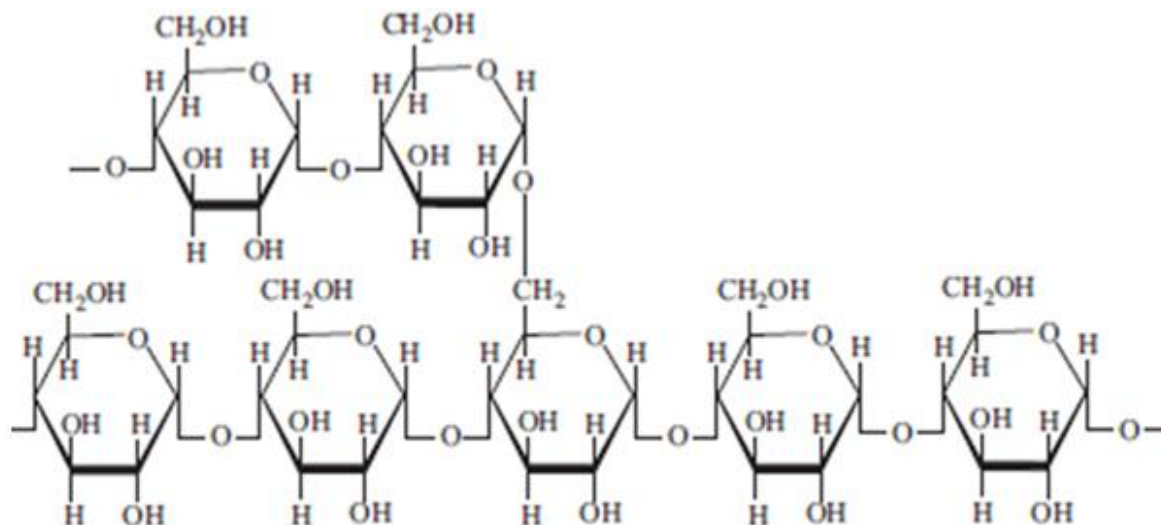
Starch or amyllum is the major form of stored carbohydrate in plants. Starch is composed of a mixture of two substances: amylose which is a linear polysaccharide, and amylopectin that is a highly branched polysaccharide. Both forms of starch are polymers of α -D glucose which is joined by 1, 4- α bonds. Natural starches contain 10-20% amylose and 80- 90% amylopectin. Amylose forms a colloidal dispersion in hot water (which helps to thicken gravies) whereas amylopectin is completely insoluble. Starch consist two polysaccharides component water soluble amylose and a water insoluble amylopectin.

Amylose molecules consist typically of 200 to 20,000 D-glucose units held by (α 1 $\rightarrow$ 4) glycosidic linkage.



Amylose

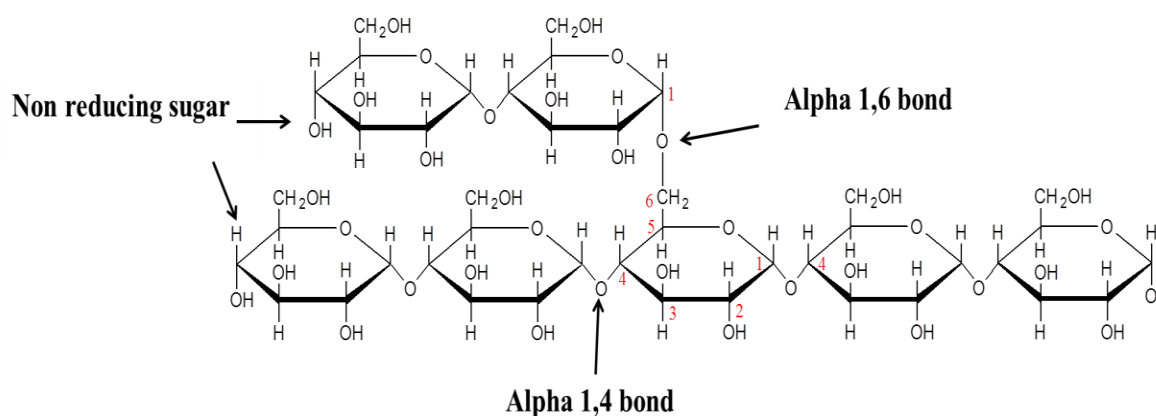
Amylopectin is a branched chain with α 1 $\rightarrow$ 6 glycosidic bond at the branching points and α 1 $\rightarrow$ 4 linkage everywhere else.



Amylopectin

Glycogen

Glucose is stored as glycogen in animal tissues by the process of glycogenesis. The excess glucose which cannot be stored as glycogen or used immediately for energy is converted to fat. Glycogen is a polymer of α D-Glucose which is identical to amylopectin, but the branches in glycogen are shorter (about 13 glucose units) and more common. The glucose chains are organized globularly and look like branches of a tree originating from a pair of molecules of glycogenin which an enzyme and convert glucose to glycogen. Glycogen is easily converted back to glucose to give energy.

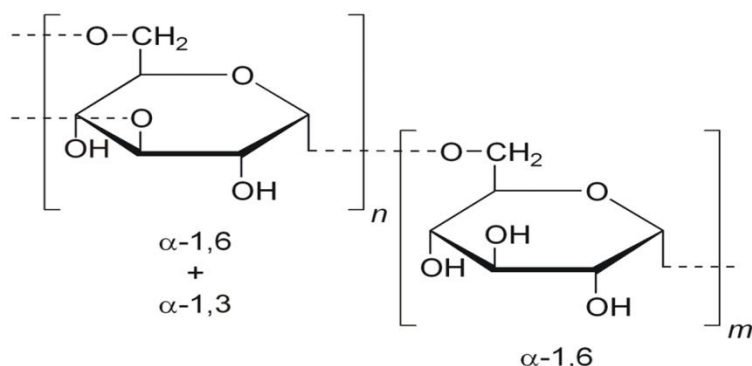


Glycogen

Dextran

Dextran is a polysaccharide similar to amylopectin, but the main chains are formed by α -1,6 glycosidic linkages and the side branches are attached by α -1,3 linkages. Dextran found in

oral bacterial help to adhere to the teeth, creating a film called plaque. It is also used in confections, in lacquers, as food additives, and as plasma volume expanders.

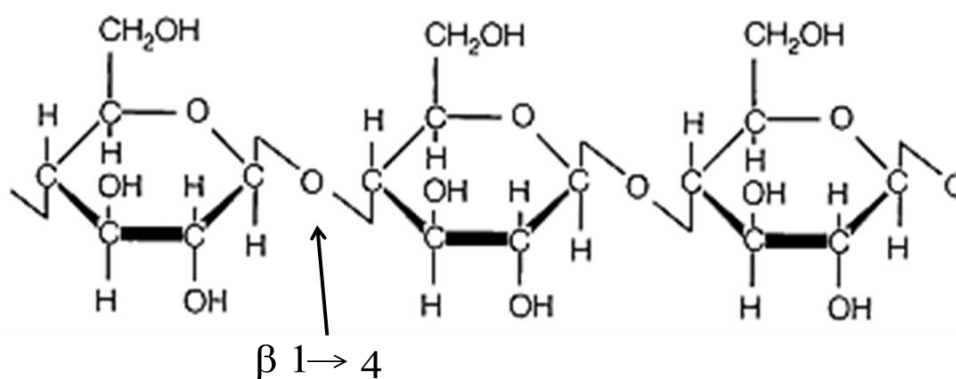


Dextran unit

Cellulose

Cellulose is a polymer of β -D-Glucose joined by β 1, 4 bond. It in contrast to starch is oriented with $-\text{CH}_2\text{OH}$ groups alternating above and below the plane of the cellulose molecule thus producing long, unbranched chains. $\text{C}_6\text{H}_{10}\text{O}_5$ is the general formula of cellulose. The absence of side chains allows cellulose molecules to lie close together and form rigid structures.

It is the major structural material of plants. The cotton is almost pure cellulose. Cellulose can be hydrolyzed to its constituent glucose units by microorganisms that are found in the digestive tract of termites and ruminants. Cellulose could be may be modified by nitric acid (HNO_3) to replace all the hydroxyl groups with nitrate groups ($-\text{ONO}_2$) resulting cellulose nitrate (nitrocellulose or guncotton) which is an explosive. Pyroxylin is partially nitrated cellulose that is used in the manufacture of collodion, plastics, lacquers, and nail polish.

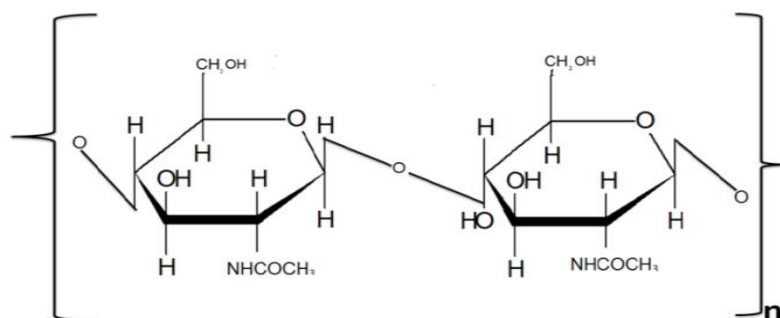


Cellulose

Chitin

Chitin is an unbranched polymer of N-Acetyl-D-glucosamine. It is found in fungi and is the principal component of arthropod and lower animal exoskeletons, e.g., insect, crab, and shrimp

shells. It may be regarded as a derivative of cellulose, in which the hydroxyl groups of the second carbon of each glucose unit have been replaced with acetamido ($-\text{NH}(\text{C}=\text{O}) \text{CH}_3$) groups.



Chitin

Heteropolysaccharides

Heteropolysaccharides contain two or more different kind of monosaccharides. Generally, they provide extracellular support for organisms of all kingdoms. They give protection, specific shape, and support to cells, tissues and organs. Together with fibrous proteins, like collagen, elastin, fibronectin, laminin and others, heteropolysaccharides are the most important components of the extracellular matrix. Example; Hyaluronic acid, chondroitin sulfates and dermatansulfates. They are found in the extracellular matrix. The heteropolysaccharides usually are made up by the repetition of a disaccharide unit of an aminosugar and an acid sugar.

Heteropolysaccharides combine with proteins to form proteoglycans, glycosaminoglycans or mucopolysaccharides. Such heteropolysaccharides are found abundantly in mucous secretions. They are involved in diverse functions: structural, water metabolism regulation cellular cement, biological sieve, biological lubricant, docking sites for growth factors, among other functions.

Hyaluronic Acid (Hyaluronate): It acts as lubricant in the synovial fluid of joints, provides consistency to vitreous humor, contributes to tensile strength and elasticity of cartilages and tendons.

Chondroitin Sulfates: It contributes to tensile strength and elasticity of cartilages, tendons, ligaments and walls of the aorta.

Dermatan sulfate: It is found mainly in skin, but also is present in lungs, vessels, and heart. It is considered being involved in coagulation and vascular diseases and other conditions.

Keratan sulfate: Present in the cornea, cartilage bone and a variety of other structures as nails and hair.

10.3.2-Properties

Physical Properties of Monosaccharides

Most monosaccharides have a sweet taste (fructose is sweetest; 73% sweeter than sucrose).

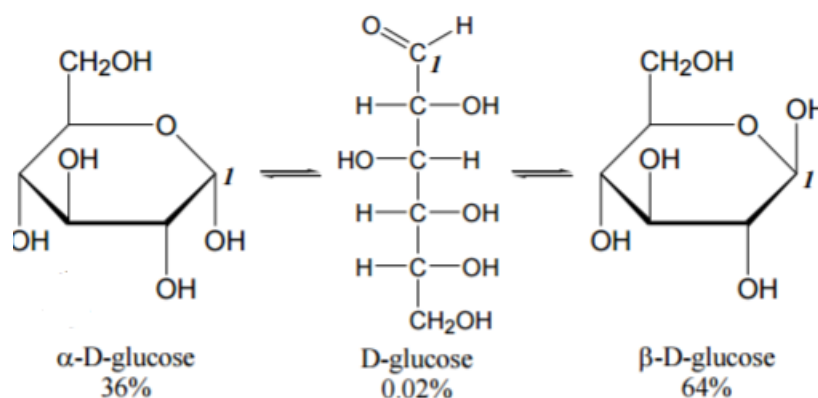
They are solids at room temperature.

They are extremely soluble in water: – Despite their high molecular weights, the presence of large numbers of OH groups make the monosaccharides much more water soluble than most molecules of similar MW.

Glucose can dissolve in minute amounts of water to make syrup (1 g /1 ml H₂O).

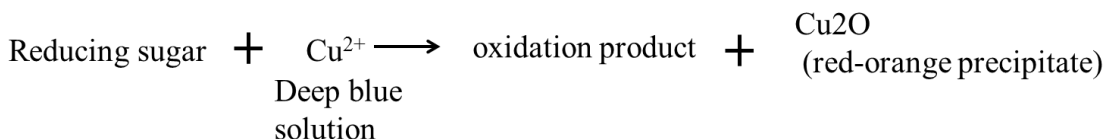
Chemical Properties of Monosaccharides

Monosaccharides do not usually exist in solution in their “open-chain” forms: an alcohol group can add into the carbonyl group in the same molecule to form a pyranose ring containing a stable cyclic hemiacetal or hemiketal.



Oxidation of Monosaccharides

Aldehydes and ketones that have an OH group on the carbon next to the carbonyl group react with a basic solution of Cu^{2+} (Benedict's reagent) to form a red-orange precipitate of copper(I) oxide (Cu_2O). • Sugars that undergo this reaction are called reducing sugars. (All of the monosaccharides are reducing sugars.)



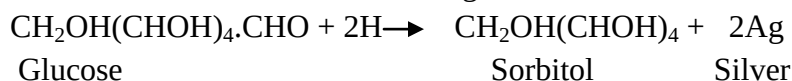
Chemical properties of Carbohydrates

Monosaccharides have carbonyl ($>\text{C}=\text{O}$) group and hydroxyl ($-\text{OH}$) group.

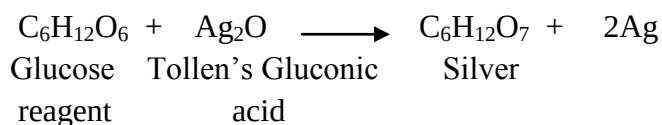
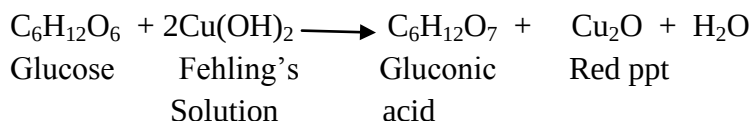
The properties are as follows:-

1) Reduction

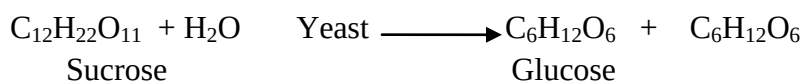
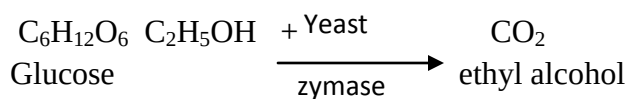
They are reduced with sodium amalgam and water.



2) Oxidation



3) Fermentation- Monosaccharides form ethyl alcohol on fermentation.



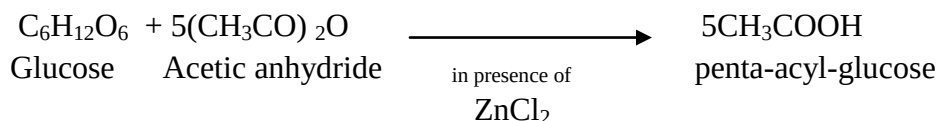
4) Optical activity

Usually, the soluble carbohydrate shows optical activity in solution. Optical activity of carbohydrates refers to its property of rotating the plane of polarized light. The expression of rotating power of solution is explained in terms of the number of degree of angular rotation of the plane of polarized light.

Dextrorotatory compounds are such compounds can rotate in the plane of polarized light to the right or clockwise direction while **Levorotatory** compound can rotate left in anticlockwise direction. The optical activity of fresh glucose solution is $+113^{\circ}$ but this value gradually falls to $+50^{\circ}$. This change of rotation is known as **mutarotation**. **This occurs** due to the establishment of an equilibrium between two anomers (α and β).

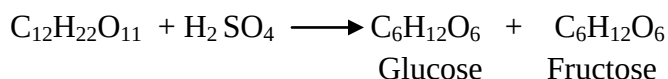
5) Acetylation

Monosaccharide when treated with acetic anhydride forms penta acyl derivatives.

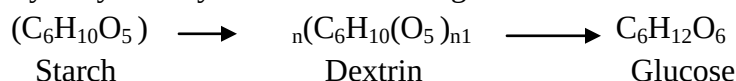


6) Hydrolysis

1. The hydrolysis oligosaccharides forms glucose and fructose.



2. The hydrolysis Polysaccharides forms glucose and



10.3.3 Biological role of Carbohydrates

1. Carbohydrate like glucose is source of energy for the body. Glucose is a simple sugar and found in many basic foods.
2. Carbohydrate are starting material in biosynthesis
3. Carbohydrates may be soluble and insoluble. The insoluble carbohydrate which is like fiber promotes regular bowel movement, regulates the rate of consumption of blood glucose, and also helps to remove excess cholesterol from the body.
4. To provide immediate source of energy, glucose is broken down during the process of cellular respiration, which produce ATP, the energy currency of the cell.
5. Carbohydrates are an important part of the human nutrition.
6. Since carbohydrates are main source of energy, they are essential to all animal life.
7. Carbohydrates are immediate source of energy while lipids are long-term source of energy. Carbohydrates work as energy stores of animals and plants.
8. Carbohydrates are instant source of energy while lipids are long-term source of energy.
9. Glucose is a free sugar and circulates in blood. Therefore it is an important substance for normal cell functioning.
10. Regulation of glucose metabolism is l for survival. Usually carbohydrates content of most of the plant is about 60-80% of its dry mass.
11. In plants they are used for storage of energy in the form of starch.
12. Cellulose is a polysaccharide which is an important structural component in the cell wall of plants.
13. Sucrose, a disaccharide is formed by photosynthesis and is transported internally.
14. Carbohydrates are an important component of diet in animals.
15. Carbohydrates act as fuel to physical body parts on daily basis.
16. They help in breakdown of fatty acids and prevent ketosis.
17. They minimize the use of proteins for energy.
18. They also act as flavor and sweeteners.

10.4 LIPIDS

The word lipid is derived from a greek word “lipos” which means FAT. Lipids are biological molecules and commonly known as fats. They are largely hydrocarbon in composition. They represent highly reduced forms of carbon. Lipids can be defined as either the esters of fatty acids and glycerol, or as fatty acid triglycerides. They yield large amounts of energy upon oxidation in metabolism in the cells. They act as building blocks of the structure and function of living cells.

Examples of lipids include fats, oils, waxes, certain vitamins, hormones and most of the non-protein membrane of cells

Lipids are non-polar and are thus soluble in nonpolar environments like in chloroform but not soluble in polar environments like water.

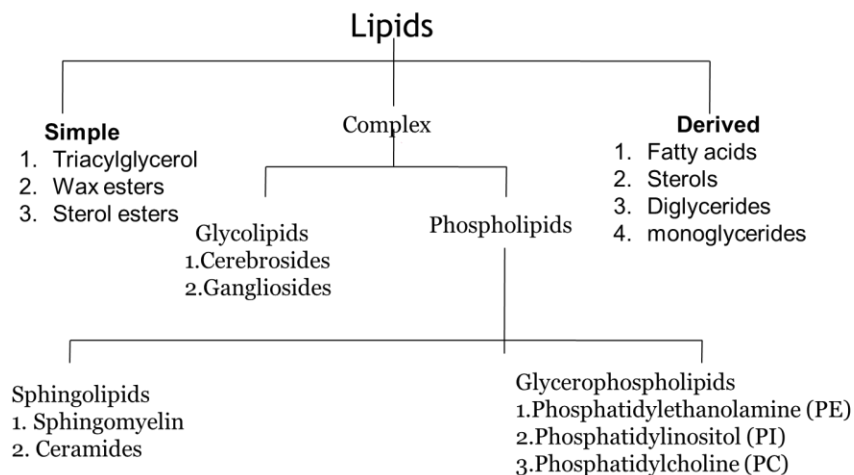
Lipids can be extracted from plants and animals using nonpolar solvents such as ether, chloroform and acetone. Fats (and the fatty acids from which they are made) belong to this group as do other steroids, phospholipids forming cell membrane components etc.

Lipids that contain a functional group ester are hydrolysable in water. These include neutral fats, waxes, phospholipids, and glycolipids. Nonhydrolyzable lipids lack such functional groups and include steroids and fat-soluble vitamins (e.g. A, D, E, and K).

Fats and oils are composed of triacylglycerols or triglycerides. These are composed of glycerol (1,2,3-trihydroxypropane) and 3 fatty acids to form a triester. Triglycerides are found in blood tests. Complete hydrolysis of triacylglycerols yields three fatty acids and a glycerol molecule.

10.4.1 Classification

Lipids can be classified in following major groups based on structure



A-Simple lipids

They are esters of fatty acids with various alcohols. Simple lipids belong to a heterogeneous class of nonpolar compounds, mostly insoluble in water. They are but soluble in nonpolar organic solvents such as chloroform and benzene.

They include:

1-Fat and oils: They are esters of glycerol and fatty acids. Fatty acids may be saturate or unsaturated. They may be same or mixed. Fats are also known as triglycerides because all the three hydroxyl groups of glycerol are esterified. Oils are fats in the liquid state.

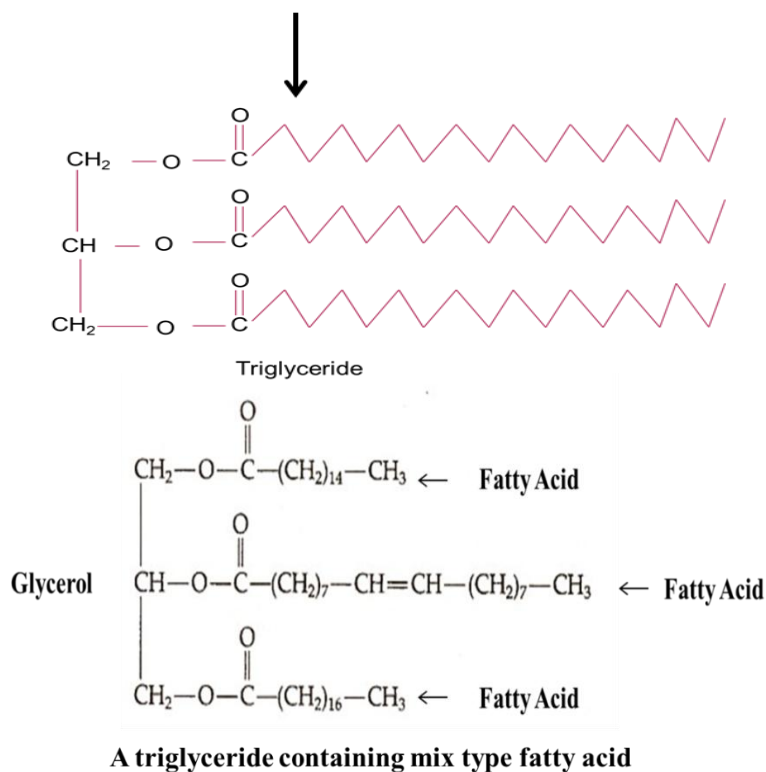
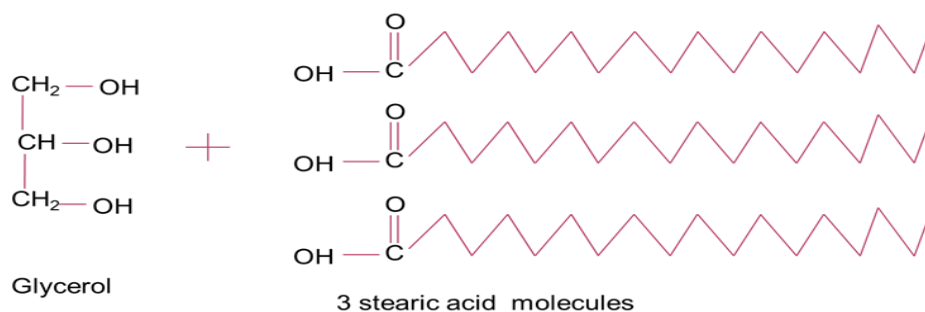


Table-1: Some saturated and unsaturated fatty acids, formula and melting point

Saturated Fatty Acids	Formula	Melting Point (°C)
Butyric	C ₄ H ₈ O ₂	Liquid
Palmitic	C ₁₆ H ₃₂ O ₂	63
Stearic	C ₁₈ H ₃₆ O ₂	70
Unsaturated Fatty Acids	Formula	Melting Point (°C)
Oleic	C ₁₈ H ₃₄ O ₂	Liquid
Linoleic	C ₁₈ H ₃₂ O ₂	Liquid
Linolenic	C ₁₈ H ₃₀ O ₂	Liquid

2-Waxes-They are esters of long-chain alcohols and fatty acids. The alcohol may contain from 12-32 carbon atoms. Many of the waxes are used in ointments, hand creams, and cosmetics.

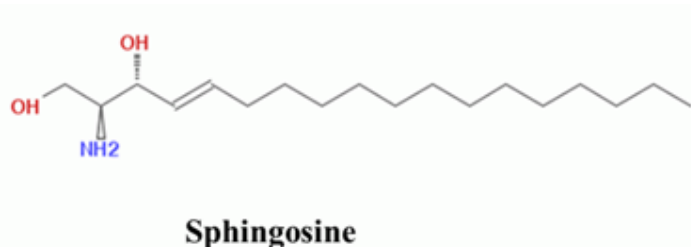
Table-2: The waxes with their component alcohols and fatty acids are listed in the table-2 on the left.

Wax		Alcohol	Fatty Acid
Carnuba	found on the leaves of Brazilian palm trees	$\text{CH}_3(\text{CH}_2)_{28}\text{CH}_2\text{-OH}$	$\text{CH}_3(\text{CH}_2)_{24}\text{COOH}$
Beeswax	secreted by bees to make cells for honey and eggs	$\text{CH}_3(\text{CH}_2)_{28}\text{CH}_2\text{-OH}$	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$
Spermacetic	found in the head cavities and blubber of the sperm whale	$\text{CH}_3(\text{CH}_2)_{14}\text{CH}_2\text{-OH}$	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$

Paraffin wax is used for making some candles. Ear wax is a mixture of phospholipids and esters of cholesterol.

The waxes with their component alcohols and fatty acids are listed in the table on the left.

3-Ceramides-They are amides of fatty acids with long-chain di- or trihydroxy bases containing 12–22 carbon atoms in the carbon chain: e.g. sphingosine.



4- Cholesteryl esters. They are dietary lipids and are esters of cholesterol and fatty acids. Cholesteryl esters have a lower solubility in water due to their increased hydrophobicity. They are hydrolyzed by pancreatic enzymes, cholesterol esterase resulting free fatty acids and cholesterol. They have been found associated with atherosclerosis. Example- Cholesteryloleate

B-Complex lipids

Lipids which contain parts other than or including fatty acids and glycerol are called complex lipids. They are found in most cell membranes, in blood platelets and especially brain tissue.

Example-

1. Phosphatidic acid that is diacylglycerol esterified to phosphoric acid.
2. Phosphatidylcholine that is phosphatidic acid linked to choline, also called lecithin.
3. Phospholipids that is glycerol esters of fatty acids.
4. Phosphoric acid and other groups containing nitrogen.

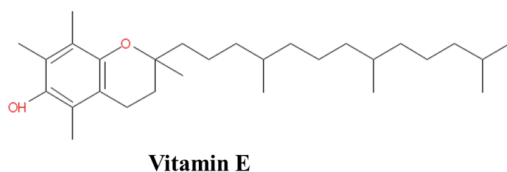
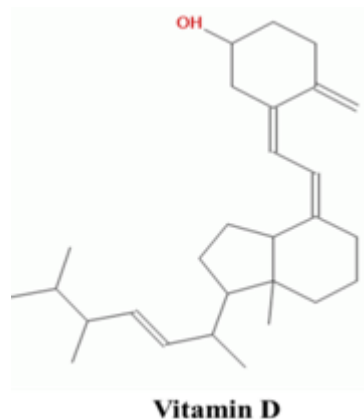
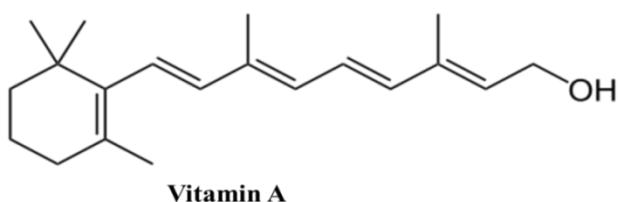
5. Phosphatidylethanolamine.
6. phosphatidylserine;
7. phosphatidylinositol;
8. phosphatidylacylglycerol in which more than one glycerol molecule is esterified to phosphoric acid: e.g. cardiolipin and diphosphatidylacylglycerol;
9. gangliosides that are glycolipids that are structurally similar to ceramide polyhexoside and also contain 1-3 sialic acid residues; most contain an amino sugar in addition to the other sugars;
10. sphingolipids, derivatives of ceramides;
11. sphingomyelin that is ceramide phosphorylcholine;
12. cerebroside: they are ceramide monohexoside that is ceramide linked to a single sugar moiety at the terminal hydroxyl group of the base);
13. ceramide di- and polyhexoside that is linked respectively to a disaccharide or a tri- or oligosaccharide;
14. Cerebroside sulfate that is ceramide monohexoside esterified to a sulfate group.

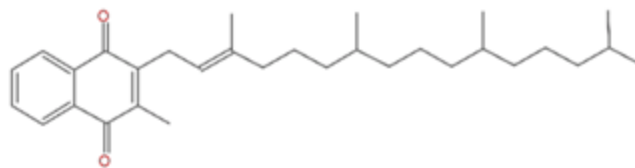
C- Derived lipids

They are produced from simple and compound lipids through the process of hydrolysis.

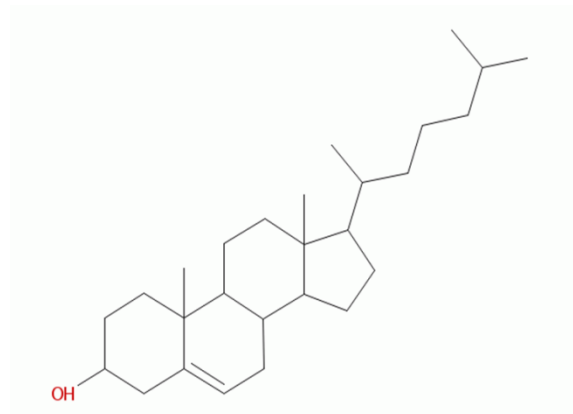
They include:

1. Fatty acids and alcohols
2. Fat soluble vitamins A, D, E and K;
3. Sterols, e.g., Cholesterol.



**Vitamin K**

Cholesterol is the precursor to our sex hormones and Vitamin D. Vitamin D is formed by the action of UV light in sunlight on cholesterol molecules that have "risen" to near the surface of the skin. Our cell membranes contain a lot of cholesterol, in between the phospholipids, to help keep them fluid. Excess cholesterol levels can exceed the saturation level in bile, causing gallstones to form. Gallstones are almost all cholesterol with a small amount of minerals, like calcium. Maximum cholesterol levels in the blood would be 220 mg/dl of blood plasma.

**Cholesterol**

10.4.2 Properties of Lipids

Physical Properties of Fatty Acids

1. The physical properties of fatty acids are largely determined by the length and degree of unsaturation of the hydrocarbon chain.
2. The longer the chain and the fewer the double bonds, the lower is the solubility in water, and higher is the melting point.
3. Addition of double bonds decreases the melting point whereas, increasing the chain length increases the melting point. For example; 4:0 MP -7.9 C, 12:0 MP 44.2 C, 16:0 MP 62.7 C, 18:1 MP 10.5 C, 18:2 MP -5.0 C, 18:3 MP -11 C.
4. Trivial names of fatty acids refer to the natural sources of derivation: eg
5. Lauric (12:0) isolated from seed fat of Lauraceae
6. Myristic (14:0) –seed fat Myristaceae
7. Palmitic (16:0) –seed fat of palmae
8. Oleic (18:1) –seed fat of olive oil.

Chemical Properties of Lipids

1. Acid number: The acid number is defined as the mg of KOH necessary to neutralize the free fatty acids present in 1g of fat or oil. The acid number tells the quantity of free fatty acid present in a fat. A high acid value indicates a state oil or fat stored under improper conditions. The acid value is important because it measures hydrolytic rancidity. $\text{FFA} = \text{ml alkali} \times \text{N of alkali} \times 28.2$ mg/sample weight

2. Saponification: Saponification is the process of breaking down or degrading a neutral fat into glycerol and fatty acids by treatment of with alkali. Saponification number is defined as the mg of KOH required to saponify 1g of fat. $\text{Saponification number} = (\text{S}-\text{B}) \times \text{N} \times 56.1 / \text{sample weight}$.

3. Iodine Number: It is a measure of the degree of unsaturation, the number of carbon-carbon double bonds in relation to the amount of fat or oil. It is defined as the g iodine absorbed per 100g of the sample. $\text{Iodine number} = (\text{B}-\text{S}) \times \text{N} \times 12.69 / \text{sample weight}$

4. Peroxide value: Peroxide value is the measures the degree of lipid oxidation in fats and oils. It is used to check rancidity in unsaturated fats and oils. Peroxide value is the number of milliequivalents of peroxide per kg fat. It is a measure of the formation of peroxide or hydroxide groups that are initial products of the lipid oxidation. $\text{Peroxide value} = (\text{S}-\text{B}) \times \text{N} \times 1000 / \text{sample weight}$.

5. Riechert Messel Number: Riechert Messel Number is a measure the amount of H₂O soluble volatile fatty acids. It is defined as the number of milliliters of 0.1 N alkali necessary to neutralize the volatile H₂O soluble fatty acids in a 5g sample of fat.

6. Polenske number: Polenske number refers the required the amount of volatile insoluble fatty acids. It can be defined as the no. of millilitres of 0.1N alkali necessary to neutralize the volatile H₂O insoluble fatty acids which are present in the 5g sample.

7. Hydrolysis: When fats react with water, it results in the splitting of some of the fatty acids from the oil or fat, yielding some free fatty acids, monoglycerides and diglycerides. It is accelerated by high temperatures & pressures & an excessive amount of water.

8. Hydrogenation: Hydrogenation is used to make hydrogenated fats are unnatural fats that are detrimental to health. Hydrogenation (or, more accurately, "partial hydrogenation") is the forced chemical addition of hydrogen into omega-6 polyunsaturated oils to make them hard at room temperatures, The rate of hydrogenation depends on nature of the substance to be hydrogenated, type and concentration of the catalyst, concentration of hydrogen, temperature, pressure etc.

9. Isomerization: Isomerization is the process by which one molecule is transformed into another molecule that has exactly the same atoms, but different arrangement. The two important types of isomerism are Geometric & Positional isomerism. Geometrical isomerism: -

(i) In geometrical Isomerism a double bond can have two configurations; - Cis or Trans. When the H₂ atoms are on the same side of the carbon chain, the arrangement is called Cis. H₂ atoms are on opposite sides of the carbon chain, the arrangement is called trans. Natural fats & oils contain cis form.

(ii) Positional Isomerism: In positional Isomerism unsaturated fatty acid can be isomerised in acid or alkaline conditions or by high temperatures where the double bond move from one

position to another. Hydrogenation process can cause shifts double bonds in the fatty acid chains causing cis - trans isomerisation. Cis isomers found in food fats & oils & trans isomers occur in fats from remnants. Most Trans isomers result from the partial hydrogenation of fats & oils.

10. Esterification: Esterification is the reverse of hydrolysis which combines free fatty acids with glycerol to form triglycerides. Mono & diglycerides are produced by esterification. Monoglycerides are important emulsifying agents in food products. Emulsifying agents are called emulsifiers which are made either by alcoholysis or by direct esterification. In direct esterification, fatty acids & polyalcohol are reacted together under controlled conditions to form esters.

11. Interesterification: It takes place when the fatty acids have been moved from one triglyceride molecule to another. This process is also referred to as Randomization, rearrangement or modification. It is used for processing edible fats & oils to produce confectionery or coating fats, margarine oils, cooking fats, frying fats, shortenings & other special application products.

12. Oxidation: The oxidation reaction of an oil or fat occurs with O₂ in the air, and with the food at the double bonds or points of unsaturation. This reaction affects the flavour of the fat. The rate of oxidation increases with increase in temperature, exposure to O₂, presence of light & pro-oxidants like Metal Cu. Oxidation which takes place by air at room temperature is referred to as Autoxidation.

13. Polymerization: Excessive oxidation of fatty acid results Polymerization. The deep frying of foods at temperatures ranging from 325o F -375o F lead to polymerization due to heat stress, oxidation & presence of the radical & polar catalyst. The rate of polymerization increases with the amount of unsaturation & viscosity of fat or oil.

14. Halogenation: The halogens include Chlorine, Bromine & Iodine can readily add to the double bonds of unsaturated fatty acids. In halogenation, measured quantities of iodine are added to measure quantities of fats or oils to determine the average degree of unsaturation of fat or oil. This results in iodine number, an important analytical measurement.

10.4.3-Biological role of lipids:

Lipids perform several biological functions:

1. Lipids are storage compounds. The triglycerides serve as reserve energy of the body.
2. Lipids are important component of cell membranes structure in eukaryotic cells.
3. Lipids regulate membrane permeability.
4. They serve as source for fat soluble vitamins like A, D, E, K.
5. They act electrical insulators to the nerve fibers, where the myelin sheath contains lipids.
6. Lipids are components of some enzyme systems.
7. Some lipids like prostaglandins and steroid hormones act as cellular metabolic regulators.
8. Cholesterol is found in cell membranes, blood, and bile of many organisms.
9. As lipids are small molecules and are insoluble in water, they act as signaling molecules.

10. Layers of fat in the subcutaneous layer, provides insulation and protection from cold. Body temperature maintenance is done by brown fat.
11. Polyunsaturated phospholipids are important constituents of phospholipids, they provide fluidity and flexibility to the cell membranes.
12. Lipoproteins that are complexes of lipids and proteins, occur in blood as plasma lipoprotein, they enable transport of lipids in aqueous environment, and their transport throughout the body.
13. Cholesterol maintains fluidity of membranes by interacting with lipid complexes.
14. Cholesterol is the precursor of bile acids, Vitamin D and steroids.
15. Essential fatty acids like linoleic and linolenic acids are precursors of many different types of eicosanoids including prostaglandins, thromboxanes. These play an important role in pain, fever, inflammation and blood clotting.

10.5 SUMMARY

Carbohydrates are complex biochemical structures and have vital functions in the human body. They are the main energy source for the human body. There are two major types of carbohydrates in foods: 1-simple and 2-complex. Simple carbohydrates are found in refined sugars, white sugar. 2-Complex carbohydrates: In complex carbohydrates, more than one sugar make link together to form carbohydrates of different types. Complex carbohydrates consist of thousands of repeating sugar units. Example; starch and glycogen. In the body, carbohydrates are broken down into simple sugars and absorbed into the bloodstream. Carbohydrates come from a wide array of foods - bread, beans, milk, popcorn, potatoes, cookies, spaghetti, corn, and cherry pie.

Lipids-The lipids are a large and diverse group of naturally occurring organic compounds. They show differential solubility in nonpolar organic solvents (e.g. ether, chloroform, acetone & benzene) and are generally insoluble in water. Fatty acids are the hydrocarbon chain with one carboxylic acid (-COOH) group. Fatty acids are main components of many lipids, constitutes an even number of carbon atoms (generally 12 to 24). Lipids include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and others. Lipids that contain a functional group ester are hydrolysable in water. These include neutral fats, waxes, phospholipids, and glycolipids. Nonhydrolyzable lipids include steroids and fat-soluble vitamins (e.g. A, D, E, and K). Fats and oils are made of triacylglycerols or triglycerides which are composed of glycerol (1, 2, 3-trihydroxypropane) and 3 fatty acids to form a triester. Complete hydrolysis of triacylglycerols yields three fatty acids and a glycerol molecule. The plasma membrane is made up of proteins and lipids. The main biological functions of lipids include storing energy, functioning as structural components of cell membranes and acting as signaling molecules.

10.6 GLOSSARY

Acid number- acid value / "neutralization number"/ or "acid number" or "acidity" is the mass of potassium hydroxide (KOH) in milligrams that is required to neutralize one gram of chemical substance like a fatty acid, or in a mixture of compounds.

Aldoses- Monosaccharides with one aldehyde ($-\text{CH}=\text{O}$) group per molecule. Example- glyceraldehyde, erythrose, ribose, arabinose, xylose, glucose, mannose, glucose, etc.

Amylopectin- A water-soluble polysaccharide, highly branched polymer of glucose found in plants.

Anomers - A type of stereoisomer and epimer found in carbohydrate chemistry. Example α -D glucose and β -D glucose.

Cellulose- Cellulose is polysaccharide with the formula $(\text{C}_6\text{H}_{10}\text{O}_5)_n$ that contains a linear chain of several hundred to many thousands of $\beta(1\rightarrow4)$ linked D-glucose units.

Ceramides- It is a family of waxy lipid molecules. A ceramide is composed of sphingosine and a fatty acid.

Cholesterol- A type of fatty acid that is vital for the normal functioning of the body.

Chitin - Chitin $(\text{C}_8\text{H}_{13}\text{O}_5\text{N})_n$ is a long-chain polymer of an N-acetylglucosamine. It is a derivative of glucose, and is found in many organisms. It is a characteristic component of the cell walls of fungi.

Disaccharides- It is made up of two sugar molecules, examples are sucrose, lactose and maltose.

Enantiomers- also known as optical isomers, are two stereoisomers that are related to each other by a reflection. They rotate polarized light. Example D- glucose and L-Glucose

Glycogen- A multi-branched polysaccharide of glucose that serves as a form of energy storage in humans, animals, and fungi.

Haworth projection- A Haworth projection is a common way of writing a structural formula to represent the cyclic structure of monosaccharides with a simple three-dimensional perspective.

Hormones- A chemical substance produced in the body that controls and regulates the activity of certain cells or organs. eg insulin.

Iodine Number- The iodine value (or "iodine adsorption value" or "iodine number" or "iodine index") is the mass of iodine in grams that is consumed by 100 grams of a chemical substance. Iodine numbers are often used to determine the amount of unsaturation in fatty acids.

Mutarotation- Mutarotation is the change in the optical rotation because of the change in the equilibrium between two anomers; α and β anomeric forms.

Starch or amylum is a polymeric carbohydrate consisting of a large number of glucose units joined by glycosidic bonds.

Saponification value- Saponification value /saponification number/Koettstorfer number represents the number of milligrams of potassium hydroxide required to saponify 1g of fat under the conditions specified.

Vitamins -Vitamins are biomolecules, required to grow and develop by our body. There are 13 vitamins your body needs. Examples; vitamin A,B,C,D,K,B-6, B-12 etc.

10.7 SELF-ASSESSMENT QUESTION

10.7.1 Objective type Questions:

- The chemical formula of carbohydrate is
(a) $(\text{CH}_2\text{O})_n$ (b) $(\text{CH}_2\text{O})_{2n}$
(c) $(\text{CHO})_n$ (d) $\text{C}_n\text{H}_{2n}\text{O}$
- Which of the following is an aldotriose?
(a) Dihydroxyacetone (b) Glyceraldehyde
(c) Ribulose (d) Erythrose
- The molecular formula of sucrose is-
(a) $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ (b) $\text{C}_{10}\text{H}_{20}\text{O}_{10}$
(c) $\text{C}_6\text{H}_{12}\text{O}_6$ (d) $\text{C}_{12}\text{H}_{20}\text{O}_{11}$
- The correct glycosidic linkage between glucose molecule in maltose is
(a) β 1 – 4 (b) α 1 – 2
(c) α 1 – 4 (d) β 1 – 2
- The number of stereoisomers of a keto pentose is -
(a) 4 (b) 6
(c) 8 (d) 10
- The reserve food material of green algae is
(a) Laminarin (b) Chrysolaminarin
(c) Floridian starch (d) Starch
- The only carbohydrate which is not having any chiral carbon atom is
(a) Glyceraldehyde (b) Erythrose
(c) Dihydroxyacetone (d) Erythrulose
- Choose the odd carbohydrate from the following.
(a) Arabinose (b) Xylose
(c) Lyxose (d) Erythrose
- A pentose sugar reported to be present in heart cells
(a) Xylose (b) Arabinose
(c) Lyxose (d) Xylulose
- Which of the following is an epimeric pair?

- (a) D-glucose and D-mannose
(c) D-glucose and L-glucose
- (b) D-glucose and D-galactose
(d) Both A and B

11. Fats are abundantly present in

- (a) Reproductive tissue
(c) Both a and b
- (b) Vegetative tissue
(d) None of these

12. Lipids are readily soluble in

- (a) Oil
(c) Water
- (b) Mercury
(d) None of these

13. Select unsaturated fatty acids.

- (a) Linoleic acid
(c) Palmitoleic acid
- (b) Oleic acid
(d) All of these

14. Liquid form of triglycerides at ordinary room temperature are called

- (a) Oils
(c) Fats
- (b) Solid
(d) None of these

15. The synthesis of glucose from fat is called

- (a) Glycolysis
(c) Saponification
- (b) Krebs cycle
(d) Gluconeogenesis

16. Hydrolysis of fats by alkalies into fatty acids and glycerol is called

- (a) Coagulation
(c) Suspension
- (b) Saponification
(d) Colloidal

17. The fats and oils are respectively rich in

- (a) Unsaturated fatty acids
(c) Saturated and unsaturated fatty acids
- (b) Saturated fatty acids
(d) None of these

18. β -oxidation takes place in

- (a) Mitochondria
(c) Chloroplasts
- (b) Cytoplasm
(d) Nucleus

19. Which is a phospholipid

- (a) Lecithin
(c) Sterol
- (b) Cholesterol
(d) Steroid

20. The number of double bonds in Arachidonic acid

- (a) 1 (b) 2
(c) 3 (d) 4

10.7.1 Answers Key: 1-(a), 2-(b), 3-(a), 4-(c), 5-(a), 6-(d), 7-(c), 8-(d), 9-(c), 10-(d), 11-(a), 12-(d), 13-(d), 14-(a), 15-(d), 16-(b), 17-(c), 18-(a), 19-(a), 20-(d)

10.8 REFERENCES

- <https://en.wikipedia.org/wiki/Anomer>
- <https://en.wikipedia.org/wiki/Cellulose>
- Cellulose.(2008). In *Encyclopædia Britannica*. Retrieved January 11, 2008, from Encyclopædia Britannica Online.
- <https://en.wikipedia.org/wiki/Chitin>
- Tang, WJ; Fernandez, JG; Sohn, JJ; Amemiya, CT. "Chitin is endogenously produced in vertebrates". *Curr Biol.* 25: 897–900. doi:10.1016/j.cub.2015.01.058. PMC 4382437. PMID 25772447.
- <https://en.wikipedia.org/wiki/Glycogen>
- https://en.wikipedia.org/wiki/Haworth_projection
- <https://en.wikipedia.org/wiki/Mutarotation>
- <https://en.wikipedia.org/wiki/Starch>
- <https://en.wikipedia.org/wiki/Stereoisomerism>
- https://en.wikipedia.org/wiki/Acid_value
- https://en.wikipedia.org/wiki/Iodine_value
- <http://www.edurite.com/kbase/biological-importance-of-carbohydrates>

10.9 SUGGESTED READINGS

- Lehninger Principles of Biochemistry 5th Edition by David L. Nelson (Author), Michael M. Cox (Author)
- Biochemistry by Berg JM, Tymoczko JL, and Stryer L, published by W.H. Freeman and Company.
- Biochemistry, 4th Edition by Donald Voet, Judith G. Voet
- Biochemistry By J L Jain
- Fundamentals of biochemistry by satyanarayana
- Bios Instant Notes in Biochemistry by David Hames, Nige
- Instant Notes in Biochemistry by B.D. Hames, N.M. Hooper
- Principles and Techniques of Biochemistry and Molecular Biology by Wilson, K. & Walker, J.

10.10 TERMINAL QUESTIONS

- 1-What are carbohydrates?
- 2- Describe the classification of carbohydrates?
- 3- The molecular formula of galactose is $C_6H_{12}O_6$.What is molecular formula of lactose?
- 4-What are Monosaccharides, Oligosaccharides, and Polysaccharides?
- 5- What are difference between Monosaccharides and Disaccharides explain with example?
- 6- What is stereoisomers explain with example?
- 7- What is difference between reducing disaccharides and non-reducing disaccharides?
- 8- Write short note on biological function of carbohydrates?
- 9- What is racemic mixture?
- 10- Describe the structures and functions of any two homo and heteropolysaccharides?
- 11-What is lipids?
- 12- Describe the structure and classification of lipids?
- 13- Write the biological significance of phospholipid?
- 14- Difference between saturated and unsaturated fatty acid?
- 15-Explain different type of fatty acid and their functions?
- 16-Describe the structure of steroids with suitable example?
- 17- Write the short note on essential fatty acid?
- 18- Describe in brief the properties of fats and oils?
- 19- Distinguish between fats and steroids?
- 20-Write some difference between carbohydrates and lipids?

UNIT-11 AMINO ACIDS, PROTEINS AND VITAMINS

Content

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 - 11.3.3-Biological role
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 - 11.4.1-Classification
 - 11.4.2-Properties
 - 11.4.3-Biological role
- 11.5 Vitamins
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 - 11.5.3-Biological role
- 11.6 Summary
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- 11.11 Terminal Questions

11.1 OBJECTIVES

After reading this unit students will be able to

- Know amino acids and its properties.
- Study about proteins, their occurrence and importance.
- understand about vitamins and its classification

11.2 INTRODUCTION

Among all the possible **amino acids**, only 20 are usually found in proteins. The general structure of amino acids includes an amino group and a carboxyl group, both of which are bonded to the α -carbon (the one next to the carboxyl group). The α -carbon is also bonded to hydrogen and to the side chain group, which is represented by the letter R. The R group determines the identity of the particular amino acid. The two-dimensional formula shown here can only partially convey the common structure of amino acids because one of the most important properties of these compounds is their three-dimensional shape, or stereochemistry.

Biologically active **proteins** are polymers consisting of amino acids linked by covalent peptide bonds. Many different conformations (three-dimensional structures) are possible for a molecule as large as a protein. Of these many structures, one or (at most) a few have biological activity; these are called the native conformations. Many proteins have no obvious regular repeating structure. As a consequence, these proteins are frequently described as having large segments of “random structure” (also referred to as random coil). The term random is really a misnomer, because the same nonrepeating structure is found in the native conformation of all molecules of a given protein, and this conformation is needed for its proper function. Because proteins are complex, they are defined in terms of four levels of structure.

The term ‘**vitamin**’, in its modern sense, usually refers to the substances distinct from major components of food, required in minute quantities (i.e. oligodynamic in nature) and whose absence causes specific deficiency diseases. As the living organisms cannot synthesize most of these compounds, a steady supply of them for life. Their ultimate source is the plant or bacterial world.

This chapter deals with structure, properties and function of amino acids, proteins and vitamins.

11.3 AMINO ACIDS

Amino acids are commonly known as the building blocks of proteins and they are the key molecules that regulate structure and function of the proteins. They are organic acids that contain an amine group. Common amino acids are α -L amino acids. The dramatic diversity of the

enormous no of proteins found in nature result from the intrinsic properties of only 20 universally occurring amino acids. Some of these features include:

- (1) Polymerization capacity
- (2) Acid–base properties
- (3) Varied structure and chemical functionality in the amino acid side chains
- (4) Chirality

The general structure of amino acids consists of an amino group and a carboxyl group where both of them are bonded to the α -carbon (the carbon next to the carboxyl group).

The α -carbon forms bond with a hydrogen and to the side chain group, which is generally represented by the letter R.

The R group determines the individuality of the particular amino acid (Fig.11.1).

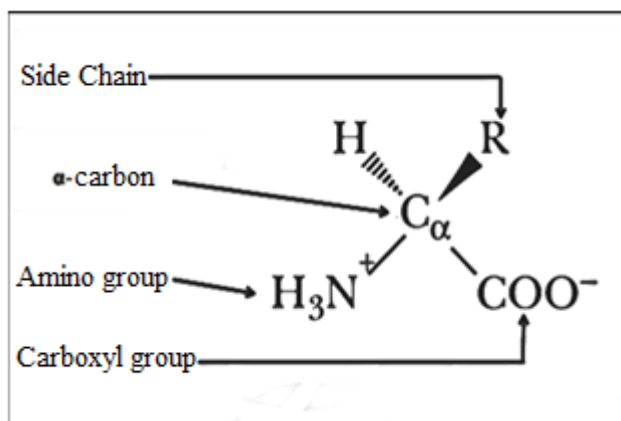


Fig.11.1-General structure of amino acids

Amino acids (except glycine) are non superimposable and four different groups attached with the chiral carbon. There are two stereoisomers of each amino acid known as **L** and **D** -amino acids on the basis of their similarity to the glyceraldehyde standard. The terminology comes from the Latin words laevus and dexter, meaning “left” and “right,” respectively, that denotes the ability of optically active compounds to rotate polarized light to the left or the right. When drawn in a certain orientation, the L form of amino acids has the hydroxyl group on the left side of the molecule, and the D form has it on the right side, as shown in perspective in Fig.11.2.

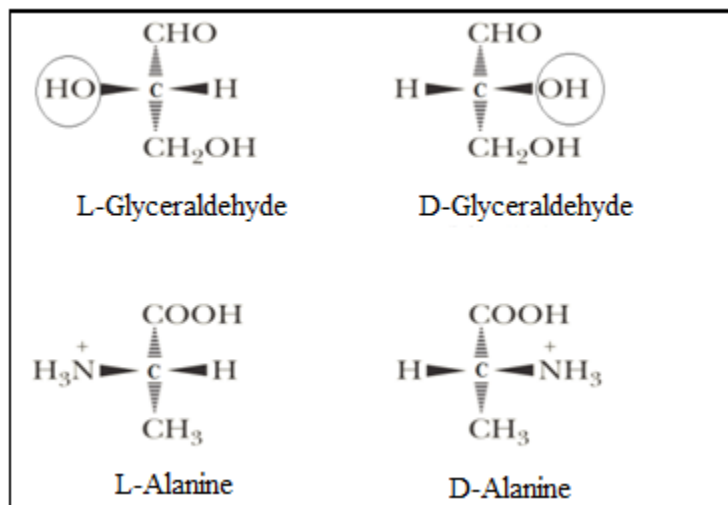


Fig.11.2- D and L configurations of amino acids

11.3.1 Classification

[I] Based on the structure: Amino acids can be classified into following seven distinct groups depending on the structure of their R groups.

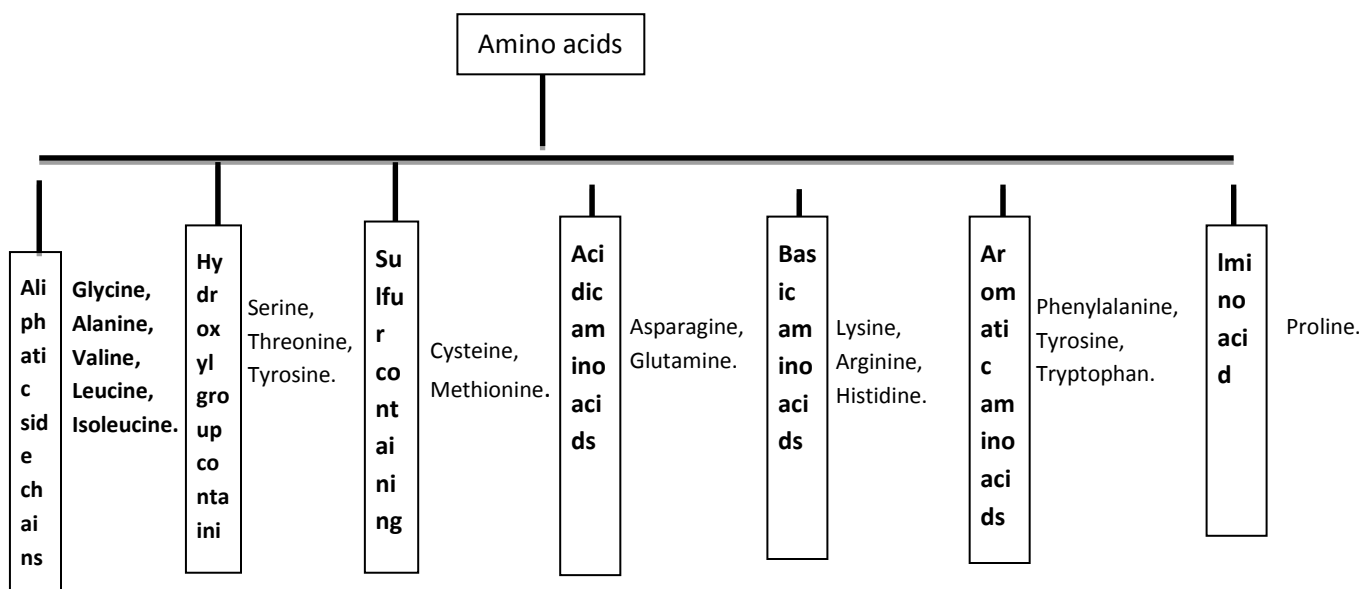


Fig-11.3-Classification of Amino acids based on R groups

[II] Based on polarity: There are four classes of amino acids based on their polarity. Polarity is a vital property of amino acids which is directly related to their functional role in the protein structure. The four classes include:

- 1. Non-polar amino acids:** Amino acids of this group are hydrophobic (water hating) in nature. Their 'R' group are uncharged. The amino acids comprise in this group are - alanine, leucine, isoleucine, valine, methionine, phenyl alanine, tryptophan and proline.
- 2. Polar amino acids with no charge on 'R' group:** Amino acids of this group contains uncharged 'R' group like hydroxyl, sulfhydryl and amide which usually involve in forming hydrogen bonds in protein. The amino acids of this group are glycine, serine, threonine, cysteine, glutamine, asparagine and tyrosine.
- 3. Polar amino acids with positive 'R' group:** Amino acids of this group are lysine, arginine and histidine.
- 4. Polar amino acids with negative 'R' group:** This group consists of dicarboxylic mono amino acids like aspartic acid and glutamic acid.

Table 1- Different Classes of Amino acids

Name	One word abbreviation	Three word abbreviation	
Hydrophobic Aliphatic Side Chain containing			
Glycine	G	Gly	
Alanine	A	Ala	
Valine	V	Val	
Leucine	L	Leu	
Isoleucine	I	Ile	
Methionine	M	Met	
Proline	P	Pro	
Cysteine	C	Cys	
Hydrophobic, aromatic side chain containing			
Phenylalanine	F	Phe	
Tyrosine	Y	Tyr	
Tryptophan	W	Trp	
Polar, charged side chain containing			
Arginine	R	Arg	
Lysine	K	Lys	
Histidine	H	His	

Aspartic acid	D	Asp	
Glutamic acid	E	Glu	
Polar, Side chain containing uncharged			
Asparagine	N	Asn	N Q S T
Glutamine	Q	Gln	
Serine	S	Ser	
Threonine	T	Thr	

[III] Based on nutritional requirement: Amino acids are categorized in following two distinct groups depending on the nutritional requirements:

- 1. Essential or indispensable amino acids:** Ten amino acids namely Arginine, Valine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan are the members of this groups. All these amino acids are necessary for proper growth of individual but cannot be synthesized by the body. Therefore they are supplied through diet. Among the ten Arginine and Histidine can be synthesized by adults but growing children cannot able to synthesize them, so they are considered as semi-essential amino acids.
- 2. Non-essential or dispensable amino acids:** Another ten amino acids that could be synthesized by the human body are called non-essential amino acids .e.g., Alanine, Serine, Cysteine, Aspartate, Asparagine, Glutamate, Glutamine, Tyrosine and Proline.

[IV] Based on metabolic fate: The carbon skeleton of amino acids is used as a precursor for the synthesis of glucose (glycogenic) or fat (ketogenic) or both. All amino acids can be following classified into following three groups depending on their metabolic fate.

Table -2 Amino acid classification based on metabolic fate

Class	Character	Example
Glycogenic amino acids	Serve as precursors for the formation of glucose or glycogen	Alanine, Aspartate, Glycine, Methionine etc.
Ketogenic amino acids	Serve as precursors for fat	Leucine And Lysine
Glycogenic and ketogenic amino acids	Serve as precursors for both glucose and fat	Isoleucine, Phenylalanine, Tryptophan, Tyrosine

11.3.2 Properties

(i) Physical properties of amino acids: Physical properties are important for determining the function of amino acids. The solubility of amino acids is a vital property and most of the amino acids are soluble in water but insoluble in organic solvents. Melting temperature of amino acids are high near above 200°C. Taste of amino acids varies from sweet (C, A, V) to tasteless (L) or bitter (R, I). All amino acids except glycine have asymmetric carbon atoms and have optical isomerism. They also act as ampholytes as they have both acidic (-COOH) and basic (-NH₂) groups.

The aromatic amino acids (F, Y, W), like other aromatic compounds carries conjugated rings, thus exhibit strong absorption of light in the near-ultraviolet region of the spectrum (Fig.11.4.). This absorption is regularly used for the analytical detection of proteins.

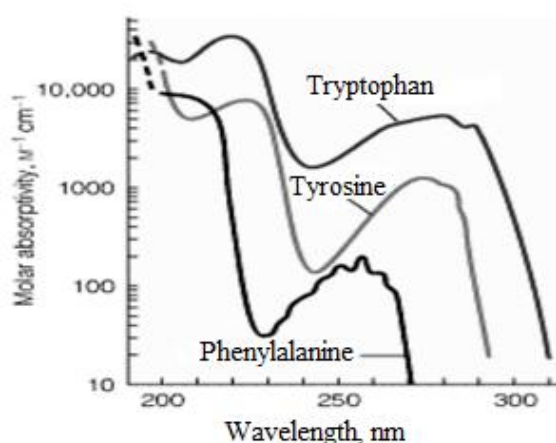
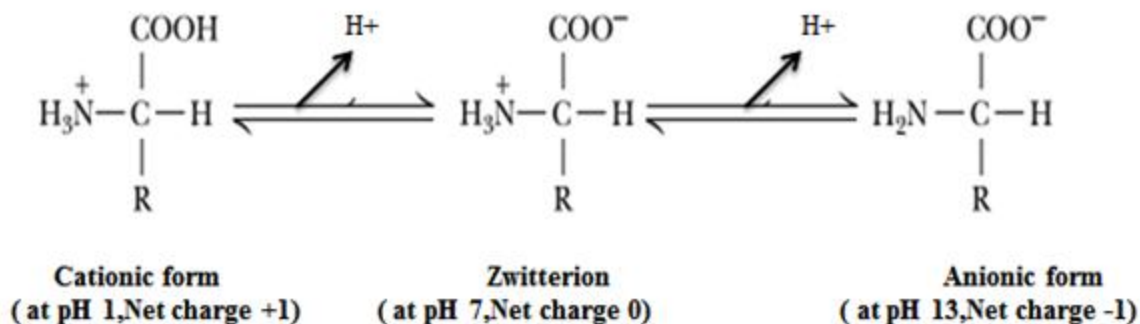


Fig.11.4 Absorption spectra of the aromatic amino acids in the near-ultraviolet region from D. Wetlaufer

(ii) Chemical properties of amino acids: The chemical properties of amino acids are mainly due to the presence of carboxyl (-COOH) group and amino (-NH₂) group. The carboxyl group of amino acids can able to form salts (-COONa) when they react with bases and esters (-COOR') with alcohols. They can also undergo decarboxylation to produce corresponding amines. The carboxyl group of dicarboxylic amino acids can also reacts with NH₃ to form amide. On the other hand the amino groups behave as bases and combine with acids (e.g. HCl) and ultimately forms salts (-NH₃⁺Cl⁻). The α -amino acids have the ability to react with ninhydrin and forms purple, blue or pink colour complex called Ruhemann's purple (exception proline and hydroxyproline give yellow colour). This reaction is efficiently used for the quantitative determination of amino acids. Amino acids can also undergo transamination reaction and oxidative deamination. All the amino acids are weak polyprotic acids.

The ionizable groups present in amino acids are not strongly dissociating ones, and the degree of dissociation mainly depends on the pH of the medium. All the amino acids contain at least two dissociable hydrogens. e.g. - Glycine, the simplest amino acid. At low pH, both the amino and carboxyl groups are protonated and the molecule has a net positive charge.

**Fig.10.5 Ionization of an Amino Acid**

If the pH is increased, the carboxyl group is the first to dissociate, yielding the neutral zwitterionic species Gly⁰ (Fig.11.5).

A further increase in pH eventually results in dissociation of the amino group to yield the negatively charged glycinate Gly⁻.

If dissociation constant is calculated then

First dissociation constant
$$K_1 = \frac{[\text{Gly}^0][\text{H}_3\text{O}^+]}{[\text{Gly}^+]}$$

Values for K₁ for the common amino acids are typically 0.4 to 1.0 × 10⁻² M, so that typical values of pK₁ center on values of 2.0 to 2.4 (Table.3).

Second dissociation constant
$$K_2 = \frac{[\text{Gly}^-][\text{H}_3\text{O}^+]}{[\text{Gly}^0]}$$

Typical values for pK₂ are in the range of 9.0 to 9.8. At physiological pH, the α-carboxyl group of a simple amino acid (with no ionizable side chains) become completely dissociated, but the α-amino group has not really begun its dissociation. The titration curve for such an amino acid is shown in Figure.

Table.3- pK_a Values of Common Amino Acid

Amino Acid	α-COOH pK _a	α-NH ₃ ⁺ pK _a	R group pK _a
Alanine	2.4	9.7	
Arginine	2.2	9	12.5
Asparagine	2	8.8	
Aspartic acid	2.1	9.8	3.9
Cysteine	1.7	10.8	8.3
Glutamic acid	2.2	9.7	4.3

Glutamine	2.2	9.1	
Glycine	2.3	9.6	
Histidine	1.8	9.2	6
Isoleucine	2.4	9.7	
Leucine	2.4	9.6	
Lysine	2.2	9	10.5
Methionine	2.3	9.2	
Phenylalani	1.8	9.1	
Proline	2.1	10.6	
Serine	2.2	9.2	~13
Threonine	2.6	10.4	~13
Tryptophan	2.4	9.4	
Tyrosine	2.2	9.1	10.1
Valine	2.3	9.6	

11.3.3 Biological role: Amino acids are mainly involved in the formation of protein structure. The α -amino acids polymerize, through the elimination of a water molecule and forms peptide bond. Polymers composed of two, three, a few (3–10), and many amino acid residues are known, as dipeptides, tripeptides, oligopeptides, and polypeptides respectively. These substances, however, are often referred to simply as “peptides.” Proteins are molecules that consist of one or more polypeptide chains.

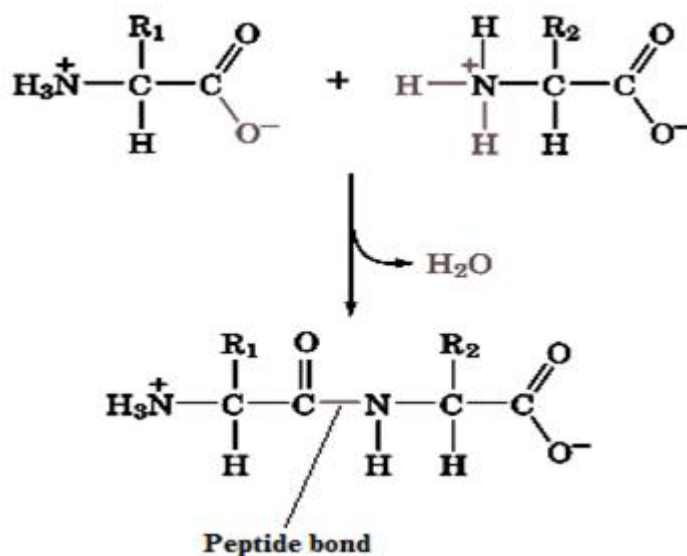


Fig.11.6- Formation of peptide bond

The main role of amino acids is mainly involved in the protein structure formation. Some of the amino acids are vital intermediates of metabolic pathways like citrulline and ornithine,

intermediates in urea biosynthesis, homocysteine, an intermediate in amino acid metabolism. Some other important biological properties of amino acid involve:

1. Amino acids and their derivatives act as chemical messengers in the communications between cells. For example, glycine, γ -aminobutyric acid (GABA; a glutamate decarboxylation product), and dopamine (a tyrosine derivative) are neurotransmitters (substances released by nerve cells to alter the behavior of their neighbors);
2. Histamine (the decarboxylation product of histidine) is a potent local mediator of allergic reactions;
3. Thyroxine (a tyrosine derivative) is an iodine-containing thyroid hormone that generally stimulates vertebrate metabolism
4. S-adenosylmethionine, acts as a biological methylating reagent .
5. Azaserin can act as antibiotics.
6. Creatine is derived from muscle and excreted in urine.
7. β -Alanine is a component of vitamin pantothenic acid and coenzyme A.

11.4 PROTEINS

Proteins are the macromolecules responsible for the biological processes in the cell. They consist at their most basic level of a chain of amino acids, determined by the sequence of nucleotides in a gene. Depending on the amino acid sequence (different amino acids have different biochemical properties) and interactions with their environment, proteins fold into a three-dimensional structure, which allows them to interact with other proteins and molecules and perform their function. They are naturally occurring polypeptides made up of 40 to 4000 amino acids. Proteins serve many functions in living systems. More than 28 million proteins are known.

Examples of the Diverse Functions of Proteins in Living Systems:

Structural Proteins -These proteins impart strength to biological structures or protect organisms from their environment. For example, collagen is the major component of bones, muscles, and tendons; keratin is the major component of hair, hooves, feathers, fur, and the outer layer of skin.

Protective Proteins- Snake venoms and plant toxins are proteins that protect their owners from predators.

Blood-clotting proteins protect the vascular system when it is injured. Antibodies and peptide antibiotics protect us from disease.

Enzymes- Enzymes are proteins that catalyze the reactions that occur in cells.

Hormones -Some hormones, compounds that regulate the reactions that occur in living systems, are proteins.

Proteins with Physiological Functions-These proteins include those that transport and store oxygen in the body, store oxygen in the muscles, and contract muscles.

11.4.1 Classification

Different methods of protein classification have been proposed, but currently none of them is universally valid. Below, some examples based on chemical composition, structure, functions, and solubility in different solvents.

Protein classification based on chemical composition

On the basis of their chemical composition, proteins may be divided into two classes: simple and complex.

1. Simple proteins: Also known as homoproteins, they are made up of only amino acids. Examples are plasma albumin, collagen, and keratin.

2. Conjugated proteins: Sometimes also called heteroproteins, they contain in their structure a non-protein portion. Three examples are glycoproteins, chromoproteins, and phosphoproteins.

(a) Glycoproteins- They are proteins that covalently bind one or more carbohydrate units to the polypeptide backbone. Typically, the branches consist of not more than 15-20 carbohydrate units, where you can find arabinose, fucose (6-deoxygalactose), galactose, glucose, mannose, N-acetylglucosamine (GlcNAc, or NAG), and N-acetylneuraminic acid (Neu5Ac or NANA). Examples of glycoproteins are: glycophorin, the best known among erythrocyte membrane glycoproteins fibronectin, that anchors cells to the extracellular matrix through interactions on one side with collagen or other fibrous proteins, while on the other side with cell membranes; all blood plasma proteins, except albumin; immunoglobulins or antibodies.

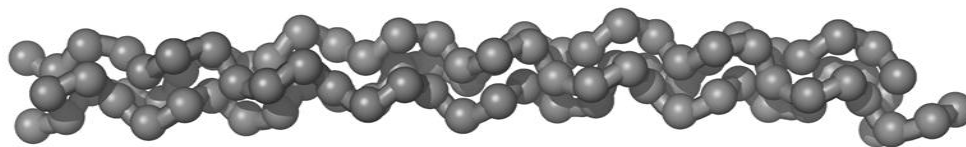
(b) Chromoproteins -They are proteins that contain colored prosthetic groups.

Typical examples are: hemoglobin and myoglobin, which bind, respectively, one and four heme groups; chlorophylls, which bind a porphyrin ring with a magnesium atom at its centre; rhodopsins, which bind retinal.

(c) Phosphoproteins- They are proteins that bind phosphoric acid to serine and threonine residues. Generally, they have a structural function, such as tooth dentin, or reserve function, such as milk caseins (alpha, beta, gamma and delta), and egg yolk phosvitin.

Protein classification based on shape

On the basis of their shape, proteins may be divided into two classes: fibrous and globular.

Fibrous proteins**Fig.11.7 – Collagen**

They have primarily **mechanical and structural functions**, providing support to the cells as well as the whole organism. These proteins are insoluble in water as they contain, both internally and on their surface, many hydrophobic amino acids. The presence on their surface of hydrophobic amino acids facilitates their packaging into very complex supramolecular structures.

In this regard, it should be noted that their polypeptide chains form long filaments or sheets, where in most cases **only one type of secondary structure, that repeats itself, is found**. In vertebrates, these proteins provide external protection, support and shape; in fact, thanks to their structural properties, they ensure flexibility and/or strength. Some fibrous proteins, such as α -keratins, are only partially hydrolyzed in the intestine. Here are some examples.

1. **Fibroin:** It is produced by spiders and insects. An example is that produced by the silkworm, *Bombyxmori*.
2. **Collagen:** The term “collagen” indicates not a single protein but a family of structurally related proteins (at least 29 different types), which constitute the main protein component of connective tissue, and more generally, the extracellular scaffolding of multicellular organisms.
3. In vertebrates, they represent about 25-30% of all proteins. They are found in different tissues and organs, such as tendons and the organic matrix of bone, where they are present in very high percentages, but also in cartilage and in the cornea, In the different tissues, they form different structures, each capable of satisfying a particular need. For example, in the cornea, the molecules are arranged in an almost crystalline array, so that they are virtually transparent, while in the skin they form fibers not very intertwined and directed in all directions, which ensure the tensile strength of the skin itself. Note: the different types of collagen have low nutritional value as deficient in several amino acids (in fact, they contain no tryptophan and low amount of the other essential amino acids). The gelatin used in food preparation is a derivative of collagen.
4. **α -Keratins:** They constitute almost the entire dry weight of nails, claws, beak, hooves, horns, hair, wool, and a large part of the outer layer of the skin. The different stiffness and flexibility of these structures is a consequence of the number of disulfide bonds that contribute, together with other binding forces, to stabilize the protein structure. And this is the reason why wool keratins, which have a low number of disulfide

bonds, are flexible, soft and extensible, unlike claw and beak keratins that are rich in disulfide bonds.

5. **Elastin:** This protein provides elasticity to the skin and blood vessels, a consequence of its random coiled structure that differs from the structures of the α -keratins and collagens.

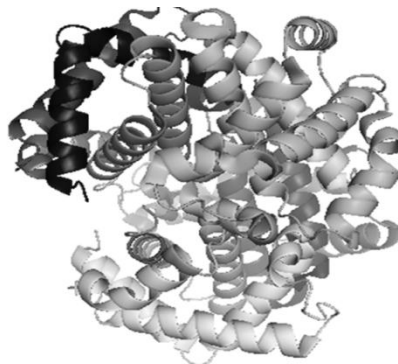


Fig. 11.8 Haemoglobin

Globular proteins

Most of the proteins belong to this class. They have a compact and more or less spherical structure, more complex than fibrous proteins. In this regard, motifs, domains, tertiary and quaternary structures are found, in addition to the secondary structures. They are generally soluble in water but can also be found inserted into biological membranes (transmembrane proteins), thus in a hydrophobic environment.

Unlike fibrous proteins, that have structural and mechanical functions, they act as:

1. enzymes
2. hormones
3. membrane transporters and receptors
4. transporters of triglycerides, fatty acids and oxygen in the blood
5. immunoglobulins or antibodies
6. grain and legume storage proteins

Examples of globular proteins are myoglobin, hemoglobin, and cytochrome c. At the intestinal level, most of the globular proteins of animal origin are hydrolyzed almost entirely to amino acids.

Protein classification based on biological functions

The multitude of functions that proteins perform is the consequence of both the folding of the polypeptide chain, therefore of their three-dimensional structure, and the presence of many different functional groups in the amino acid side chains, such as thiols, alcohols, thioethers, carboxamides, carboxylic acids and different basic groups.

From the functional point of view, they may be divided into several groups.

1. Enzymes (biochemical catalysts): In living organisms, almost all reactions are catalyzed by specific proteins called enzymes. They have a high catalytic power, increasing the rate of the reaction in which they are involved at least by factor 10^6 . Therefore, life as we know could not exist without their “facilitating action”.

Almost all known enzymes, and in the human body they are thousand, are proteins (except some catalytic RNA molecules called ribozymes, that is, ribonucleic acid enzymes).

2. Transport proteins: Many small molecules, organic and inorganic, are transported in the bloodstream and extracellular fluids, across the cell membranes, and inside the cells from one compartment to another, by specific proteins.

Examples are: hemoglobin, that carries oxygen from the alveolar blood vessels to tissue capillaries; transferrin, which carries iron in the blood; membrane carriers; fatty acid binding proteins (FABP), that is, the proteins involved in the intracellular transport of fatty acids; Proteins of plasma lipoproteins, macromolecular complexes of proteins and lipids responsible for the transport of triglycerides, which are otherwise insoluble in water; albumin, that carries free fatty acids, bilirubin, thyroid hormones, and certain medications such as aspirin and penicillin, in the blood.

Many of these proteins also play a protective role, since the bound molecules, such as fatty acids, may be harmful for the organism when present in free form.

3. Storage proteins: Examples are: ferritin that stores iron intracellularly in a non-toxic form; milk caseins that act as a reserve of amino acids for the milk; egg yolk phosvitin that contains high amounts of phosphorus; prolamins and glutelins, the storage proteins of cereals.

Protein classification based on solubility

The different globular proteins can be classified based on their **solubility in different solvents**, such as water, salt and alcohol.

11.4.2 Properties

A protein is a biological macro molecule composed of one or more chain of amino acids linked by peptide bonds. In general, we speak of protein when the string contains more than 50 amino acids. For smaller sizes, we speak of peptide and polypeptide, but more often they are simply "small protein".

The Dutch chemist Gerhard Mulder (1802-1880) discovered proteins. The word protein comes from the Greek “protos” which means first, essential. This probably refers to the fact that proteins are essential to life and they often constitute the majority share (60%) of the dry weight of cells. Another theory that would make reference protein as the adjective protean, with the Greek God Proteus who could change shape at will. The proteins indeed adopt many forms and provide multiple functions. But, this was not discovered until much later, during the twentieth century.

Solubility in Water

1. The relationship of proteins with water is complex. The secondary structure of proteins depends largely on the interaction of peptide bonds with water through hydrogen bonds.
2. Hydrogen bonds are also formed between protein (alpha and beta structures) and water. The protein-rich static balls are more soluble than the helical structures.
3. At the tertiary structure, water causes the orientation of the chains and hydrophilic radicals to the outside of the molecule, while the hydrophobic chains and radicals tend to react with each other within the molecule (cf. hydrophobic effect).
4. The solubility of proteins in an aqueous solution containing salts depends on two opposing effects on the one hand related to electrostatic interactions ("salting in") and other hydrophobic interactions (salting out).

Denaturation

A protein is denatured when its specific three-dimensional conformation is changed by breaking some bonds without breaking its primary structure. It may be, for example, the disruption of helix areas. The denaturation may be reversible or irreversible. It causes a total or partial loss of biological activity. This is an important property of protein.

There are a number of Denaturing agents as follows.

(a) Physical agents: Heat, radiation, pH

(b) Chemical agents: Urea solution which forms new hydrogen bonds in the protein, organic solvents, detergents.

11.4.3 Biological role

Proteins have a pivotal role in the stabilization of many structures. Examples are α -keratins, collagen and elastin. The same cytoskeletal system, the scaffold of the cell, is made of proteins. They generate movement. They are responsible, among others, for the contraction of the muscle fibers (of which myosin is the main component); the propulsion of spermatozoa and microorganisms with flagella; the separation of chromosomes during mitosis. They are involved in nerve transmission. An example is the receptor for acetylcholine at synapses.

1. They control development and differentiation: Some proteins are involved in the regulation of gene expression. An example is the nerve growth factor (NGF), discovered by Rita Levi-Montalcini, that plays a leading role in the formation of neural networks.

2. Hormones: Many hormones are proteins. They are regulatory molecules involved in the control of many cellular functions, from metabolism to reproduction. Examples are insulin, glucagon, and thyroid-stimulating hormone (TSH).

3. Protection against harmful agents: The antibodies or immunoglobulins are glycoproteins that recognize antigens expressed on the surface of viruses, bacteria and other infectious agents. Interferon, fibrinogen, and factors of blood coagulation are other members of this group.

4. Storage of energy: Proteins, and in particular the amino acids that constitute them, act as energy storage, second in size only to the adipose tissue, that in particular conditions, such as prolonged fasting, may become essential for survival. However, their reduction of more than 30% leads to a decrease of the contraction capacity of respiratory muscle, immune function, and organ function that are not compatible with life. Therefore, proteins are an extremely valuable fuel.

11.5 VITAMINS

Vitamins are organic molecules that an animal is unable to synthesize and must therefore obtain from its diet. Vitamins can be divided into two groups, water-soluble vitamins and fat-soluble vitamins.

Vitamins are required by the body in small amounts for metabolism, to protect health, and for proper growth in children. Vitamins also assist in the formation of hormones, blood cells, nervous-system chemicals, and genetic material. They generally act as catalysts, combining with proteins to create metabolically active enzymes that in turn produce hundreds of important chemical reactions throughout the body. Without vitamins, many of these reactions would slow down or cease.

The vitamins are required in small amounts, since their degradation is relatively slow.

The first vitamin to be discovered was vitamin A, or retinol.

General characteristics

The vitamins are characterized for some general facts, which are listed below:

1. Vitamins are of widespread occurrence in nature, both in plant and animal worlds.
2. All common foodstuffs contain more than one vitamin.
3. The plants can synthesize all the vitamins whereas only a few vitamins are synthesized in the animals.
4. Human body can synthesize some vitamins, e.g., vitamin A is synthesized from its precursor carotene and vitamin D from ultraviolet irradiation of ergosterol and 7-dehydrocholesterol. Some members of the vitamin B complex are synthesized by microorganisms present in the intestinal tract. Vitamin C is also synthesized in some animals such as rat.
5. Most of vitamins have been artificially synthesized.
6. All the cells of the body store vitamins to some extent.
7. Vitamins are partly destroyed and are partly excreted.
8. Vitamins are nonantigenic.
9. Vitamins carry out functions in very low concentrations. Hence, the total daily requirement is very small.

11.5.1- Classification

Vitamins are classified according to their ability to be absorbed in fat or water. Two categories of vitamins are usually recognized: fat-soluble and water-soluble.

A. Fat-soluble vitamins: These are oily and hydrophobic compounds. These are stored in the liver and are not excreted out of the body. Bile salts and fats are required for their absorption. Vitamin A, D, E and K are fat soluble vitamins. Because these vitamins can be stored, their excessive intake may have toxic effect and can result in Hypervitaminosis. They contain only carbon, hydrogen and oxygen. They, however, play more specialized roles in certain group of animals and in particular type of activities. For instance, they function in the formation of a visual pigment (vitamin A), in the absorption of calcium and phosphorus from the vertebrate intestine (vitamin D), in protecting mitochondrial system from inactivation (vitamin E) or in the formation of a blood clotting factor in vertebrates (vitamin K).

B. Water-soluble vitamins: Vitamin B complex and vitamin C are water soluble. They are compounds of carbon, hydrogen, oxygen and nitrogen. They are not stored in the body therefore they required daily in small amount. Most of these are universally vitamins since they perform the same general functions wherever they occur. Besides C, H and O, they also contain nitrogen. They are catalytic factors and as such form vital links in the chains of biochemical reactions characteristic of all living objects.

Many nutritionists, however, do not consider them as true vitamins, although their dietary deficiencies in animals lead to the development of characteristic symptoms. Moreover, none of them except lipoic acid is a part of the coenzyme system. The B-series of vitamins, being water-soluble and excretable, are required daily in meagre amounts (in milligrams or even less) for the normal growth and good health of humans and many other organisms. It is virtually impossible to 'overdose' on them.

Storage of vitamins in the body

The vitamins can be stored in the body to a slight extent. The liver cells are, however, rich in certain fat-soluble vitamins. For instance, the amount of vitamin A contained in the liver is sufficient enough to meet its requirement without any additional intake for about 6 months. Similarly, the quantity of vitamin D stored ordinarily in the liver is sufficient to maintain a person without any additional intake of vitamin D for about 2 months. The storage of vitamin K is, however, relatively slight.

The water-soluble vitamins are stored even in lesser amounts in the cells. Evidently, in cases of deficiency of vitamin B compounds, clinical symptoms appear rather early, that is within a few days. Similarly, absence of vitamin C can induce deficiency symptoms within a few weeks. Vitamin C is stored in the adrenal cortex.

11.5.2 Properties

Vitamin	Solubility	Chemical Name	Formula	Molar Mass	Melting Point	Boiling Point	Density	ChemSpider	CAS Registry
Vitamin A	Fat	Retinol	$C_{20}H_{30}O$	286.45	62-64°C	421.2°C	0.954	393012	68-26-8
		Retinal	$C_{20}H_{28}O$	284.44	61-64°C	421.428°C	0.949	553582	116-31-4
		beta-Carotene	$C_{40}H_{56}$	536.87	180-182°C	654.7°C	0.941	556	7235-40-7
Vitamin B1	Water	Thiamine	$C_{12}H_{17}ClN_4OS$	300.81	248.00°C	N/A	N/A	5819	59-43-8
Vitamin B2	Water	Riboflavin	$C_{17}H_{20}N_4O_6$	376.36	290 °C	N/A	1.65	431981	83-88-5
Vitamin B3	Water	Niacin	$C_6H_5NO_2$	123.11	236-239°C	292.467°C	1.293	913	59-67-6
		Niacinamide	$C_6H_6N_2O$	122.12	128-131°C	334.411°C	1.205	911	98-92-0
Vitamin B5	Water	Pantothenic Acid	$C_9H_{17}NO_5$	219.23	183.83°C	551.5°C	1.266	6361	137-08-6
Vitamin B6	Water	Pyridoxine	$C_8H_{11}NO_3$	169.18	159-162°C	491.9°C	1.353	1025	65-23-6
		Pyridoxamine	$C_8H_{12}N_2O_2$	168.19	N/A	460.1°C	1.282	1023	85-87-0
		Pyridoxal	$C_8H_9NO_3$	167.16	165 °C	412.8°C	1.36	1021	66-72-8
Vitamin B7	Water	Biotin	$C_{10}H_{16}N_2O_3S$	244.31	232-233°C	573.58°C	1.268	149962	58-85-5
Vitamin B9	Water	Folic Acid	$C_{19}H_{19}N_7O_6$	441.4	250 °C	N/A	1.689	5815	59-30-3
Vitamin B12	Water	Cyanocobalamin	$C_{63}H_{88}CoN_{14}O_{14}P$	1355.38	> 300°C	> 300°C	N/A	10469504	68-19-9
		Hydroxycobalamin	$C_{64}H_{93}CoN_{13}O_{17}P$	1406.46	N/A	N/A	N/A	21160115	13422-51-0
		Methylcobalamin	$C_{63}H_{91}CoN_{13}O_{14}P$	1344.40	N/A	N/A	N/A	3994	13422-55-4
Vitamin C	Water	Ascorbic Acid	$C_6H_8O_6$	176.12	190-192°C	552.672°C	1.954	10189562	50-81-7
Vitamin D	Fat	Cholecalciferol	$C_{27}H_{44}O$	384.64	83–86°C	496.4°C	0.96	9058792	67-97-0
Vitamin E	Fat	Alpha-Tocopherol	$C_{29}H_{50}O_2$	430.71	2.5-3.5°C	485.856°C	0.931	14265	59-02-9
		Gamma-Tocopherol	$C_{28}H_{48}O_2$	416.68	N/A	516.3°C	0.933	5256784	7616-22-0
Vitamin K	Fat	Phylloquinone	$C_{31}H_{46}O_2$	450.7	-20 °C	546.44°C	0.964	4447652	84-80-0

11.5.3 Biological role

1. Vitamin A

A. History. It was first recognized as an essential nutritional factor by Elmer McCollum in 1915 and then isolated from fish-liver oil by Holmes in 1917. On account of its established role in the visual process, it is often called as **antixerophthalmic factor** or the “**bright eyes**” **vitamin**. It was first synthesized in 1946 by Milas.

B. Occurrence. Liver oils of various fishes are the richest natural sources of vitamin A. Shark and halibut contain maximum amount whereas the cod-liver has lowest amount. However, polar bear liver is an extremely concentrated source of vitamin A. Other noteworthy sources are butter, milk and eggs and, to a lesser extent, kidney. In its provitamin form (*i.e.*, ascarotenes) it is supplied by all pigmented (particularly yellow) vegetables and fruits such as carrots, pumpkins, cantaloupes, turnips, peppers, peas, sweet potatoes, papayas, tomatoes, apricots, peaches, plums, cherries, mangoes etc.

C. Structure. Vitamin A is found in two forms A1 and A2. The carotenoids that give rise to vitamin-A in animal body is named as provitamin A. These include β -carotenes and cryptoxanthin. The carotene is most potent of all these forms. A molecule of β -carotene is made of eight 5-carbon isoprenoid units, linked to form a long chain of 40 carbon atoms with an ionone ring at each end. It is an orange-red hydrocarbon and upon hydrolysis yields 2 moles of vitamin A1.

Another form of vitamin A, present in fresh-water fishes, is known as vitamin A2. It differs from vitamin A1, which is found in salt-water fishes, in possessing an additional conjugate double bond between carbon atoms 3 and 4 of the β -ionone ring. Its potency is 40% that of vitamin A1.

D. Properties. Ordinarily retinol is a viscid, colourless oil but by careful fractionation it has also been isolated as pale yellowish needles. It gives a characteristic absorption band in ultraviolet(UV) spectrum at 328 *m*. It is soluble in fat and fat solvents but insoluble in water. Loss of vitamin A in cooking, canning and freezing of foodstuffs is small; oxidizing agents, however, destroy it. It is destroyed on exposure to UV light. Vitamin A is relatively unstable in air unless protected by antioxidants including vitamin E.

E. Metabolism. In the tissues, the metabolic transformation of retinol is carried out by enzymes. The dietary carotene is split into retinal by an enzyme of the intestine. Retinal is then reduced by another enzyme to retinol which, in turn, is converted to retinyl ester by reacting with a fatty acid like palmitic. The retinyl ester, on the contrary, is enzymatically hydrolyzed to produce retinol which is re-esterified with palmitic acid to produce retinyl ester. This is absorbed

through the lymphatic system and is stored in the liver. Liver stores vitamin A in large quantities mainly in the form of retinyl ester. But the vitamin A that circulates in the blood is in the form of retinol and is bound to a specific carrier protein called retinol binding protein (RBP). The retinyl ester present in the liver, therefore, has to be converted to retinol before it mixes with the blood. Liver can also successively convert retinol to retinal and retinal to retinoic acid. Retinoic acid is quickly absorbed from the intestine through the portal system and is rapidly excreted back into the intestine through the bile. Vitamin A helps maintain the epithelial cells of the skin and the linings of the digestive, respiratory and genito-urinary systems. These linings play a protective role against cancer-causing agents (or carcinogens), viruses and bacteria and are rendered vulnerable by a deficiency of vitamin A. Vitamin A guards against cancer by protecting cell walls from undesirable oxidation, and scavenging the products of oxidation— free radicals, which are linked to the development of cancer.

F. Deficiency. Vitamin A is perhaps the most important as it affects the various metabolic processes in the body. It has profound effect on epithelial structures, in general. Vitamin A deficiency leads to the onset of many diseases like nyctalopia or night blindness (inability to see in night), xerophthalmia (scaly condition of the delicate membrane covering the eyes), keratomalacia (softening of the cornea), phrynoderma or “toad skin” (hard and horny skin) and stunted growth.

2. Vitamin D

A. History. The first demonstration of the existence of vitamin D was shown by Elmer McCollum in 1922 who found that cod liver oil was effective in preventing rickets, a disease induced in rats by providing low calcium diet. On account of its preventive action on rickets, vitamin D is often called as **antirachitic factor**. It is also known as ‘**sunshine vitamin**’ as its provitamin form present in human skin is easily converted to the active form by irradiation with ultraviolet light. At least 10 different compounds are known to have antirachitic properties and are designated as D₂, D₃ etc., but the two, namely, vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol) are more important. Vitamin D₃ was, however, first isolated by Brockmann and others.

B. Occurrence. The best natural sources of vitamin D are the liver oils of many fishes such as cod and halibut. The flesh of oily fishes (*e.g.*, sardine, salmon, herring) is also excellent source. Egg yolks are fairly good but milk, butter and mushrooms are poor. The diets of infant may contain only small amounts of vitamin D; cow’s milk contains only 0.1 to 1 µg/quart (1 µg = 40 IU). Cereals, vegetables and fruits contain only negligible amounts. Most marketed cow’s milk is fortified with 10 µg of vitamin D per quart and most commercially-prepared milks for infant formulae are also fortified. Vitamin D₂ is of plant origin and is produced commercially by irradiation with ultraviolet light of a provitamin known as ergosterol which is found in plants, especially in ergot (hence so named) and yeast. Vitamin D₃, on the contrary, is of animal origin

and can be produced from 7-dehydrocholesterol also by irradiating with ultraviolet light. The 7-dehydrocholesterol is also a provitamin found naturally occurring in animals. Vitamins D₂ and D₃ both have about the same degree of activity in the human beings. In nature, these vitamins occur as esters. Like vitamin A, vitamin D is absent from vegetable fats and oils and is added to margarine during its manufacture.

C. Properties. Vitamin D is a white and almost odour less crystalline substance, soluble in fat and fat solvents. It is fairly heat resistant and also relatively resistant to oxidation. It is not affected by acids and alkalis.

D. Metabolism. The provitamin D₃ can be synthesized within the human body so that it may, in fact, not be required in the diet. This may, henceforth, not be treated as a vitamin. In the past when man lived mainly outdoors and with minimum clothing, there was no hindrance for the penetration of ultraviolet light from the sun to convert it into the active form. In the far northern areas, however, the amount of light is not adequate for conversion and as such fish liver oils serve as excellent source of vitamin D in these areas. The increased need of this vitamin is usually felt in growth and in pregnancy to provide for the needs of the foetus. Vitamin D plays an important role in calcification of bones and teeth. It encourages the absorption, into the blood, of calcium salts and phosphates. Calcium passage across duodenum occurs mainly by diffusion and active transport of Ca²⁺ occurs across the ileal mucosa. Both these processes are related in deficiency of vitamin D. The subsequent release of bound calcium is also markedly stimulated by vitamin D but only in the presence of parathyroid hormone. On the whole, the function of vitamin D is to cause increased absorption, longer retention and better utilization of calcium and phosphorus in the body.

E. Deficiency. The most characteristic symptom of vitamin D deficiency is the childhood disease known as rickets. Deficiency of it in human adults leads to osteomalacia, a condition that might also be termed “adult rickets”. **Rickets** (derived from an old English word, *wrickken*= to twist) is primarily a disease of growing bones. In it, the deposition of inorganic materials on the matrix of bones (*i.e.*, calcification) fails to occur, although matrix formation continues. Clinical manifestations of rickets in children usually manifest in the first year or in the second year. One of the early signs of rickets is **craniotabes**, which is due to thickening of the outer table of the skull and is detected by pressing firmly over the occiput or posterior parietal bones. A ping-pong ball like sensation will be felt. Craniotabes near the suture line may, sometimes, be present in normal premature infants. Costochondral junctions become prominent to give appearance of a beaded rib, the **rachitic rosary**. Thickening of the wrists and ankles are other early evidences of osseous changes. Increased sweating, especially around the head, may also be present.

3. Vitamin E

A. History. The presence of this active principle was first demonstrated in vegetable oils by Evans and Mattill independently in 1920. This was designated as vitamin E or **antisterility factor** on account of the development of sterility in animals in its absence. In 1936, two compounds with vitamin E activity were isolated from wheat germ oil by Evans and his associates and given the name, tocopherol (*tokos* G = childbirth ; *pheros* G = to bear; *ol*= an alcohol). Subsequently, five other tocopherols were obtained from various cereal grains like wheat germ, corn oil, rice etc.

B. Occurrence. The tocopherols are of widespread occurrence in many plant oils such as wheat germ, rice, corn, cottonseed, soybean and peanut but not olive oil. They are also present in small amounts in meat, milk, eggs, leafy plant and some fruits. *Fish liver oils, so abundant in vitamin A and D, are devoid of vitamin E.* Of all the tocopherols discovered so far, the form has the widest distribution and greatest biologic activity.

C. Structure. Vitamin E is the collective name for a group of closely related lipids called tocopherols. The tocopherols are derivatives of 6-hydroxychroman (also known as tocol) bearing an isoprenoid side chain at carbon 2.

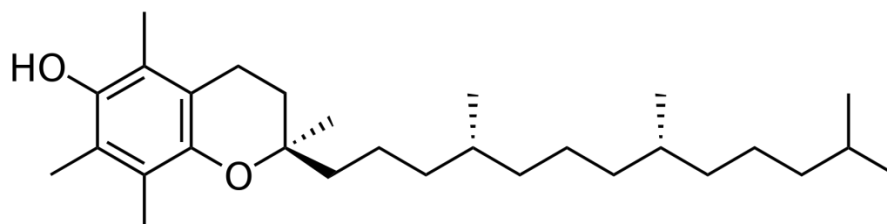


Fig.11.9 Vitamin E

D. Properties. Vitamin E is a light yellow oil. It is resistant to heat (up to 200°C) and acids but acted upon by alkalies. It is easily but slowly oxidized and is destroyed by UV rays. The tocopherols are excellent antioxidants. They prevent other vitamins presents in food (*e.g.*, vitamin A) from oxidative destruction. It is found in the non saponifiable fraction of the vegetable oils.

E. Metabolism. Tocopherols act as antioxidants, i.e., they can prevent the oxidation of various other easily oxidized substances such as fats and vitamin A. It is for this reason that they are commercially added to foods to retard their spoilage. It may be recalled that vitamin A is essential for reproduction. Whereas the beneficial action of vitamin A is mainly on the ectoderm and endoderm, that of vitamin E is on the mesodermal tissue. But, very likely, vitamin E influences all the 3 germinal layers of the embryo by preventing the too rapid destruction of vitamin A. Certain substances such as phenols and vitamin C (ascorbic acid) stimulate the antioxidant property of vitamin E.

F. Deficiency. The characteristic symptoms of experimentally-induced vitamin E deficiency vary from animal to animal. In mature female rats, **sterility** develops because of reabsorption of fetus after conception while in males, the germinal epithelium of the testes degenerates and the spermatozoa become nonmotile. A vitaminosis E in herbivorous animals like rabbits and guinea pigs leads to acute **muscular dystrophy** (atrophy of muscle fibres), which ultimately results in creatinuria ; young chicks exhibit capillary damage and **encephalomalacia**; hen eggs show **low hatchability** and monkeys reveal **hemolytic anemia**. There is, however, little evidence that man is ever short of vitamin E. Finally, as is true for almost all the vitamins, a vitaminosis E prevents normal growth. It also sometimes causes degeneration of the renal tubular cells.

G. Human requirements. Vitamin E is not a problem in human nutrition because it is ubiquitous in foods. However, the minimum daily requirement of vitamin E for adults is 30 I.U. for men and 25 I.U. for women. The pregnant and lactating mothers, however, require 30 I.U. daily. For infants and children, the vitamin E requirement is at the rate of 1 to 1.25 I.U. per kilogram of body weight. One International Unit of dl-tocopherol is equivalent to the biologic activity of 1.1 mg of pure compound or 0.67 mg of d-tocopherol.

4. Vitamin K

A. History: Henrick Dam (1929), a Danish investigator, found that newly-hatched chicks, fed on artificial diets, develop hemorrhage, a fatal disease characterized by prolonged blood-clotting period. The term vitamin K (K for Danish koagulations) was then proposed by Dam (1934) himself to designate the active factor which cured or prevented this disease. On account of its blood-clotting power, it is also called as **antihemorrhagic factor** or coagulation **vitamin**. Of the 2 naturally-occurring forms of this vitamin, vitamin K1 was first isolated by Dam *et al* from alfalfa in 1939 and the other form, vitamin K2 from fish meal by Doisy *et al*, also in 1939.

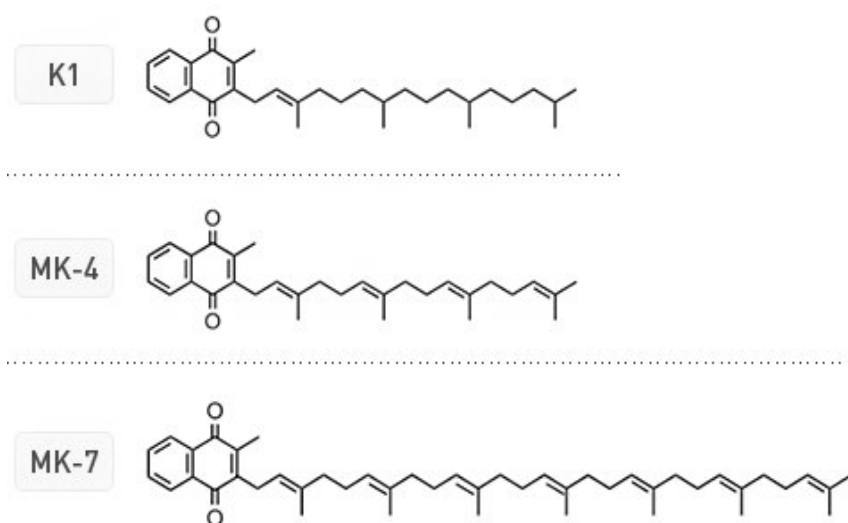


Fig.11.10-Three vitamins of K group

B. Occurrence. Vitamin K₁ occurs in green vegetables like spinach, alfalfa, cabbage etc. Fruits and cereals are poor sources. Vitamin K₂ is found in some intestinal bacteria. A rich source of K₂ is putrefied fish meal. Their relative *biologic potencies* are:

Vitamin K₁—100

Vitamin K₂—80

C. Structure. Chemically, the two forms of vitamin K (Fig. 33–10) are derivatives of quinones and differ from each other in the composition of their side chain present at carbon 3 of the naphthoquinone ring. It is a phytyl radical in vitamin K₁ (C₃₁H₄₆O₂) and a difarnesyl radical in vitamin K₂ (C₄₁H₅₆O₂). Vitamin K₁, found in plants, has 4 isoprene units in its side chain whereas vitamin K₂, found in animals, contains in its side 6 isoprene units, each with a double bond.

D. Properties. Vitamin K₁ is a yellow viscid oil but vitamin K₂ is a yellowish crystalline solid. It is sensitive to light and is, therefore, kept in dark bottles. It is destroyed by irradiation, strong acids, alkalies and oxidizing agents.

E. Metabolism. Vitamin K plays an essential role in the biosynthesis of prothrombin— a blood plasma protein needed in the process of blood clotting and produced in liver.

F. Deficiency. Deficiency of vitamin K causes loss of blood-clotting power. The infants may also show signs of vitamin K deficiency by developing **hemorrhage**. This disease persists by the time the bacteria develop in the intestine. Administration of this vitamin to pregnant mothers before parturition decreases the onset of this disease. In man, however, a vitaminosis K results in **steatorrhea** with diminished intestinal absorption of lipids. In general, vitamin K deficiency is rarely found in higher animals as this is provided by food and also synthesized by intestinal bacteria.

5. Vitamin B₁ (Thiamine): Thiamine, or vitamin B₁, a colorless, crystalline substance. It is readily soluble in water and slightly in ethyl alcohol.

Source: Vitamin B₁ is abundantly found in germinating seeds, un-milled cereals, beans, orange juice, tomato, egg, meat, fish, organ meats (liver, heart, and kidney), leafy green vegetables, nuts, and legumes.

Physiological Significance:

(i) Acts as a catalyst in carbohydrate metabolism, enabling pyruvic acid to be absorbed and carbohydrates to release their energy.

(ii) Thiamine also plays a role in the synthesis of nerve-regulating substances.

Deficiency: Deficiency in thiamine causes beriberi, which is characterized by muscular weakness, swelling of the heart, and leg cramps.

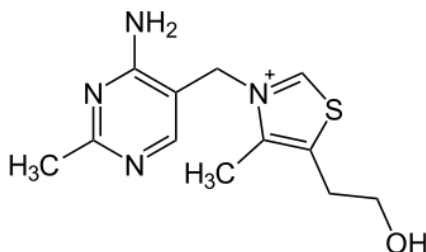


Fig.11.11-Vitamin B1 (Thiamine Chloride)

6. Vitamin B₂ (Riboflavin):

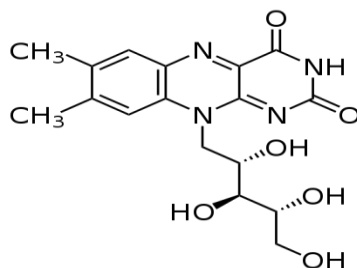


Fig. 11.12-Vitamin B2 (Riboflavin)

Source: The best sources of riboflavin are liver, milk, meat, dark green vegetables, whole grain and enriched cereals, pasta, bread, and mushrooms.

Physiological Significance:

- (i) It is essential for carbohydrate metabolism. Enzyme containing riboflavin is called Flavoproteins.
- (ii) It acts as coenzyme for enzyme catalyzing oxidation-reduction reaction.

Deficiency:

- (i) Its deficiency causes **Glossitis** (inflammation of tongue).
- (ii) Lack of thiamine causes skin lesions, especially around the nose and lips, and sensitivity to light.

7. Vitamin B₃ (Niacin or Nicotinic Acid):

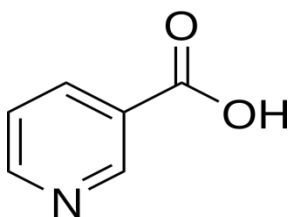


Fig.11.13-Vitamin B3 (Niacinamide)

Source:

- (i) The best sources of niacin are liver, poultry, meat, canned tuna and salmon, whole grain and enriched cereals, dried beans and peas, and nuts.
- (ii) The body also makes niacin from the amino acid tryptophan.

Physiological Significance:

- (i) Nicotinic acid is essential for the normal functioning of skin, intestinal tract and the nervous system.
- (ii) Vitamin B₃ works as a coenzyme in the release of energy from nutrients.

Deficiency:

- (i) A deficiency of niacin causes **pellagra**, the first symptom of which is a sunburn like eruption that breaks out where the skin is exposed to sunlight.
- (ii) Later symptoms are a red and swollen tongue, diarrhea, mental confusion, irritability, and, when the central nervous system is affected, depression and mental disturbances.

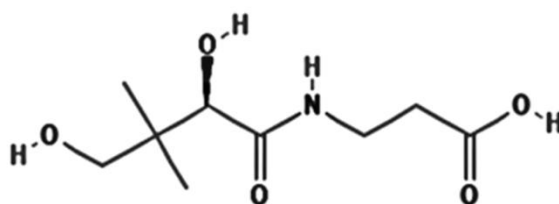
8. Pantothenic acid or Vitamin B5:

Fig.11.14-Vitamin B5 (Pantothenic Acid)

Source: Its main sources are liver, milk, meat, eggs, wheat germ, wheat bran, potatoes, sweet potatoes, tomatoes, cabbage, cauliflower and broccoli. Fruit and other vegetables also have pantothenic acid.

Physiological Significance:

- (i) Pantothenic acid is essential for growth of infants and children,
- (ii) It plays a major role in the metabolism of proteins, carbohydrates, and fats.

Deficiency: Its deficiency causes nausea, vomiting, gastrointestinal disorders, improper growth and fatty liver.

9. Vitamin B₆ (Pyridoxine):

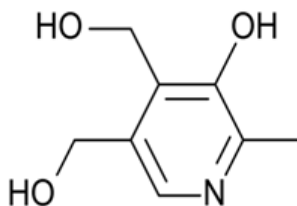


Fig.11.15-Vitamin B6 (Pyridoxal) Chemical Structure

Source:

- (i) The best sources of pyridoxine are whole (but not enriched) grains, cereals, bread, liver, avocados, spinach, green beans, and bananas.
- (ii) It is also found in milk, eggs, fish, chicken, beef, pork and liver.

Physiological Significance:

- (i) Pyridoxine, or vitamin B₆, is necessary for the absorption and metabolism of amino acids.
- (ii) It also plays roles in the use of fats in the body and in the formation of red blood cells.

Deficiency: Pyridoxine deficiency is characterized by skin disorders, cracks at the mouth corners, smooth tongue, convulsions, dizziness, nausea, anemia, and kidney stones.

10. Vitamin b₇ (Biotin):

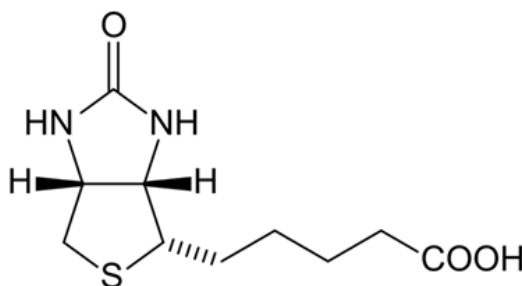


Fig.11.16-Vitamin B7 (Biotin)

Biotin is also known as “anti-egg white injury factor” or as H-factor.

Source: Biotin occurs in combined state as biocytin. It is found in yeast, liver, kidney, milk and molasses.

Physiological Significance:

- (i) Biotin serves as prosthetic group for many enzymes which catalyze fixation of CO₂ into organic molecules.
- (ii) It helps in synthesis of fatty acids.

Deficiency: Its deficiency caused the destruction of intestinal bacteria. It leads to nausea and muscular pain.

11. Vitamin B₉ or M or Bc (Folic Acid):

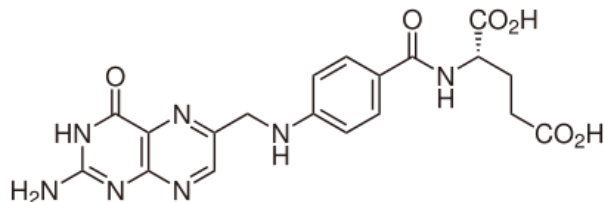


Fig.11.17-Vitamin M or Folic Acid

Source:

- (i) Folic acid is found in yeast, liver and kidney.
- (ii) Fish meat and green leafy vegetables, milk and fruits also provide folic acid.

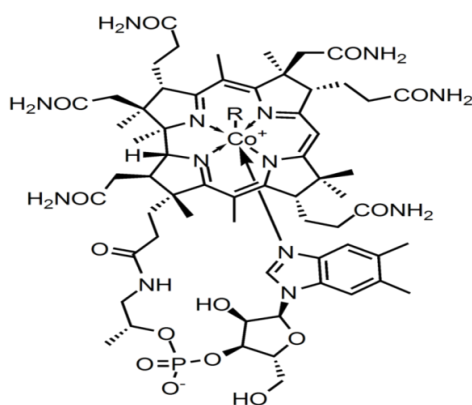
Physiological Significance:

- (i) Folic acid acts as a coenzyme and help in synthesis of purines and thymine during DNA synthesis.
- (ii) It helps in formation and maturation of red blood cells.

Deficiency:

- (i) Folic acid deficiency gives rise to megaloblastic anemia.
- (ii) The patient suffers from retarded growth, weakness, infertility, inadequate lactation in females and gastrointestinal disorders.

12. Vitamin B₁₂ (Cynocobalamin):



R = 5'-deoxyadenosyl, Me, OH, CN

Fig.11.18-Vitamin B₁₂

Vitamin B₁₂ or Cobalamin, or Anti-Pernicious Anaemic Factor (APA), one of the most recently isolated vitamins.

Source: Cobalamin is obtained only from animal sources—liver, kidneys, meat, fish, eggs, and milk. Vegetarians are advised to take vitamin B₁₂ supplements.

Physiological Significance:

- (i) It is necessary in minute amounts for the formation of nucleoproteins, proteins, and red blood cells.
- (ii) It is necessary for the functioning of the nervous system.
- (iii) It stimulates the appetite of the subject.

Deficiency: Due to its deficiency **Pernicious Anemia** results which is characterized by symptoms of ineffective production of red blood cells, faulty myelin (nerve sheath) synthesis, and loss of epithelium (membrane lining) of the intestinal tract.

13. Vitamin C (Ascorbic Acid or Antiscorbutic Vitamin):

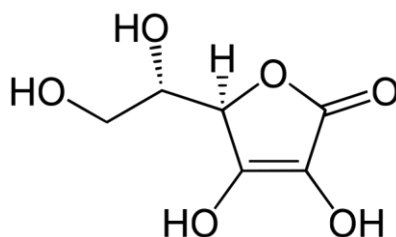


Fig.11.19-Vitamin C - Ascorbic Acid

Source:

- (i) Sources of vitamin C include citrus fruits, fresh strawberries, cantaloupe, pineapple, and guava.
- (ii) Good vegetable sources are Broccoli, Brussels sprouts, Tomatoes, Spinach, Kale, Green Peppers, Cabbage, and Turnips.

Physiological Significance:

- (i) Vitamin C is important in the formation and maintenance of collagen, the protein that supports many body structures and plays a major role in the formation of bones and teeth.
- (ii) It also enhances the absorption of iron from foods of vegetable origin.
- (iii) The connective tissue fibrils and collagen are synthesized with the help of vitamin C.
- (iv) It play important role in wound repair.
- (v) It protects body against stress.

Deficiency: This well-known Scurvy is the classic manifestation of severe ascorbic acid deficiency. Its symptoms are loss of the cementing action of collagen and include hemorrhages which lead to loosening of teeth and cellular changes in the long bones of children.

11.6 SUMMARY

All peptides and polypeptides are polymers of α -amino acids. There are 20 α -amino acids that are relevant to the make-up of mammalian proteins (see below). Several other amino acids are found in the body free or in combined states (e.g. not associated with peptides or proteins). These non-protein associated amino acids perform specialized functions such as citrulline and ornithine in the disposal of waste nitrogen via the urea cycle. Several of the amino acids found in proteins also serve functions distinct from the formation of peptides and proteins, e.g., tyrosine in the formation of thyroid hormones or glutamate acting as a neurotransmitter.

Protein, highly complex substance that is present in all living organisms. Proteins are of great nutritional value and are directly involved in the chemical processes essential for life. The importance of proteins was recognized by chemists in the early 19th century, including Swedish chemist Jöns Jacob Berzelius, who in 1838 coined the term protein, a word derived from the Greek *proteios*, meaning “holding first place.” Proteins are species-specific; that is, the proteins of one species differ from those of another species. They are also organ-specific; for instance, within a single organism, muscle proteins differ from those of the brain and liver.

Vitamins are organic molecules that function in a wide variety of capacities within the body. The most prominent function of the vitamins is to serve as cofactors (co-enzymes) for enzymatic reactions. The distinguishing feature of the vitamins is that they generally cannot be synthesized by mammalian cells and, therefore, must be supplied in the diet. The vitamins are of two distinct types, water soluble and fat soluble.

11.7 GLOSSARY

Acid: a molecule or chemical group that donates a proton, either to water or to some other base.

Active site: An asymmetric pocket on or near the surface of a Enzyme that promotes chemical catalysis when the appropriate **ligand** (substrate) binds.

Affinity: the tightness of a protein–ligand complex.

Allosteric activator: a ligand that binds to a protein and induces a conformational change that usually increases the protein’s activity.

Alpha /beta domain: a protein domain composed of beta strands connected by alpha helices.

Amphipathic: Molecules having both polar and nonpolar character and therefore a tendency to form interfaces between **hydrophobic** and **hydrophilic** molecules.

Antiparallel beta sheet: a beta sheet found in polypeptide chain of a protein, in which each strand runs in the opposite direction from its immediate neighbors.

Base: (in a nucleic acid) the aromatic group attached to the sugar of a **nucleotide**.

Beta sheet: a **secondary structure** element formed by backbone hydrogen bonding between segments of extended polypeptide chain.

Catalyst: a substance that accelerates the rate of a reaction without itself being permanently altered.

Co -repressor: a regulatory molecule that binds to a gene repressor protein and assists its binding to DNA.

Codon: They are found in genetic code where three consecutive **nucleotides** in a strand of DNA or RNA that represent either a particular amino acid or a signal to stop translating the transcript of the gene.

Coenzyme: a cofactor that is an organic or organometallic molecule and that assists catalysis.

Cofactor: a small, non-protein molecule or ion that is bound in the functional site of a protein and assists in ligand binding or catalysis or both. Some cofactors are bound covalently, others are not.

Denaturant: a chemical capable of unfolding a protein in solution at ordinary temperatures.

Denatured state: the partially or completely unfolded form of a biological macromolecule in which it is incapable of carrying out its biochemical and biological functions.

Dimer: an assembly of two identical (homo-) or different (hetero-) subunits. In a protein, the subunits are individual folded .polypeptide chains.

Electrostatic interactions: noncovalent interaction between atoms or groups of atoms due to attraction of opposite charges.

Entropy: a measure of the disorder or randomness in a molecule or system.

Free energy: a function, designed to produce a criterion for spontaneous change, that combines the **entropy** and **enthalpy** of a molecule or system. Free energy decreases for a spontaneous process, and is unchanged at equilibrium.

G protein: a member of a large class of proteins with GTPase activity that act as molecular switches in many different cellular pathways, controlling processes such as sensory perception, intracellular transport, protein synthesis and cell growth and differentiation. They undergo a large conformational change when a bound GTP is hydrolyzed to GDP.

GTPase-activating protein (GAP): a protein that accelerates the intrinsic GTPase activity of switch GTPases.

Hydrogen bond: a noncovalent interaction between the **donor atom**, which is bound to a positively polarized hydrogen atom, and the acceptor atom, which is negatively polarized. Though not covalent, the hydrogen bond holds the donor and **acceptor atom** close together.

Hydrolysis: **breaking** a covalent bond by addition of a molecule of water.

Hydrophilic: tending to interact with water. Hydrophilic molecules are polar or charged and, as a consequence, are very soluble in water.

Hydrophobic: The property of a molecule that tends to avoid water. Hydrophobic molecules are nonpolar and uncharged and, as a consequence, are relatively insoluble in water. In polymers, hydrophobic **side chains** tend to associate with each other to minimize their contact with water or polar side chains.

Hydrophobic effect: the tendency of nonpolar groups in water to self-associate and thereby minimize their contact surface area with the polar solvent.

Ligand: A small molecule or macromolecule that recognizes and binds to a specific site on a macromolecule.

Ligand -binding site: site on the surface of a protein at which another molecule binds.

Motif: characteristic sequence or structure that in the case of a **structural motif** may comprise a whole domain or protein but usually consists of a small local arrangement of secondary structure elements which then coalesce to form domains. **Sequence motifs**, which are recognizable amino-acid sequences found in different proteins, usually indicate biochemical function. Structural motifs are less commonly associated with specific biochemical functions.

Peptide bond: another name for **amide bond**, a chemical bond formed when a carboxylic acid condenses with an amino group with the expulsion of a water molecule. The term peptide bond is used only when both groups come from amino acids.

Protomer: the asymmetric repeating unit (or units) from which an oligomeric protein is built up.

11.8 SELF ASSESSMENT QUESTION

11.8.1 Multiple choice Questions:

1- Glycine and proline are the most abundant amino acids in the structure of

- | | |
|--------------|----------------|
| (a) Collagen | (b) Myoglobin |
| (c) Insulin | (d) Hemoglobin |

2- Some proteins contain additional amino acids that arise by modification of an amino acid already present in a peptide, examples includes

- | | |
|------------------------------|-----------------------|
| (a) hydroxy proline | (b) 5- hydroxy Lysine |
| (c) Gamma Amino Butyric Acid | (d) All of the above |

3- Select the wrong statement out of the followings-

- (a) Only L amino acids are found in the biological system
- (b) Glycine is optical inactive
- (c) Tyrosine is a modified amino acid
- (d) Seleno cysteine is 21st amino acid

4- The amino acid that carries a net positive charge at the physiological pH?

- | | |
|----------------|----------------------------|
| (a) Valine | (b) Leucine |
| (c) Isoleucine | (d) None of the followings |

5- Which out of the following amino acids is a precursor for a mediator of allergies and inflammation?

- (a) Histidine
- (b) Tyrosine
- (c) Phenyl Alanine
- (d) Tryptophan

6- Which out of the followings proteins should be recommended for a mal nourished child?

- (a) Pulses
- (b) Wheat
- (c) Soy Protein
- (d) Milk

7- The amino acids can participate in hydrogen bonding except one

- (a) Serine
- (b) Cysteine
- (c) Threonine
- (d) Valine

8- Amino acid which is a precursor of niacin (Vitamin)?

- (a) Tyrosine
- (b) Threonine
- (c) Tryptophan
- (d) Phenylalanine

9- The greatest buffering capacity at physiological pH would be provided by a protein rich in which of the following amino acids?

- (a) Serine
- (b) Cysteine
- (c) Alanine
- (d) Histidine

10-Choose the correct category for milk protein casein out of the followings

- (a) Nucleoprotein
- (b) Phospho protein
- (c) Lipoprotein
- (d) Glycoprotein

11- Which of the amino acids below is the uncharged derivative of an acidic amino acid?

- (a) Cystine
- (b) Tyrosine
- (c) Glutamine
- (d) Serine

12- Which of the following amino acids in a globular protein, is highly likely to be localized within the interior of the molecule?

- (a) Arginine
- (b) Valine
- (c) Aspartic acid
- (d) Lysine

13- Which of the characteristics below is found in Glycine?

- (a) Optically inactive
- (b) Hydrophilic, basic and charged
- (c) Hydrophobic
- (d) Hydrophilic, acidic and charged

14- The amino acid that may be considered a hydrophobic amino acid at physiological pH of 7.4?

- | | |
|-------------------|---------------|
| (a) Isoleucine | (b) Arginine |
| (c) Aspartic acid | (d) Threonine |

15-Choose amino acid which is most compatible with an α - helical structure?

- | | |
|----------------|-------------|
| (a) Tryptophan | (b) Alanine |
| (c) Leucine | (d) Proline |

11.8.1 Answers Key-

1-(a), 2-(d), 3-(c), 4-(d), 5-(a),6-(d),7-(d), 8-(c), 9-(d),10-(b),11-(c),12-(b),13-(a),14-(a),15-(b)

11.9 REFERENCES

- E. E. Conn and P. K. Stumpf, Outlines of Biochemistry, John Wiley & Sons, New York.
- A. L. Lehninger, Principles of Biochemistry, CBS Publishers and Distributors.
- R. K. Murry, D. K. Granner, P.A. Mayes, V. W. Rodwell, Harper's Biochemistry, Prentice Hall International Inc., Latest Edition.
- S. C. Rastogi, Biochemistry, Tata McGraw Hill, New Delhi, Latest Edition.
- M. Cohn, K. S. Roth, Biochemistry and Disease, William and Wilkins Co., Baltimore, Latest Edition.
- U. Satyanarayana, Biochemistry, Books and Allied (P) Ltd., Calcutta, Latest Edition.
- G. F. Zubay, W. W. Parson, D. E. Vance, Principles of Biochemistry, WBC Publishers, England, Latest Edition.
- S. Ramakrishnan, K. G. Prasanna, R. Rajan, Textbook of Medical Biochemistry, Orient Longman, Madras, Latest Edition.
- S. K. Sawhney, Randhir Singh Eds, Introductory Practical Biochemistry, Narosa Publishing House, New Delhi.
- D. T. Plummer, An Introduction to Practical Biochemistry, Tata McGraw Hill, New Delhi.
- J. Jayaraman, Laboratory Manual in Biochemistry, Willey, Eastern Limited, New Delhi.
- Lehninger Principles of Biochemistry, 3rd ed London : Macmillan Press Ltd., 2000
- Harper's Biochemistry, 25th ed New York : McGraw-Hill, Inc., 2002
- A Text Book of Biochemistry for Medical Students, 9th ed. New Delhi : UBS Publisher's Distributors Ltd., 2003
- Textbook of Medical Biochemistry, 5th ed. New Delhi : Jaypee Brothers Medical Publishers (P) Ltd , 2002

11.10 SUGGESTED READINGS

- Lehninger Principles of Biochemistry 5th Edition by David L. Nelson (Author), Michael M. Cox (Author)
- Biochemistry by Berg JM, Tymoczko JL, and Stryer L, published by W.H. Freeman and Company.
- Biochemistry, 4th Edition by Donald Voet, Judith G. Voet
- Biochemistry By J L Jain
- Fundamentals of biochemistry by satyanarayana
- Bios Instant Notes in Biochemistry by David Hames, Nige
- Instant Notes in Biochemistry by B.D. Hames, N.M. Hooper
- Principles and Techniques of Biochemistry and Molecular Biology by Wilson, K. & Walker, J.

11.11 TERMINAL QUESTIONS

1. What are properties of amino acids?
2. What are amino acids? Describe classification of amino acids?
3. What are the secondary structures of protein?
4. How can amine groups be classified?
5. What is the importance of proteins for living organisms?
6. What is the flat structural representation of an amino acid molecule?
7. What is the difference between alpha-helix and beta-sheet protein conformations?
8. What is the difference between essential and nonessential amino acids?
- 9-What is the primary structure of a protein? What is the importance of the primary structure?
10. What are the different types of vitamins?
11. What role do vitamins play?
12. What are water soluble and fat soluble vitamins?

UNIT-12 ENZYMES

Content

- 12.1 Objectives
- 12.2 Introduction
- 12.3 Discovery
- 12.4 Nomenclature
- 12.5 Characteristics of enzymes
- 12.6 Concept of enzyme
 - 12.6.1 Active site
 - 12.6.2 Parts of enzymes
 - 12.6.3 Types of enzymes
 - 12.6.4 Mechanism of the enzyme action
 - 12.6.5 Mode of Action
 - 12.6.6 Enzyme Kinetics
 - 12.6.7 Enzyme Inhibitors
- 12.7 Summary
- 12.8 Glossary
- 12.9 Self Assessment Question
- 12.10 References
- 12.11 Suggested Readings
- 12.12 Terminal Questions

12.1 OBJECTIVES

After reading this unit students will be able to -

- Explain the enzymes and their role.
- Explain about discoveries related to enzymes.
- Describe old and new pattern of enzyme nomenclature, and EC Code.
- Classify the enzymes on the basis of their catalytic properties.
- Describe the characteristic features and properties of the enzymes.
- Explain the basic concept of enzyme and its type.
- Describe the mechanism and kinetics of the enzymes.

12.2 INTRODUCTION

In this unit you will understand, what enzymes are and how they are able to play role in all metabolic processes of living organisms. The cell may be like a minute laboratory which is capable of carrying out various processes like synthesis and breakdown of various substances. These processes carried out by an **enzyme** at normal body temperature, low ionic strength, low pressure and narrow range of pH. The study of enzyme is called **enzymology**.

Enzymes are **biocatalyst**, macromolecules of biological origin, which speeds up a chemical reaction but remain, unchanged itself at the end, so that it can be used again and again. They have extraordinary catalytic power, often far greater than that of synthetic or inorganic catalysts. Some enzymes can make their conversion of substrate to product occur millions of time faster. They have a high degree of specificity for their substrates. They accelerate chemical reactions tremendously and they function in aqueous solutions under very mild conditions of temperature and pH.

Enzymes are central to every biochemical process and are also involved in many regulatory mechanisms which allow the metabolism to adapt to changing conditions. The wide majority of enzymes are proteins and necessary for all living organisms. Like other proteins, enzymes are of high molecular weight compound and linear chain of various amino acids that fold to produce three-dimensional or quaternary structure. The enzymes are most remarkable and highly specialized proteins, for fulfilling fundamental requirements of the cell that is to convert food into energy and necessary material for growth and repair of the organism.

12.3 DISCOVERY

French chemist **Anselme Payen** was first to discover an enzyme, diastase in 1833. But biological catalysis was recognized and described in the 1850s by **Louis Pasteur**. He revealed that the

‘living intact’ yeast cells were responsible for fermentation of sugar into alcohol and used the term ‘ferments’ for such catalysts. Then in 1897 **Edward Buchner** discovered that yeast extracts could ferment sugar to alcohol, because of this work he is credited for the discovery of enzyme and **Frederick W. Kuhne** coined the word enzymes (en= in, zyme= yeast). **James B. Sumner** (1926) isolated enzyme urease for the first time in crystalline form from Jack bean, *Canavalia ensiformis* at the Cornell University. Sumner found that urease crystals completely made up of proteins and he postulated that all enzymes are proteins. But Sumner’s conclusion was widely accepted, only after **John Northrop** crystallized pepsin, trypsin and other digestive enzymes and found them also to be proteins. On the basis of all these findings, he determined the proteinaceous nature of enzymes. For such a pioneer and innovative work, Sumner and Northrop share the Nobel Prize in 1947. The discovery that enzyme could be crystallized eventually helped in the X-ray crystallography of enzyme lysozyme by **D.C. Phillips** in 1965. **J.B.S. Haldane** first time suggested that weak bonding interactions between an enzyme and its substrate might be used to catalyze a reaction.

12.4 NOMENCLATURE

According to the older system, the enzymes are usually named by adding suffix ‘ase’ to the name of substrate, example - sucrase acting on sucrose, lipase acting on lipid etc. The names of some enzymes indicate the nature of reactions they catalyze, example - isomerase, dehydrogenase, phosphatase, carboxylase etc. Some enzymes are named arbitrarily, example - pepsin, renin etc. An enzyme known to act in the digestion of foods was named pepsin, from the Greek used pepsis, “digestion,” and lysozyme was named for its ability to lyse bacterial cell walls. Still others were named for their source: trypsin, named in part from the Greek tryein, “to wear down,” was obtained by rubbing pancreatic tissue with glycerin.

The name of enzymes according to the older system has often been haphazard and confusing. Therefore, a systematic approach of nomenclature of the enzymes has been recommended by the **Commission on enzymes of the International Union of Biochemistry** (1961), according to which the various enzymes are designated by code numbers consisting of four digits (*E.C. number* or *Enzyme Commission number*).

Characteristic features of International Union of Biochemistry (IUB) system

- Enzymes are divided into six major classes, each with 4-13 subclasses.
- The name enzyme has two parts: the first part indicates the name of substrate and second part indicates the type of reaction ending with suffix –ase.
- Each enzyme has a systematic four-digit code number (EC number).

The first digit of **E.C. number** indicates the major class (Table – 11.1), the second digit indicates the sub-class, and the third digit indicates its sub-sub class, while the fourth digit denotes the systematic specific name of the enzymes, the first part of which indicates the name of the substrate and the second part the nature of the reaction. For example - the code no. 1.1.1.1. stands for the enzyme *alcohol dehydrogenase* where -

1. Stands for **oxidoreductase**.
- 1.1. Stands for enzyme which utilizes substrate as – **CHOH group**.
- 1.1.1. Stands for those enzymes which utilize **NAD** as an acceptor.

12.4.1 -Major classes:

1. **Oxidoreductase** – catalyze oxidation-reduction reaction (transfer of electrons or protons).
2. **Transferases** – catalyze reaction which involves transfer of functional groups from one molecule to another molecule.
3. **Hydrolases** – catalyze breaking of one molecule in to two molecules by adding water molecule (transfer of functional group to water).
4. **Lyases** – catalyze reactions in which either a double bond is established due to the removal of a group or a group is added to the double bond.
5. **Isomerases** – catalyze isomerisation reactions (transfer of functional group within the molecule).
6. **Ligases** – also called as synthetases, catalyze those reactions in which linking of two molecules are coupled with the breakdown of pyrophosphate bond of ATP or similar triphosphate.

Table - 12.1: The major classes of enzymes with an E.C. number and reaction catalysed

Sr. No	Class	Reaction Type	Example	Reaction Catalysed
1	Oxidoreductase	Oxidation-reduction reaction	Alcohol dehydrogenase <i>E.C. no 1.1.1.1</i>	$A_{red} + B_{ox} \leftrightarrow A_{ox} + B_{red}$
2	Transferase	Involve group transfer	Glycerokinase <i>E.C. no 2.4.3.2</i>	$A-B + C \leftrightarrow A + B-C$
3	Hydrolases	Hydrolytic reactions	Carboxypeptidase <i>E.C. no 3.4.17.1</i>	$A-B + H_2O \leftrightarrow A-H + B-OH$
4	Lyases	Rearrangements of electrons	Pyruvate decarboxylase <i>E.C. no 4.1.1.1</i>	$A + B \leftrightarrow A-B$
5	Isomerases	Rearrangements of functional group	Maleate isomerase <i>E.C. no 5.2.1.1</i>	$A \leftrightarrow A_{isomer}$

6	Ligases	Joining of two molecules	Pyruvate carboxylase <i>E.C. no 6.4.1.1</i>	$A + B + A/G/U/C-TP \leftrightarrow A-B + A/G/U/C-DP$
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12.5 CHARACTERISTICS OF ENZYMES

Enzymes possess the following major characteristics -

1. Enzymes are proteinaceous in nature.
2. Enzymes remain unchanged qualitatively and quantitatively at the end of reaction they catalyze.
3. Enzymes increase the rate of reactions but they do not initiate reaction.
4. Enzymes do not alter the chemical equilibrium point of a chemical reaction.
5. Enzymes are required in very minute quantity in respect to substrate.
6. Enzymes are very efficient; an average enzyme undergoes about 1000 reactions per second.
7. Enzymes are highly specific, that is an enzyme will generally catalyze only a single reaction.
8. Enzymes lower the activation energy of the reactions they catalyze.
9. Enzymes form complexes with substrates.
10. Enzymes activity is affected by pH, temperature, substrate and enzyme concentration.

12.5.1 - Properties of Enzymes

1. **Catalytic Property** - Enzymes are capable of catalyzing biochemical reactions. They transform large number of substrate into products without undergoing any change themselves. Catalytic power of enzyme is measured in terms of “**turn over number**”. Turn over number is the number of substrate molecules converted into product per unit time, when the enzyme is fully saturated with substrates.
2. **Colloidal Nature** - Enzymes are partly or totally proteins hence they are colloidal in nature. They have high molecular weight and low diffusion rate and form colloidal system in water.
3. **Reversibility** - In most of the cases the reactions catalyzed by the enzymes are reversible depending upon the requirements of the cell.
4. **Specificity** - Enzymes are highly specific in action with few exceptions, i.e. particular enzyme can catalyze only a particular type of reaction.
5. **Heat Sensitivity (Thermostability)** - The enzymes are proteinaceous in nature, hence they are thermo labile. They work in narrow range of temperature (20°C – 40°C). Beyond 45°C enzymes get **denatured** i.e. they lose their activity due to change in 3-D structure of protein and their properties are not restored even after giving suitable temperature.
6. **pH Sensitivity** - Each enzyme acts best at certain pH. Most of the enzymes act in neutral pH. Any increase or decrease in medium pH leads to slow down or inhibition of enzymatic activity.

12.6 CONCEPT OF ENZYME

In this topic, you have learnt about enzymes, its types and mechanisms. The basic requirement for life is, the organism must be able to catalyze chemical reactions efficiently and selectively. Most chemical reactions require an initial input of energy to get started. This initial investment of energy is known as the **energy of activation**. A catalyst is a substance that lowers the activation energy required for a reaction. Enzymes are biological catalyst that speeds up reaction without being consumed by the reaction. Thus enzymes are typically effective in very small amounts.

The enzyme catalyzed reactions differ from ordinary chemical reactions in following ways-

1. The rates of enzyme catalysed reactions are very high, approximately 10^6 to 10^{12} times greater than ordinary unanalyzed reactions.
2. Enzymes are highly specific, to recognize the minute and specific differences in substrate and product molecules, even able to discriminate between mirror images of the same molecule.
3. Enzyme reactions typically occur at normal body temperature, low ionic strength, low pressure and narrow range of pH near neutrality.
4. Enzymes activities have regulatory control on metabolic reactions of living organism in balance by various activators and inhibitors.

The molecule on which an enzyme acts is known as its **substrate**. The substrate binds with the enzymes active site by weak interactions. The **active site** is the location on the enzyme where the catalysis of chemical reaction and product formation takes place. Thus, active site both confines the substrate molecule and orients it in correct manner (Fig.12.1).

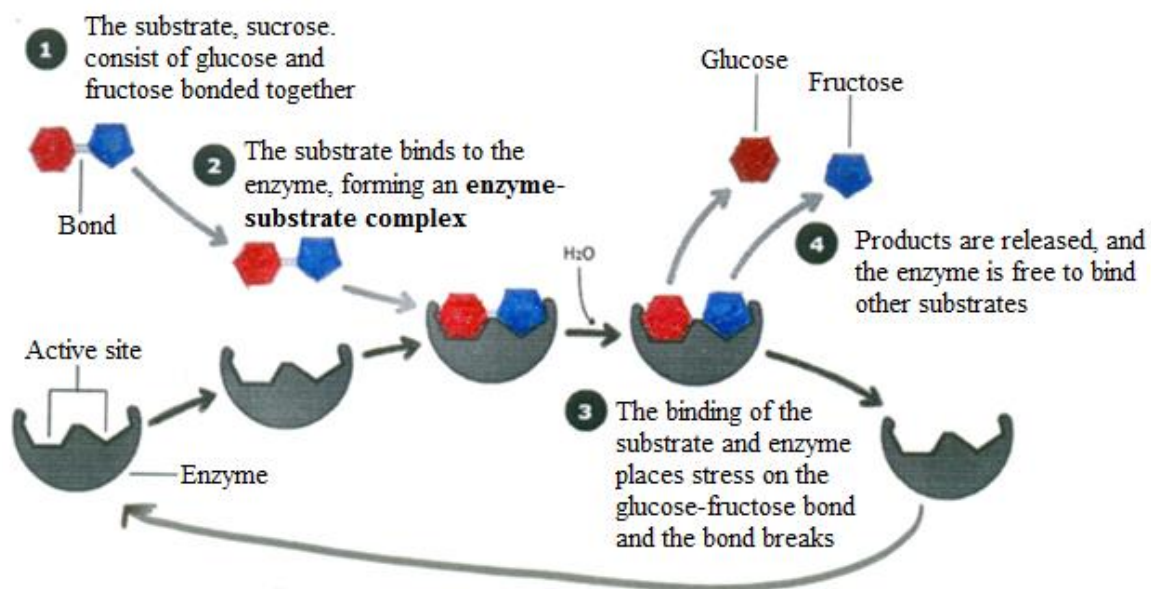


Fig.12.1: Sucrose, a disaccharide is hydrolyzed to produce one molecule of glucose and fructose. The enzyme sucrose is involved and specific for this process. The active site of the enzyme fits the two subunits of the sucrose molecule.

12.6.1- Active site: The active site of an enzyme is the region that binds the substrates and contains residues that directly participate in the making and breaking of bonds. These residues are called as catalytic group, although, enzymes differ widely in structure, specificity and mode of catalysis, a number of generalization concerning their active sites are –

1. The active site takes up a relatively small part of the total volume of an enzyme
2. The active site is a three dimensional structure
3. Substrates are bound to the enzymes by multiple weak bonds
4. Active sites are cleft or crevices
5. The specificity of binding depends on the precisely defined arrangement of atoms in an active site.

12.6.2 - Parts of enzymes: Enzymes are proteinaceous in nature and made up of a hundred to a millions of amino acids with its own unique sequence. These amino acids are coiled and folded many times to form a complex three dimensional structure. The structure and function of the enzyme is determined by the unique sequences of amino acids and its 3-D structure. Some enzymes appear to depend only on their proteinaceous structure and others require an additional non protein component for their catalytic activity. On such basis, some enzymes are purely made up of proteins and others are conjugated enzymes of protein and non protein parts -

- a) Simple proteinaceous enzyme
- b) Conjugated enzymes (Holoenzyme)

Enzymes have molecular weights ranging from about 12,000 to more than 1 million. The catalytic activity of many enzymes depends upon the presence of small non proteinaceous molecules termed **cofactor** (Table-12.2), unlike enzymes, they were stable at relatively high temperatures. There are either one or more inorganic ions (such as Fe, Mg, Mn or Zn) or a complex organic or metallo-organic molecule called a **coenzyme**, it often serves as electron carriers and generally derived from vitamins. Some enzymes require both a coenzyme and one or more metal ions for activity, even in some cases these ions serve to hold the enzyme protein in its proper three dimensional structures. A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called as **prosthetic group**. A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions is called a **holoenzyme**. The protein part of such an enzyme is called the **apoenzyme** or **apoprotein**.



Table - 12.2: ENZYME COFACTORS

Coenzymes component	Name of enzyme (example)
1. Inorganic molecule (Metal ions)	
Copper (Cu^{2+})	Cytochrome oxidase
Iron ($\text{Fe}^{2+}/\text{Fe}^{3+}$)	Catalase

Potassium (K^+)	Pyruvate kinase
Magnesium (Mg^{2+})	Hexokinase
Manganese (Mn^{2+})	Superoxide dismutase
Molybdenum (Mo)	Nitrate reductase
Nickel (Ni^{2+})	Urease
Zinc (Zn^{2+})	Carbonic anhydrase
2. Organic molecule	
Biotin	Pyruvate carboxylase
Coenzyme A	Acetyl CoA carboxylase
Flavin adenine nucleotide	Monoamine oxidase
Nicotinamide adenine dinucleotide	Lactate dehydrogenase
Thiamine pyrophosphate	Pyruvate dehydrogenase

Function of Cofactors:

1. In some enzymes, cofactors are required for completion of the active site.
2. Cofactor acts as a donor of electrons in the enzymatic reactions.
3. Cofactors may serve as temporary recipients of either one of the reaction products/ electrons/ protons.

SIMILARITIES BETWEEN COENZYMES AND PROSTHETIC GROUPS

- They are required in small amounts.
- They help enzyme in catalysis, but do not have their own catalytic properties.
- They are not proteins and are much smaller than the enzymes with which they are associated.
- They may undergo a temporary change during catalysis, but after reaction they get restored.

12.6.3 - Types of Enzymes

1. **Abzyme:** An abzyme is a monoclonal antibody with catalytic activity and commonly called as catalytic antibody. Abzyme have only weak, modest catalytic activity and have not proved to be of any practical use. Besides this, it has academic importance to understand the mechanism and catalytic behavior of enzymes.
2. **Ribozymes:** This enzyme is made up of ribonucleic acid (RNA) instead of protein and extracted from protozoa – *Tetrahymena thermophila* in 1981. Thomas J. Cech and Sydney Altman won the Nobel Prize of Chemistry in 1989 for this discovery.
Ribozymes (also called RNA enzyme) catalyze trans-esterification and finally hydrolysis of phosphodiester bonds in RNA molecules. It has a significant role in the intron splicing events

essential for the conversion of pre-mRNA in a mature mRNA, example - peptidyl transferase.

3. **Zymogens:** Some enzymes are synthesized in the cells in inactive state in which condition they called as zymogens or pro-enzymes or pre-enzymes. Zymogens are proteins which are inactive precursors of enzymes and are primarily concerned with proteolytic activity, example - pepsin, trypsin, chemotrypsin etc.
4. **Isoenzymes:** Some enzymes occur in multiple or more than one form in the same species, tissue or even in the same cell which catalyze the same biochemical reaction but have different molecular structure and kinetic properties, example - lactic dehydrogenase. Some reactions are common to many pathways of cell; such identical reactions that occur in different pathways are catalyzed by different enzymes. Such enzymes are known as isoenzymes. Isoenzymes differ in their physio-chemical properties, which mainly due to differences in a few amino acids constituents in the polypeptide chain. Isoenzymes are product of different genes, and adapted to the specific pathway and cellular location where they worked.
5. **Allosteric enzymes:** The term allosteric derives from the Greek words *allos* means 'other' and *stereos* means 'shape'. So, allosteric enzymes contain two or more receptor sites, which are geometrically different and non-overlapping. One is an active site and other is allosteric site or regulatory site. However, this site is occupied by activators or inhibitors. Substances which bind to allosteric site and cause reversible change in enzyme structure are called allosteric substances or modulators and the phenomenon is called as **allosteric transition**.

Modulators (allosteric substances) are of two types-

- I. Positive modelators (allosteric activator)
- II. Negative modulators (allosteric inhibitor)

The binding of a molecule to the allosteric site induces a conformational change in the active site which enhances the rate at which the substrate is converted to product. Such molecules are called **allosteric activator**. While, another molecule's binding to allosteric site induces a conformational change in the active site which reduces the catalytic activity of the enzymes. Such molecules are called **allosteric inhibitor**.

The allosteric enzymes are the first enzymes in a series of enzymatic reactions of metabolic pathway. The end product of the pathway combines with the allosteric site of the enzyme and blocks its catalytic activity quite effectively. This phenomenon of inhibition is known as **feedback inhibition** (Figure – 11.2).

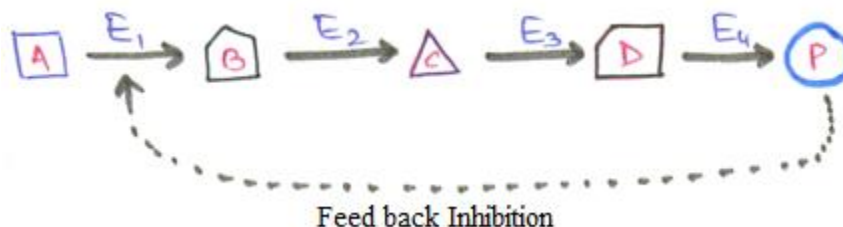


Fig. 12.2: Feedback inhibition by end product of the pathway to regulate the activity of the enzyme.

12.6.4 - Mechanism of the enzyme action

In this section you will understand, how enzymes bind substrate and turn them into products. Enzyme-catalyzed reactions are characterized by the formation of a complex between substrate and enzyme (an ES complex). Substrate binding occurs in a pocket on the enzyme called the **active site**. The function of enzymes and other catalysts is to lower the activation energy, for a reaction and thereby enhance the reaction rate. The rate of a reaction is dependent on the activation energy required for the formation of the transition state which further decays into products. Enzymes increase reaction rate by lowering the energy of the transition state, ES complex. For a reaction, a molecule must possess enough energy to collide with sufficient force to overcome their mutual repulsion and to break existing chemical bonds (Figure – 11.3). A reaction without enzyme requires more activation energy than an enzymatic reaction. However, that the overall energy changes from the initial state to the final state is the same with and without the enzyme.

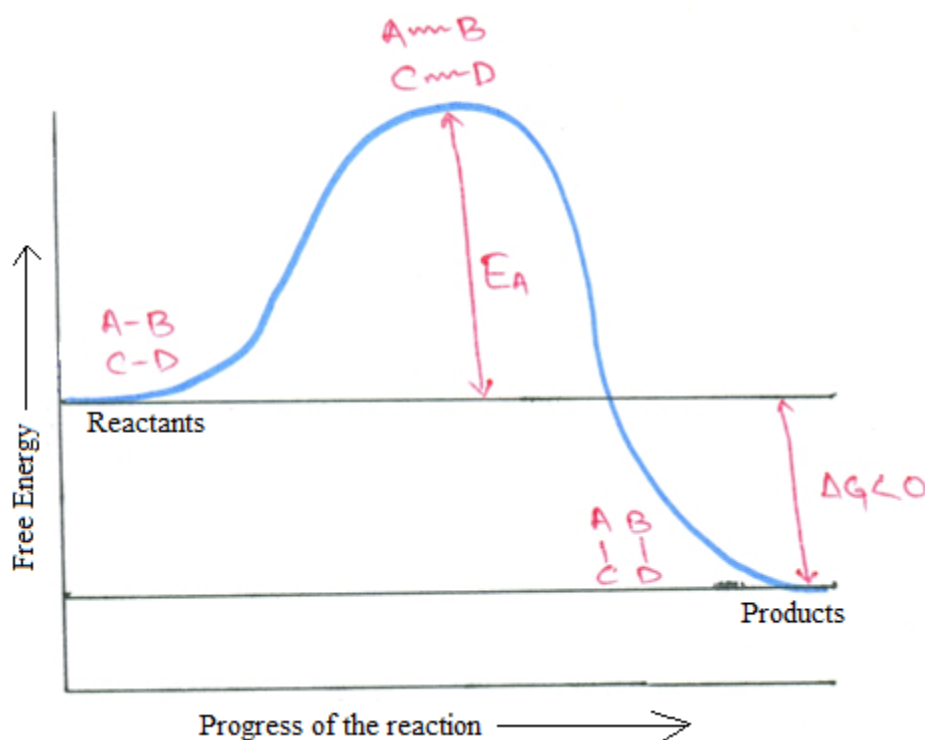
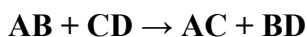


Fig. 12.3: The reactant AB & CD must absorb enough energy from the surroundings to reach the unstable transition state, where bonds can break and new bonds form, releasing energy to the surroundings.



Enzymes speed up the rate of a reaction by lowering the energy barrier between substrates (reactants) and products but are not themselves used up in the reaction, and are regenerated at end. Thus, an enzyme increases the rate of a reaction but does not affect the equilibrium ratio of reactants and products, because the rate of the reaction in both directions are increased to the

same extent. Enzymes act as catalysts because they lower the free energy of activation for a reaction. They do this by a combination of raising the ground state ΔG of the substrate and lowering the ΔG of the transition state of the reaction, thereby decreasing the barrier against the reaction. The energy derived from enzyme-substrate interaction is called **binding energy**. Its significance extends beyond a simple stabilization of the enzyme-substrate interaction. Binding energy is a major source of free energy used by enzymes to lower the activation energies of reactions. Weak binding interactions between the enzyme and the substrate provide a substantial driving force for enzymatic catalysis. The presence of the enzyme leads to a new reaction pathway that is different from that of the uncatalysed reaction (Fig-12.4).

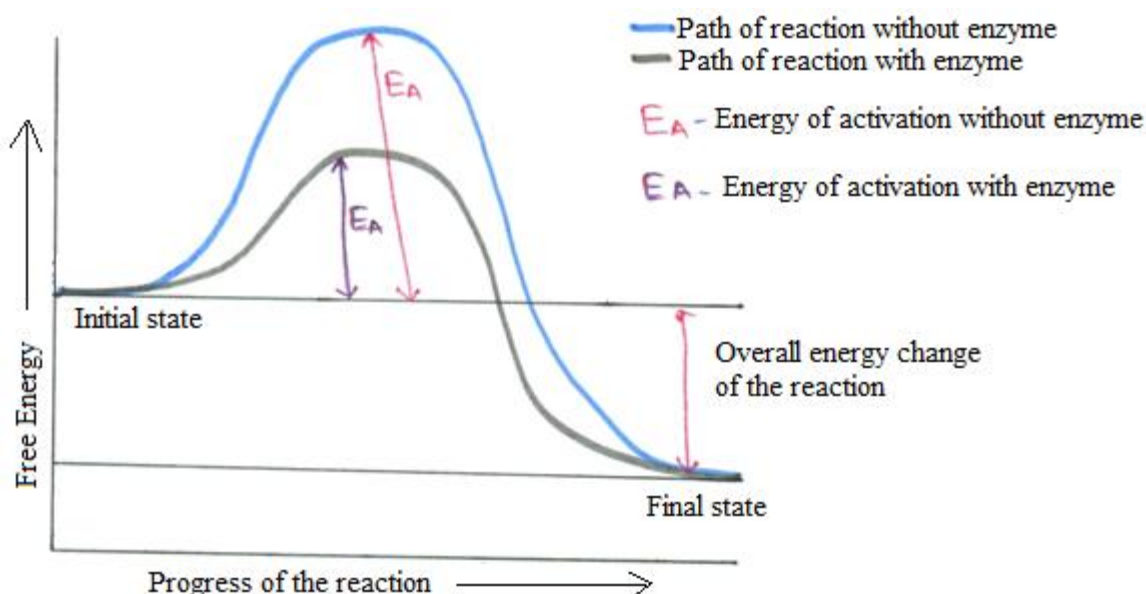


Fig.12.4: Free energy curves for the same reaction, either uncatalysed or enzyme catalysed. An enzyme catalysis lowers the E_A between substrate and products compared with the uncatalysed reaction

ENZYME ACTIVITY

Enzymes are usually present in very small quantities. So, an easy method of enzyme quantification is a measurement of catalytic activity. There are two standard units to express enzymatic activity-

1. **Enzyme unit (U):** The amount of enzyme causing conversion of a $1\mu\text{mole}$ of substrate per minute at 25°C . { **1 enzyme unit = $1\mu\text{mol min}^{-1}$** }
2. **Katal (Kat):** The katal is the most accepted SI unit of the enzyme activity. One katal is that amount of enzyme that catalysed the conversion of 1mol of substrate per second. { **1 Kat = 1mol sec^{-1}** }

12.6.5 - Mode of Action

1. Lock and Key model: This model was proposed by a German chemist **Emil Fischer** in 1898. According to this model, lock is analogous to enzymes and its socket (in which key fits) is analogous to active site, while key is analogous to substrate. It is believed that the enzyme and substrate both have strictly complementary structures which during complex formation fit to each other like a specific key in a particular lock. This model explains enzyme specificity in which a substantially rigid active site is likened to a 'lock' and the substrate to a 'key' that fits the lock. The enzyme-substrate complex dissociates only after the conversion of substrate into products and the enzyme becomes free and available for further reactions.

2. Induced Fit model: This model was proposed by **Daniel Koshland** in 1966. According to this model, the enzyme and substrate do not have strictly complementary structures but the enzyme has flexible active site structure which is changed according to substrate configuration (Fig.-12.5). This model explains enzyme specificity in which a flexible active site is induced, by a substrate, to change its conformation to an orientation properly fitting the substrate's geometry. Enzyme-substrate complex brings about conformational change in active site, in such a fashion so that catalytic group lies opposite the substrate bonds to be broken. ES binding forces exert strain to form products.

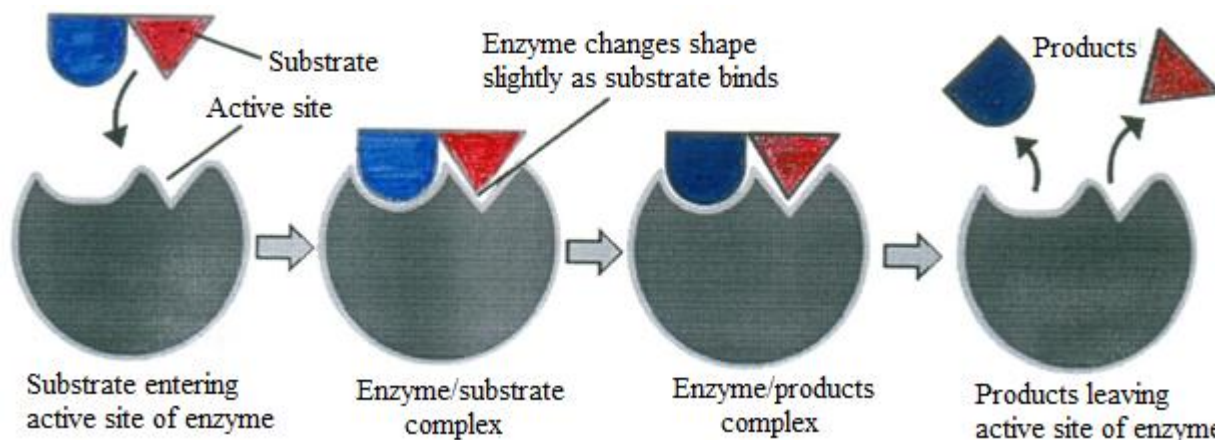
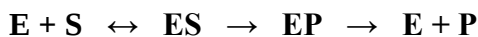


Fig.12.5: Diagram of induced fit model, representing the flexible nature of the active site of the enzymes

12.6.6 - Enzyme Kinetics

In 1913, **Leonor Michaelis** and **Maud Menten** proposed a simple model to account for the kinetic characteristics of enzymes. The enzyme kinetics is characterized by a formation of hyperbolic relationship between reaction velocity, v and substrate concentration $[S]$. An enzyme that exhibits such reaction are said to follow **Michaelis–Menten kinetics**. This type of plot is known as a **saturation plot** because when the enzyme becomes saturated with substrate with increasing substrate concentration at constant enzyme concentration. Enzymes kinetics is the quantitative study of enzyme catalysis which depends on the substrate concentration. At the maximum speed of reaction ($V_{\max}$), all the enzymes active sites are bound to substrate and the

amount of ES complex is the same as the total number of enzymes. According to this model enzyme catalysis takes place in two stages. In the first stage, formation of a specific ES complex and this complex is also called as **Michaelis-Menten complex** in their honor. In the second stage, enzyme catalyses the reaction and form the product.



Where, E represents the enzyme, S the substrate, P the product, and ES the enzyme–substrate complex. Thus, as the substrate concentration is increased, a point will reach at which all the enzyme molecules are in the form of the ES complex, and the enzyme is saturated with substrate. Since the rate of the reaction depends on the concentration of ES, the rate will not increase further, because there can be no higher concentration of ES. When an enzyme is mixed with excess of substrate, there will be an initial very short time period (usually milliseconds) during which the concentration of enzyme–substrate complexes and intermediates build up to certain level; this is known as the **pre–steady-state period**. Once the intermediate levels have been built up, they remain relatively constant until the substrate is depleted; this period is known as the **steady state**.

Normally enzyme kinetic values are measured under steady-state conditions, and such conditions usually prevail in the cell. For many enzyme-catalyzed reactions the kinetics under steady-state conditions can be described by a simple expression known as the **Michaelis–Menten equation**:

$$v = V_{\max} [S] / K_m + [S]$$

Where, v is the initial rate of the reaction, $V_{\max}$ is the maximum substrate-saturated rate of the reaction and the K_m is the substrate concentration that provides the half of the maximal substrate-saturated rate of the reaction ($1/2 V_{\max}$). The maximal rate $V_{\max}$, occurs when all the active site of the enzyme molecule are fully occupied by substrate and Michaelis constant K_m , represents the affinity of enzyme for the substrate. Smaller the value of K_m means higher will be the affinity of enzyme to substrate i.e. K_m is inversely proportional to the affinity.

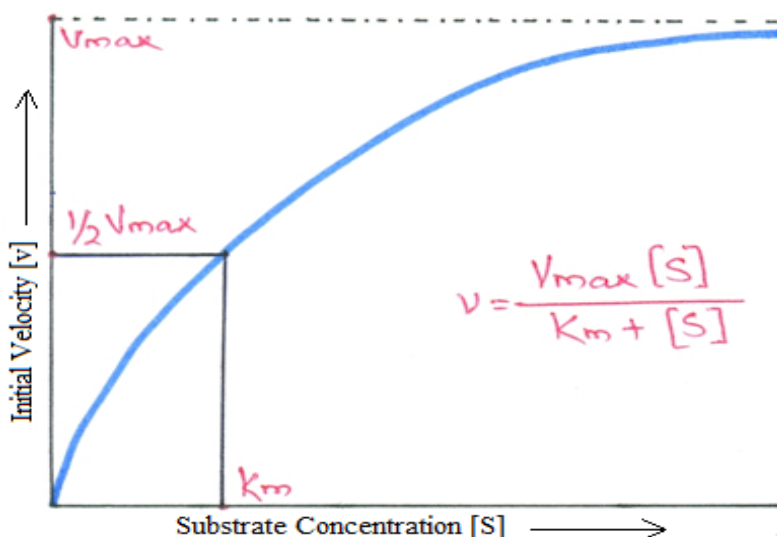


Fig.12.3: Plot of initial velocity versus substrate concentration, for an enzyme catalysed reaction. The curve is hyperbolic.

12.6.7 - Enzyme Inhibitors

A variety of small molecules exists which can reduce the rate of an enzyme controlled reaction. They are called enzyme inhibitors. Enzyme inhibitors may be reversible or irreversible. Irreversible inhibitors bound covalently to the enzymes and destroy the functional group of the active site. Most of the irreversible inhibitors are toxic substances or antibiotics and are tightly bound to the enzymes.

Reversible inhibitors can dissociate from the enzyme because they bind non-covalently to the enzymes. Reversible inhibitors are of three different types; competitive, non-competitive and mixed type.

1. Competitive inhibition: Competitive inhibition is the simplest and most common form of reversible inhibition. It binds to the enzyme at active site with an affinity similar to or stronger than that of the substrate. The competitive inhibitor forms an enzyme-inhibitor complex [EI] that is equivalent to enzyme-substrate complex [ES]. Competitive inhibition is usually based on the fact that the structure of the inhibitor resembles that of the substrate; hence the strong affinity of the inhibitor for the active site. The effect of competitive inhibitor is reversed by increasing the substrate concentrations. At high substrate concentration, all the active sites are filled with the substrates and reaction velocity similar to the value observed without an inhibitor. The diagnostic property of this type of inhibition is that $V_{\max}$ is same and K_m increases, but such inhibitors does not affect the 'turn over number'.

2. Non-competitive inhibition: In noncompetitive inhibition, the inhibitor does not compete with the substrate for binding to the active site. Instead, it may bind to another site and obstruct the substrate's access to the active site because binding alters the 3-D structure of the enzyme, or it may bind to the enzyme-substrate complex and thus alter catalysis. Noncompetitive inhibition is not reversed by increasing substrate concentration. The diagnostic property of this type of inhibition is that K_m is unaffected, whereas $V_{\max}$ decreases in the presence of increasing amounts of inhibitor.

3. Mixed inhibition: Mixed inhibition is characterized by effects on both $V_{\max}$ (which decreases) and K_m (which increases). Mixed inhibition is very common and results from the formation of a complex consisting of the enzyme, the substrate, and the inhibitor that does not break down to products.

12.7 SUMMARY

An enzyme is a protein that catalyzes a chemical reaction by lowering the activation energy. They are not changed or used in reactions, so only a small amount of enzyme is needed. They are highly specific for their substrate and functionally depend on their three-dimensional structure, which is sensitive to temperature and pH. The actual catalytic process takes place at the active site via formation of enzyme-substrate complex. The 'lock and key' hypothesis provide reason

for the specificity of the active site, according to which the shape of the active site is complementary to that of the substrate. While 'induced-fit' hypothesis revealed that the enzyme-substrate interaction may cause a conformational change in the active site. Enzymes adjust their shape to the substrate so there is a better fit. Some enzymes require cofactors for their catalytic activity. Such enzymes use inorganic molecule or complex organic molecules, which become bound during the reaction to activate the enzyme. The velocity of the enzyme-catalyzed reactions depends on the substrate concentration. The characteristic curve described in this reaction is a hyperbola that reaches a maximum velocity ($V_{\max}$) when all the enzyme active sites are saturated. Enzymes activity is regulated by reversible and irreversible inhibition in the cells. Irreversible inhibitors typically form covalent bonds while reversible inhibitors form non-covalent bonds with the enzyme and may have competitive, non-competitive and mixed effect. The competitive inhibitors compete for the enzyme's active site, while noncompetitive inhibitor binds to the allosteric site of the enzyme.

12.8 GLOSSARY

Abzyme:	The catalytic antibody.
Activation energy:	The minimum energy required to convert a normal reactant molecule into a reactive intermediate.
Active site:	The region on the surface of an enzyme where catalytic activity occurs.
Allosteric enzyme:	Enzymes which contains two or more receptor sites.
Amino acids:	A group of organic compounds having the general formula $\text{NH}_4\text{C}_2\text{O}_2\text{R}$.
Apoenzyme:	It is a protein part of holoenzyme.
Binding energy:	Amount of energy required to separate particles from a system.
Biocatalyst:	A macromolecules of biological origin.
Catalyst:	Substance that facilitates a chemical reaction without being consumed or modified.
Cofactor:	It is a non-protein chemical compound or metallic ion of some enzymes.
Denature:	The loss of normal spatial arrangement of enzymes.
E.C. number:	Every enzyme is designated by a four digit code numbers, Enzyme Commission number.
Fermentation:	It is a metabolic process that converts sugar to alcohol.
Holoenzyme:	It is a conjugate enzyme consisting of both protein and non-protein components.
Inhibitor:	A molecule that binds to an enzyme and decreases its activity, the process is called inhibition.
Isoenzyme:	Enzymes that differ in amino acid sequences but catalyze the same reaction.
Katal:	Enzyme activity that converts one mole of substrate per second under standard reaction condition.

Kinetics:	The study of motion and its causes.
Macromolecules:	A very large molecule, created by polymerization of smaller subunits.
Modulators:	A substance which indirectly influence the activity of enzymes.
Oxidation:	A reaction in which an element combines with oxygen (O ₂).
Prosthetic group:	It may be either organic or inorganic molecule which tightly bound to apoenzyme.
Proteins:	Polymers composed of amino acids.
Quaternary structure:	The overall spatial structure of a protein containing more than one polypeptide chain.
Reduction:	A reaction in which an element combines with hydrogen (H ₂).
Ribonucleic acid:	It is a polymeric molecule made up of one or more ribonucleotides.
Ribozyme:	Catalytic RNA molecule.
Substrate:	A molecule on which enzyme acts.
Tertiary structure:	Overall three-dimensional folding of a protein chain.
Thermo labile:	Substances which is change or degrade in response to heat.
Turn over number:	The number of moles of substrate converted to product per mole of enzyme per second.
Zymogen:	Inactive precursors of enzymes or proenzymes.

12.9 SELF-ASSESSMENT QUESTION

12.9.1 – Very short answer questions:

1. What are enzymes?
2. What is the chemical nature of enzymes?
3. Who discovered the enzyme?
4. Which enzyme was first crystallized?
5. What is the full form of E.C. number?
6. Name the factors, which affect the enzyme catalysis?
7. Why enzymes are colloidal in nature?
8. What is the common name of inactive form of enzyme?
9. Who proposed 'Induced fit model' of enzymology?
10. Which major class of enzymes is responsible for transfer of functional group from one molecule to another?

12.9.2 – Multiple choice questions:

- 1- Which of the following statements about enzymes is true-
 - (a) Enzymes are biocatalyst.
 - (b) Enzymes are mostly proteins.
 - (c) Enzymes speed up reactions by lowering activation energy.

(d) All of the above

2- Enzymes increases the rate of reactions by-

- (a) Shifting the equilibrium point.
- (b) Lowering the activation energy
- (c) Using allosteric site along with active site
- (d) None of the above

3-Enzyme catalyzed reactions differ from ordinary reaction by-

- (a) Slow rate of reaction
- (b) Low specificity
- (c) Their regulatory control
- (d) None of the above

4-Which of the following statements is not true regarding the active site of an enzyme-

- (a) An active site is three dimensional structures
- (b) Active site bound to substrate by multiple strong bonds
- (c) An active site is place for making and breaking of bonds
- (d) Active sites are cleft or crevices

5-Ribozymes are-

- (a) Proteins
- (b) Antibodies
- (c) Ribonucleic acid
- (d) Lipids

6-False regarding K_m is-

- (a) K_m = concentration of substrate at $\frac{1}{2} V_{max}$
- (b) K_m is inversely proportional to the affinity
- (c) K_m is increased in non-competitive inhibition
- (d) K_m is increased in competitive inhibition

7-The enzymes where catalysis involves transfer of electrons are named as-

- (a) Oxidoreductase
- (b) Transferases
- (c) Hydrolases
- (d) Isomerases

8-Which one of the following is a cofactor and not a coenzyme-

- (a) Biotin
- (b) FAD
- (c) NAD
- (d) Zn^{2+}

9-An allosteric modulator influences enzyme activity by-

- (a) Competing for the active site with the substrate
- (b) Binding to a site on the enzyme molecule distinct from the active site
- (c) Changing the specificity of the enzyme for its substrate
- (d) None of the above

10-Which of the following is true about competitive enzyme inhibitors-

- (a) Inhibitor and substrate are similar in structure
- (b) Inhibitor has strong affinity for active site
- (c) Higher substrate concentration decreases the efficiency of inhibitor
- (d) All the above

11- $A-B + H_2O \leftrightarrow A-H + B-OH$, this reaction is catalyzed by the enzyme-

- (a) Oxidoreductase
- (b) Transferases
- (c) Hydrolases
- (d) Isomerases

12- At high temperature enzyme loses its catalytic activity because-

- (a) Enzymes denatured
- (b) Enzymes loses its three dimensional structure
- (c) Enzyme's active site unable to bind substrate
- (d) All of the above

13-If E.C. number of any enzyme is 6.4.1.1, then enzyme belong to class-

- (a) Oxidoreductase
- (b) Lyases
- (c) Isomerases
- (d) Ligases

14-Which is the correct Michaelis–Menten equation-

- (a) $v = V_{\max} [S] / K_m + [S]$
- (b) $v = K_m + [S] / V_{\max} [S]$
- (c) $v = K_m [S] / V_{\max} + [S]$
- (d) $v = V_{\max} [S] / [S] + [S]$

15-Which of the following is not true about non-competitive enzyme inhibitors-

- (a) $V_{\max}$ is unaffected.
- (b) Inhibitors does not resembles to the substrate.
- (c) Binds to the enzyme at another site.
- (d) Not reversed by increasing substrate concentration.

12.9.3 Fill up the following blanks:

- 1-The word enzyme discovered by
- 2- Enzymes are in nature.
- 3- Binding energy released in enzyme-substrate complex formation causes the in the activation energy.
- 4- Enzyme commission numbers consisting of digits.
- 5- Enzymes do not alter the of a chemical reaction.
- 6- Lower value of Michaelis constant (K_m) shows affinity of the enzyme for the substrate.
- 7- Catalytic power of enzyme is measured in terms of
- 8- Catalytic antibodies function as enzymes are known as

- 9- The K_m of an enzyme is the substrate concentration that gives maximum velocity.
10- Isoenzymes can be characterized by the differences in their sequences.
11- Lock & key model was proposed by and Induced fit model was proposed by
12- ES complex also known as
13- Protein part of holoenzymes are called as
14- The active site is a dimensional structure.
15- In feedback inhibition blocks enzyme catalysis.

12.9.1 Answer Key – 1. Biocatalyst, 2. Proteins, 3. Edward Buchner, 4. Urease, 5. Enzyme Commission number, 6. Temperature & pH, 7. High Molecular Weight, 8. Zymogen, 9. D. Koshland, 10. Transferase

12.9.2 Answer Key – 1. (d), 2. (d), 3. (c), 4. (b), 5. (c), 6. (c), 7. (a), 8. (d), 9. (b), 10. (d), 11. (c), 12. (d), 13. (b), 14. (a), 15. (a)

12.9.3 Answer Key – 1. F.W. Kunhe, 2. Proteinaceous, 3. Decrease, 4. Four, 5. Chemical equilibrium point, 6. Greater, 7. Turn over number, 8. Abzymes, 9. Half, 10. Amino acid, 11. E. Fischer, D.Koshland, 12. Michaelis-Menten complex, 13. Apoenzyme, 14. Three, 15. End product

12.10 REFERENCES

- A.L. Lehninger, D.L. Nelson and M.M. Cox (1993), *Principles of Biochemistry* (second edition), CBS Publishers & Distributors, Delhi.
- D.B. Northrop (1999), Rethinking of enzyme activity. *Adv. Enzymol.* 73: 25.
- D.J. Taylor, N.P.O. Green and G.W. Stout (editor: R. Scoper) (1997), *Biological Science* (third edition), Cambridge University Press.
- G.G. Hammes (2008), How do enzymes really work? *J. Biol. Chem.* 283: 22337.
- H. Suzuki (2015), *How enzyme work : from structure to function*, CRC Press.
- J.B. Losos, K.A. Mason and S.R. Singer (2010), *Biology* (eighth edition), Tata McGraw Hill Education Private Limited, New Delhi.
- J.M. Berg, J.L. Tymoczko, L. Stryer and G.J. Gatto (2012), *Biochemistry* (seventh edition), W.H. Freeman & Company, New York.
- T. Palmer (1985), *Understanding enzymes*, Ellis Horwood, London.
- T.R. Cech (1987), The chemistry of self-splicing RNA and RNA enzymes. *Science* 236: 1532.
- W.M. Becker, L.J. Kleinsmith and J. Hardin (2005), *The world of the cell* (sixth edition), Pearson Benjamin Cummings Publication, San Francisco.
- W.W. Cleland (1977), Determining the chemical mechanisms of enzyme-catalysed reactions by kinetic studies. *Adv. Enzymol.* 45: 273.

12.11 SUGGESTED READINGS

- A.L. Lehninger, D.L. Nelson and M.M. Cox (1993), *Principles of Biochemistry* (second edition), CBS Publishers & Distributors, Delhi.
- D.J. Taylor, N.P.O. Green and G.W. Stout (editor: R. Scoper) (1997), *Biological Science* (third edition), Cambridge University Press.
- D.L. Purich and R.D. Allison (2002), *The Enzyme Reference: A Comprehensive Guidebook to Enzyme Nomenclature, Reactions and Methods*, Academic Press, Boston.
- E.D.P. De Robertis and E.M.F. De Robertis (2001), *Cell and Molecular Biology* (eighth edition), Wolters Kluwer (India) Pvt. Ltd., New Delhi
- J.B. Losos, K.A. Mason and S.R. Singer (2010), *Biology* (eighth edition), Tata McGraw Hill Education Private Limited, New Delhi.
- J.M. Berg, J.L. Tymoczko, L. Stryer and G.J. Gatto (2012), *Biochemistry* (seventh edition), W.H. Freeman & Company, New York.
- R.A. Copeland (2000), *Enzymes: A Practical Introduction to Structures, Mechanisms and Data Analysis*, Wiley Publications, New York.
- T. Palmer (1985), *Understanding enzymes*, Ellis Horwood, London.
- W.M. Becker, L.J. Kleinsmith and J. Hardin (2005), *The world of the cell* (sixth edition), Pearson Benjamin Cummings Publication, San Francisco.
- <http://en.wikipedia.org/wiki/enzyme>
- www.rsc.org/education/teachers/resources/cfb/enzymes.htm
- www.users.rcn.com/jkimball.ma.ultranet/biologypages/e/enzymes.html

12.12 TERMINAL QUESTIONS

12.12.1 - Long answer type questions:

- 1 – Describe the mechanism of enzyme's reaction?
- 2 – Write a note on holoenzymes and give the table of cofactors of enzymes?
- 3 – Why enzymes are able to catalyse the reactions and justify your answer with diagrams?
- 4 – What do you understand by enzyme inhibitors and describe its type?
- 5 – Comments on the mode of action of enzymes with diagrams?

12.12.2 - Short answer type questions:

- 1 – Write short notes on enzymes?
- 2 – Comments on the major classes of the enzymes?
- 3 – Describe the properties of enzymes?
- 4 – Differentiate the 'Lock & Key' and 'Induced Fit' Model?
- 5 – What is the Ribozymes?
- 6 – Write down the characteristics of the enzymes?

- 7 – What do you understand from 'allosteric enzymes'?
- 8 – Comments on the enzyme kinetics?
- 9 – What is 'isoenzyme'?
- 10 – What is the difference between competitive and non-competitive inhibitors?

UNIT-13 BIOCHEMICAL TECHNIQUES

Content

13.1 Objectives

13.2 Introduction

13.3 Biochemical techniques

13.3.1 Chromatography

13.3.2 Spectrophotometry

13.3.3 Centrifugation

13.3.4 Colorimetry

13.3.5 Autoradiography

13.3.6 X-Ray Diffraction

13.3.7 Electrophoresis

13.4 Summary

13.5 Glossary

13.6 Self Assessment Question

13.7 References

13.8 Suggested Readings

13.9 Terminal Questions

13.1 OBJECTIVES

After reading this unit student will be able-

- To study about Biochemical techniques which are used in biological Science
- to know about Chromatography, Spectrophotometry, Centrifugation, Colorimetry, Autoradiography, X-Ray Diffraction, Electrophoresis

13.2 INTRODUCTION

Biochemical techniques are various types of methods, assays, and procedures that are used to analyze the substances found in living organisms and to identify chemical reactions underlying life processes. They are essential tools to carry out research and diagnostic procedures in laboratories. They can be used to isolate biomolecules in pure form or can be used to detect biomolecules in vivo or in vitro conditions. Using these techniques thousands of molecules could be identified and characterized in an extracts from a biological sample and analyzed their function. The common Biochemical techniques are microscopy, chromatography, electrophoresis, colorimetric assays etc.

13.3 BIOCHEMICAL TECHNIQUES

The common Biochemical techniques are described below-

13.3.1 Chromatography

Chromatography is an analytical technique commonly used for separating a mixture of chemical substances into its individual components.

In this method, the separation of the components of a mixture is a function of their different affinities for a fixed or stationary phase (such as a solid or a liquid) and their differential solubility in a moving or mobile phase (such as a liquid or a gas). Separation starts to occur when one component is held more firmly by the stationary phase than the other which tends to move on faster in the mobile phase.

Chromatographic methods can be classified according to:

1-Mobile phase arrangement

- (a) Liquid chromatography (LC) - mobile phase is a liquid
- (b) Gas chromatography (GC) - mobile phase is a gas.

2- Stationary phase arrangement

- (a) Column chromatography – stationary phase is placed in a column
- (b) Planar techniques:

(i) **Paper chromatography (PC)** – stationary phase is a special paper, either as such or modified with other compounds.

(ii) **Thin-layer chromatography (TLC)** – stationary phase is spread on a solid flat support (e.g., glass plate or aluminum foil)

Types of chromatography

1-Paper chromatography

Paper chromatography is a technique that involves placing a small dot or line of sample solution onto a strip of chromatography paper. When sample solution get dried, the edge of the paper is immersed in a solvent, and the solvent moves up the paper by capillary action. The distance travelled by a particular component of a mixture (or solute) is used to identify it. The ratio of the distance travelled by a component (i.e., amino acid) to that travelled by the solvent front, both measured from the marked point of application of the mixture, is called the resolution front (Rf) value for that component.

Thus,

$$R_f = \frac{\text{Distance from origin run by the compound}}{\text{Distance from origin run by the solvent}}$$

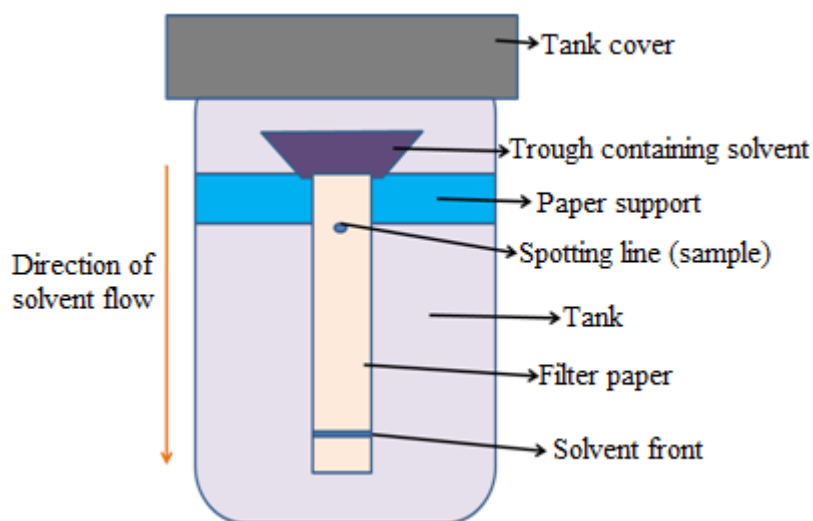


Fig.13.1- One-dimensional paper chromatography (Ascending type)

2-Thin layer chromatography

Thin-Layer chromatography (TLC) is an easy-to-use, fast and highly versatile separation technique for qualitative and quantitative analysis. Thin-Layer Chromatography is performed on a glass, plastic, or aluminum plate, which is coated with a thin layer of adsorbent material. The sample is applied on one end of the plate, and a suitable solvent is allowed to rise up the plate by capillary forces. Since different substances are moving up the Thin-Layer Chromatography plate at different rates, they can be separated, identified and analyzed.

3-Column chromatography

The most powerful methods for fractionating proteins make use of column chromatography, which takes advantage of differences in protein charge, size, binding affinity, and other properties. A porous solid material with appropriate chemical properties (the stationary phase) is held in a column, and a buffered solution (the mobile phase) percolates through it. The protein-containing solution, layered on the top of the column, percolates through the solid matrix as an ever-expanding band within the larger mobile phase. Individual proteins migrate faster or more slowly through the column depending on their properties.

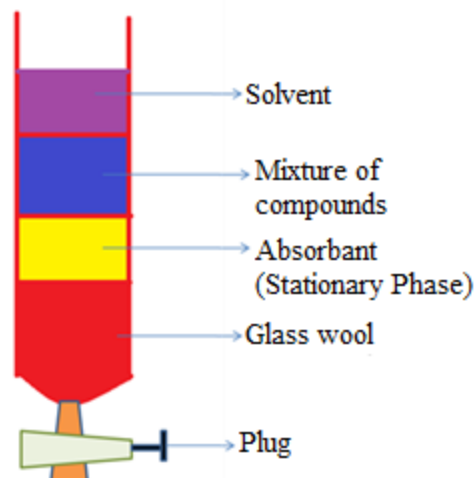


Fig.13.2- Column Chromatography

4- Size-exclusion chromatography

Size exclusion chromatography is one of the HPLC separation modes. The column used is filled with material containing many pores. When dissolved molecules of various sizes flow into the column, smaller dissolved molecules flow more slowly through the column because they penetrate deep into the pores, whereas large dissolved molecules flow quickly through the column because they do not enter the pores. Consequently, larger molecules elute from the column sooner and smaller molecules later, which effectively sorts the molecules by size. This is the separation principle of size exclusion chromatography.

The gel particles are usually in bead form and consist of one of two kinds of polymers. The first is a carbohydrate polymer, such as dextran or agarose; these two polymers are often referred to by the trade names Sephadex and Sepharose, respectively. The second is based on polyacrylamide, which is sold under the trade name Bio-Gel.

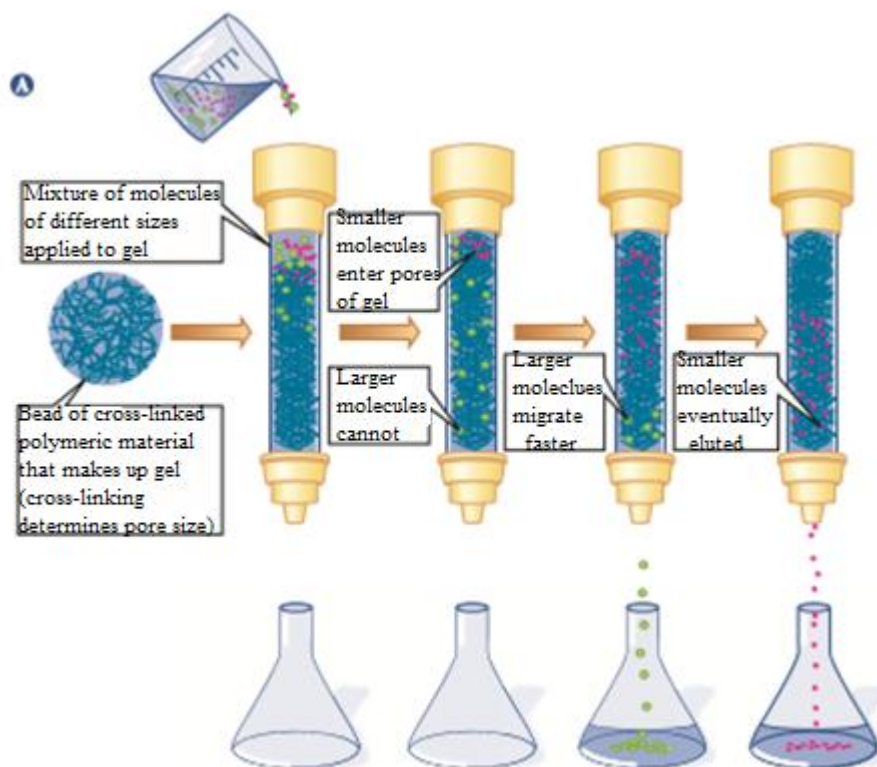


Fig.13.3-Gel-filtration chromatography, Larger molecules are excluded from the gel and move more quickly through the column. Small molecules have access to the interior of the gel beads, so they take a longer time to elute.

5-Affinity chromatography

Affinity chromatography method is based on the fact that any given biomolecule that we wish to purify usually has an inherent recognition site through which it can be bound by a natural or artificial molecule. Thus, affinity chromatography is principally based on the molecular recognition of a target molecule by a molecule bound to a column.

Affinity purification involves 3 main steps:

- (a) Incubation of a crude sample with the affinity support to allow the target molecule in the sample to bind to the immobilized ligand.
- (b) Washing away non-bound sample components from the support.
- (c) Elution (dissociation and recovery) of the target molecule from the immobilized ligand by altering the buffer conditions so that the binding interaction no longer occurs.

6-Ion-exchange chromatography

Ion-Exchange Chromatography (IEC) allows for the separation of ionizable molecules on the basis of differences in charge properties. Its large sample-handling capacity, broad applicability (particularly to proteins and enzymes), moderate cost, powerful resolving ability, and ease of

scale-up and automation have led to it becoming one of the most versatile and widely used of all liquid chromatography (LC) techniques.

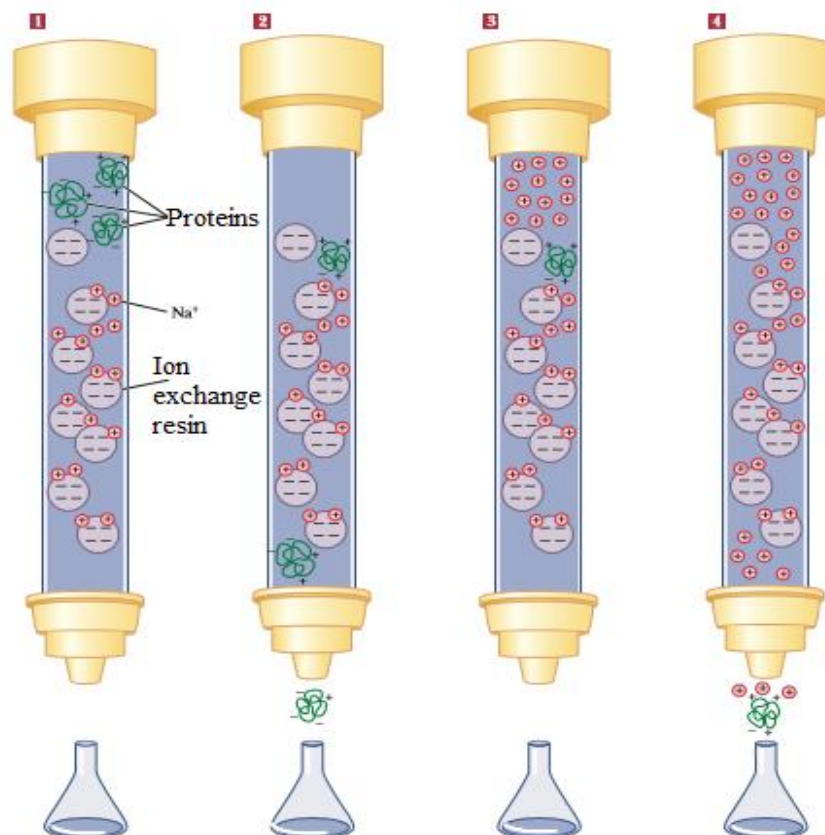


Fig.13.4- Ion-exchange chromatography using a cation exchanger. (1) At the beginning of the separation, various proteins are applied to the column. The column resin is bound to Na⁺ counterions (small red spheres). (2) Proteins that have no net charge or a net negative charge pass through the column. Proteins that have a net positive charge stick to the column, displacing the Na⁺. (3) An excess of Na⁺ ion is then added to the column. (4) The Na⁺ ions outcompete the bound proteins for the binding sites on the resin, and the proteins elute.

7-Gas chromatography (GC), is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture.

In gas chromatography, the *mobile phase* (or "moving phase") acts as a carrier gas which is usually an inert gas such as helium or an unreactive gas such as nitrogen. The *stationary phase* is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column (a homage to the fractionating column used in distillation). The

instrument used to conduct gas chromatography is known a *gas chromatograph* (or "aerograph", "gas separator").

The gaseous compounds being analyzed interact with the walls of the column, which is coated with a stationary phase. This causes each compound to elute at a different time, known as the *retention time* of the compound.

8-HPLC

High Performance Liquid Chromatography (HPLC) exploits the same principle that is applied in other chromatographic techniques, but very high resolution columns that can be run under high pressures are used. High resolution separations can be effected very quickly using automated instrumentation. A separation that might take hours on a standard column can be done in minutes with HPLC.

13.3.2-Spectrophotometry

Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. Spectrophotometry is a versatile analytical tool. The underlying principle of spectrophotometry is to shine light on a sample and to analyze how the sample affects the light.

Advantages of spectrophotometry are:

- 1) It is often non-destructive (i.e., can measure and recover sample),
- 2) It is selective (often a particular compound in a mixture can be measured without separation techniques),
- 3) It has a short time interval of measurement (10-14 seconds).

Principles of Spectrophotometry

When monochromatic light (light of a specific wavelength) passes through a solution there is usually a quantitative relationship (Beer's law) between the solute concentration and the intensity of the transmitted light, that is,

$$I = I_0 * 10^{-kcl} \quad I = I_0 * 10^{-kcl} \quad I = I_0 * 10^{-kcl} \quad I = I_0 * 10^{-kcl} \quad I = I_0 * 10^{-kcl}$$

$$I = I_0 * 10^{-kcl}$$

where I_0 is the intensity of transmitted light using the pure solvent, I is the intensity of the transmitted light when the colored compound is added, c is concentration of the colored compound, l is the distance the light passes through the solution, and k is a constant. If the light path l is a constant, as is the case with a spectrophotometer, Beer's law may be written,

$$I \div I_0 = 10^{-kc} = T$$

$$I \div I_0 = 10^{-kc} = T$$

where k is a new constant and T is the transmittance of the solution. There is a logarithmic relationship between transmittance and the concentration of the colored compound. Thus,

$$-\log T = \log 1/T = kc = \text{optical density (O.D.)}$$

The O.D. is directly proportional to the concentration of the colored compound. Most spectrophotometers have a scale that reads both in O.D. (absorbance) units, which is a logarithmic scale, and in % transmittance, which is an arithmetic scale. As suggested by the above relationships, the absorbance scale is the most useful for colorimetric assays.



Fig.13.5 -Spectrophotometer

The instrument that is used to measure O.D. is known as spectrophotometer. A spectrophotometer consists of two instruments, namely a *spectrometer* for producing light of any selected wavelength), and a *photometer* for measuring the intensity of light. The instruments are arranged so that liquid in a cuvette can be placed between the spectrometer beam and the photometer. The amount of light passing through the tube is measured by the photometer. The photometer delivers a voltage signal to a display device, normally a galvanometer. The signal changes as the amount of light absorbed by the liquid changes.

If development of color is linked to the concentration of a substance in solution then that concentration can be measured by determining the extent of absorption of light at the appropriate wavelength. For example hemoglobin appears red because the hemoglobin absorbs blue and green light rays much more effectively than red. The degree of absorbance of blue or green light is proportional to the concentration of hemoglobin.

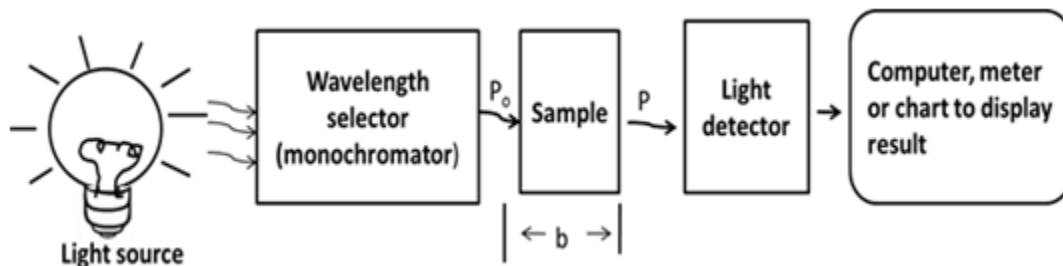


Fig.13.6-Diagram of Spectrophotometer

UV-visible spectrophotometry

The most common spectrophotometers are used in the UV and visible regions of the spectrum, and some of these instruments also operate into the near-infrared region as well.

Visible region 400–700 nm spectrophotometry is used extensively in colorimetry science. Ink manufacturers, printing companies, textiles vendors, and many more, need the data provided through colorimetry. They take readings in the region of every 5–20 nanometers along the visible region, and produce a spectral reflectance curve or a data stream for alternative presentations. These curves can be used to test a new batch of colorant to check if it makes a match to specifications, e.g., ISO printing standards.

Applications

- (i) Estimating dissolved organic carbon concentration
- (ii) Specific Ultraviolet Absorption for metric of aromaticity
- (iii) Bial's Test for concentration of pentoses

13.3.3-Centrifugation

Centrifugation is a process that involves the use of the centrifugal force for the sedimentation of mixtures with a centrifuge. It is used in industry and in laboratory settings.

Basic Principle of Sedimentation-

$$\text{Relative centrifugal force } F = M \omega^2 r$$

M: mass of particle

r: radius of rotation (cm) (i.e distance of particle from axis of rotation)

ω : Average angular velocity (radians/sec)

Rev: revolution per minute (r.p.m.)

1 revolution = 2π radians = 360

The rate of centrifugation is specified by the angular velocity measured in revolutions per minute (RPM).

The most common application is the separation of solid from highly concentrated suspensions, which is used in the treatment of sewage sludges for dewatering where less consistent sediment is produced.

Centrifuge

A **centrifuge** is a piece of equipment, generally driven by an electric motor (or, in some older models, by hand), that puts an object in rotation around a fixed axis, applying a force perpendicular to the axis. A centrifuge is also used to separate the components of blood in blood banks. The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances to separate out along the radial direction (the bottom of the tube). By the same token lighter objects will tend to move to the top (of the tube; in the rotating picture, move to the centre).

Types

Types by rotor design:

- (a) Fixed-angle centrifuges are designed to hold the sample containers at a constant angle relative to the central axis.
- (b) Swinging head (or swinging bucket) centrifuges, in contrast to fixed-angle centrifuges, have a hinge where the sample containers are attached to the central rotor. This allows the samples to swing outwards as the centrifuge is spun.
- (c) Continuous tubular centrifuges don't have individual sample vessels and are used for high volume applications.

Microcentrifuges

Microcentrifuges are used to process small volumes of biological molecules, cells, or nuclei. Microcentrifuge tubes generally hold 0.5 - 2.0 mL of liquid, and are spun at maximum angular speeds of 12,000–13,000 rpm. Microcentrifuges are small enough to fit on a table-top and have rotors that can quickly change speeds. They may or may not have a refrigeration function.

High-speed centrifuges

High-speed or superspeed centrifuges can handle larger sample volumes, from a few tens of millilitres to several litres. Additionally, larger centrifuges can also reach higher angular velocities (around 30,000 rpm). The rotors may come with different adapters to hold various sizes of test tubes, bottles, or microtiter plates.

Ultracentrifuges

Ultracentrifugation makes use of high centrifugal force for studying properties of biological particles. Compared to microcentrifuges or high-speed centrifuges, ultracentrifuges can isolate much smaller particles, including ribosomes, proteins, and viruses. Ultracentrifuges can also be used in the study of membrane fractionation. This occurs because ultracentrifuges can reach

maximum angular velocities in excess of 70,000 rpm. Additionally, while microcentrifuges and supercentrifuges separate particles in batches (limited volumes of samples must be handled manually in test tubes or bottles), ultracentrifuges can separate molecules in batch or continuous flow systems.

13.3.4-Colorimetry

In physical and analytical chemistry, **colorimetry** or **colourimetry** is a technique "used to determine the concentration of colored compounds in solution." A colorimeter is a device used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light (not to be confused with the tristimulus colorimeter used to measure colors in general).

The concentration of a sample can be calculated from the intensity of light before and after it passes through the sample by using the Beer–Lambert law. Photoelectric analyzers came to dominate in the 1960s.

The color or wavelength of the filter chosen for the colorimeter is extremely important, as the wavelength of light that is transmitted by the colorimeter has to be the same as that absorbed by the substance being measured. For example, the filter on a colorimeter might be set to red if the liquid is blue.

Colorimetric Assays

Colorimetric assays use reagents that undergo a measurable color change in the presence of the analyte. They are widely used in biochemistry to test for the presence of enzymes, specific compounds, antibodies, hormones and many more analytes. For example,

- (i) para-Nitrophenylphosphate is converted into a yellow product by alkaline phosphatase enzyme.
- (ii) Coomassie Blue once binding to proteins elicits a spectrum shift, allowing quantitative dosage. A similar colorimetric assay, the Bicinchoninic acid assay, uses a chemical reaction to determine protein concentration.
- (iii) Enzyme linked immunoassays use enzyme-complexed-antibodies to detect antigens. Binding of the antibody is often inferred from the color change of reagents such as TMB.

A colorimeter is a device used in colorimetry. It work on principle of Beer-Lambert law. This device measures the absorbance of particular wavelengths of light by a specific solution. This device is commonly used to determine the concentration of a known solute in a given solution.

13.3.5-Autoradiography

Radiography is the visualisation of the pattern of distribution of radiation in biological sample. In general, the radiation consists of X-rays, gamma (g) or beta (b) rays, and the recording medium is a photographic film. For classical X-rays, the specimen to be examined is placed between the

source of radiation and the film, and the absorption and scattering of radiation by the specimen produces its image on the film. In contrast, in autoradiography the specimen itself is the source of the radiation, which originates from radioactive material incorporated into it. The recording medium which makes visible the resultant image is usually, though not always, photographic emulsion. The image of autoradiography is called autoradiograph.

Autoradiography Method

1. Living cells are briefly exposed to a 'pulse' of a specific radioactive compound.
2. The tissue is left for a variable time.
3. Samples are taken, fixed, and processed for light or electron microscopy.
4. Sections are cut and overlaid with a thin film of photographic emulsion.
5. Left in the dark for days or weeks (while the radioisotope decays). This exposure time depends on the activity of the isotope, the temperature and the background radiation (this will produce with time a contaminating increase in 'background' silver grains in the film).
6. The photographic emulsion is developed (as for conventional photography).
7. Counterstaining e.g. with toluidine blue, shows the histological details of the tissue. The staining must be able to penetrate, but not have an adverse affect on the emulsion.
8. Alternatively, pre-staining of the entire block of tissue can be done (e.g. with Osmium on plastic sections coated with stripping film [or dipping emulsion] as in papers by McGeachie and Grounds) before exposure to the photographic emulsion. This avoids the need for individually (post-) staining each slide.
9. It is not necessary to cover slip these slides
10. The position of the silver grains in the sample is observed by light or electron microscopy
Note: the grains are in a different plane of focus in the emulsion overlying the tissue section. Often oil with a x100 objective is used for detailed observation with the light microscope.
11. These autoradiographs provide a permanent record.
12. Full details on the batch of emulsion used, dates, exposure time and conditions should be kept for each experiment.

13.3.6-X-Ray Diffraction

The spacing of atoms in a crystal lattice can be determined by measuring the locations and intensities of spots produced on photographic film by a beam of x rays of given wavelength, after the beam has been diffracted by the electrons of the atoms. For example, x-ray analysis of sodium chloride crystals shows that Na and Cl ions are arranged in a simple cubic lattice. The spacing of the different kinds of atoms in complex organic molecules, even very large ones such as proteins, can also be analyzed by x-ray diffraction methods. However, the technique for analyzing crystals of complex molecules is far more laborious than for simple salt crystals. When the repeating pattern of the crystal is a molecule as large as, say, a protein, the numerous atoms in the molecule yield thousands of diffraction spots that must be analyzed by computer.

13.3.7-Electrophoresis

Electrophoresis is a technique used in laboratories in order to separate charged macromolecules based on size under electric effect. The electrophoresis is commonly used to separate and analyze DNA, RNA and protein molecules in biology.

Electrophoresis of positively charged particles (cations) is called cataphoresis, while electrophoresis of negatively charged particles (anions) is called anaphoresis.

Specific type of Electrophoresis commonly used in Biochemistry

(i) Paper Electrophoresis-Paper electrophoresis is a commonly used electrophoretic method for analysis and resolution of small molecules. This method is not used to resolve macromolecules (e.g., proteins) because the adsorption and surface tension associated with paper electrophoresis usually alter or denature the macromolecules, causing poor resolution.

(ii) Capillary Electrophoresis- In Capillary electrophoresis, the material to be analyzed and the electrophoresis medium (a conducting liquid, usually aqueous) are placed in a long, fine-bore capillary tube, typically 50 to 100 cm long and 25 to 100 μ m inside diameter. A very small sample (in the nanoliter range) is placed at one end of the capillary and subjected to electrophoresis under fields up to 20 to 30 kV. Capillary electrophoresis offers the advantages of extremely high resolution, speed, and high sensitivity for the analysis of extremely small samples, but is obviously not useful as a preparative method. It has proven especially useful in the separation of DNA molecules that differ in size by as little as only a single nucleotide. Capillary electrophoresis can also be used for the separation of uncharged molecules by including charged micelles of a detergent (such as SDS) in the aqueous electrophoresis medium. Capillary electrophoresis is a highly adaptable method, and the range of its applications and optimal methodology are still being explored.

(iii) Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE)-Sodium dodecyl sulfate (SDS) gel electrophoresis is used to separate and determine the number and size of protein chains or protein subunit chains in a protein. Initially, the protein preparation is treated with an excess of soluble thiol (usually 2-mercaptoethanol) and SDS. Under these conditions, the thiol reduces all disulfide bonds ($-S-S-$) present within and/or between peptide units, while the SDS (an ionic or denaturing detergent) binds to all regions of the proteins and disrupts most noncovalent intermolecular and intramolecular protein interactions. These two components result negative charge and total denaturation of the proteins which are get separated according to charge and mass ration when electric current is applied

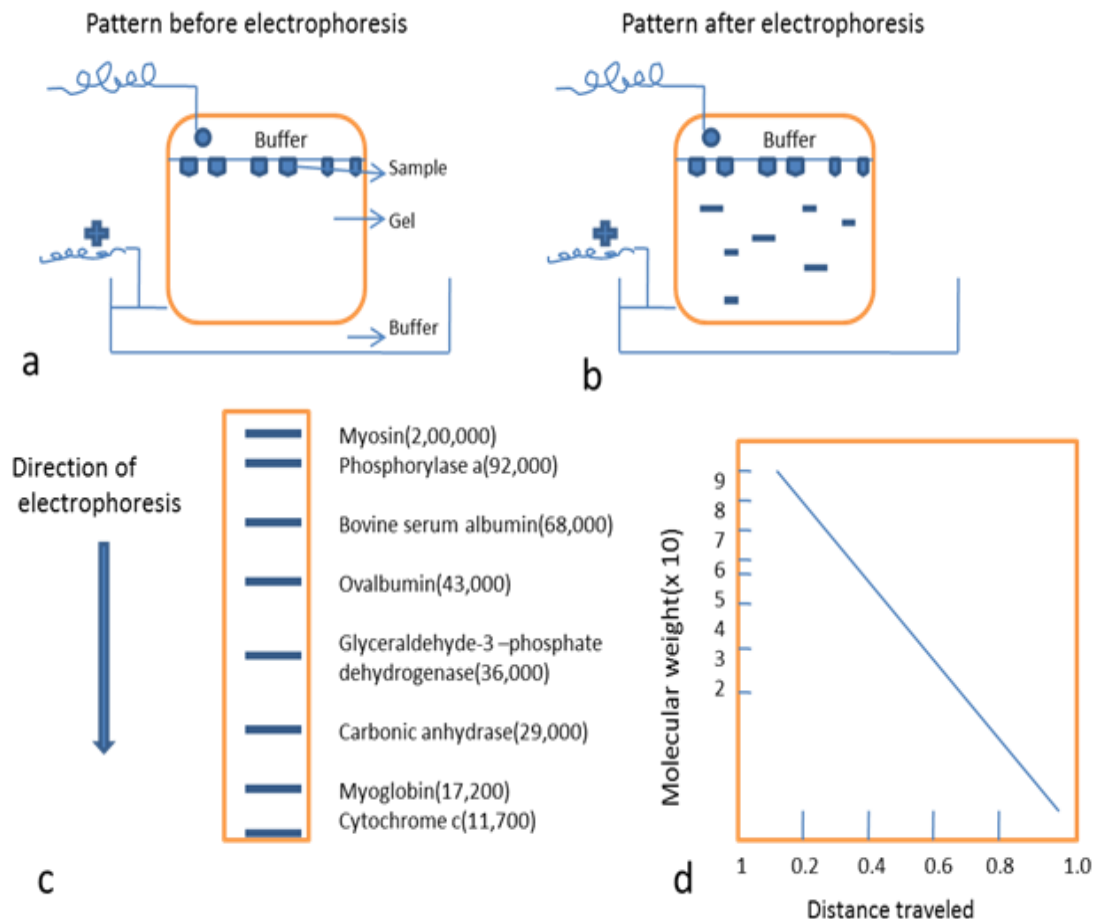


Fig.13.7-Gel electrophoresis for analyzing and sizing proteins

(iv) Isoelectric Focusing- Isoelectric focusing is an electrophoretic technique that separates macromolecules on the basis of their isoelectric points (pI, pH values at which they carry no net charge). As with SDS-PAGE, this process can be carried out in a “slab” format. A pH gradient is established in the polyacrylamide gel with the aid of ampholytes, which are small (!5000 Da) polymers containing random distributions of weakly acidic and weakly basic functional groups (e.g., carboxyls, imidazoles, amines, etc.). A polyacrylamide gel containing these ampholytes is connected to an electrophoresis apparatus that contains dilute acid solution (H⁺) in the anode chamber and a dilute base (OH⁻) solution in the cathode chamber.

(v) Agarose Gel Electrophoresis- Agarose gel electrophoresis is the principal technique which is used to separate and determine the size of high-molecular-weight nucleic acids (DNA and RNA). Agarose is a long polymer of galactose and 3,6-anhydrogalactose linked via (1n4) glycosidic bonds. This material is readily isolated from seaweed. Agarose polymers may contain up to 100 monomeric units, with an average molecular weight of around 10,000 Da. Agarose gels are cast by dissolving the white agarose powder in an aqueous buffer containing EDTA and either Tris-acetate or Tris-borate as the buffering species (TAE or

TBE buffer,). When the sample is heated to just below boiling, the agarose powder dissolves in the buffer to form a clear solution. As the solution slowly cools to room temperature, hydrogen bonding within and between the polygalactose units in the solution will cause the formation of a rigid gel with a relatively uniform pore size.

As with poly acrylamide gels, the pore size of the gel can be controlled by the percentage of the agarose dissolved in the solution. A high percent agarose gel (say, 3% wt/wt) will have a smaller pore size than a lower (0.8% wt/wt) agarose gel. The percent of agarose to be cast in the gel will be determined by the size of the various molecules to be resolved during electrophoresis; the smaller the molecular weight of the molecules to be resolved, the higher percent agarose (smaller pore size) the gel should contain

13.4 SUMMARY

The biochemical techniques are important methods for every branch of life sciences. These methods are used to understand and explain various biological phenomena. They are routinely used in diagnosis of diseases, identification of function of molecule and enzymes in living cells. In fact biochemical techniques are base for advancement in biology and new discoveries in everyday life.

13.5 GLOSSARY

Absorbance- A measure of the capacity of a substance to absorb light of a specified wavelength. It is equal to the logarithm of the reciprocal of the transmittance.

Crystal lattice - The symmetrical three-dimensional arrangement of atoms inside a crystal.

DNA (Deoxyribonucleic acid)- A self-replicating material which is present in nearly all living organisms as the main constituent of chromosomes. It is the carrier of genetic information.

Revolutions per minute- *Revolutions per minute* (abbreviated *rpm*, *RPM*, rev/*min*, r/*min*) are a measure of the frequency of rotation, specifically the number of *rotations* around a fixed axis in one *minute*.

Electric field -A region around a charged particle or object within which a force would be exerted on other charged particles or objects.

Elution- The process of extracting a substance that is adsorbed to another by washing it with a solvent.

O.D. (Optical Density)- The degree to which a refractive medium retards transmitted rays of light.

Photosensors- A *photosensor* is an electronic component that detects the presence of visible light, infrared transmission (IR), and/or ultraviolet (UV) energy.

RNA (Ribonucleic acid)- A nucleic acid present in all living cells. Its principal role is to act as a messenger carrying instructions from DNA for controlling the synthesis of proteins, although in some viruses RNA rather than DNA carries the genetic information.

Sephadex- A preparation of dextran used as a gel in chromatography, electrophoresis, and other separation techniques.

Sepharese- A preparation of agarose used as a gel in chromatography, electrophoresis, and other separation techniques.

Wavelength- It is a measure of distance between two identical peaks (high points) or troughs (low points) in a wave.

13.6 SELF ASSESSMENT QUESTION

13.6.1 Multiple choice questions:

1-Chromatography is used for separation of

- (a) solution
- (b) mixtures
- (c) molecules
- (d) atoms

2-Chromatography with solid stationary phase is called

- (a) circle chromatography
- (b) Square chromatography
- (c) solid chromatography
- (d) adsorption chromatography

3- A combination of paper chromatography and electrophoresis involves

- (a) partition chromatography
- (b) electrical mobility of the ionic species
- (c) both (a) and (b)
- (d) none of these

4-In chromatography the mobile phase can be

- (a) gas or liquid
- (b) solid or liquid
- (c) only solid
- (d) only gas

5-Thin layer chromatography is

- (a) Partition chromatography
- (b) Electrical mobility of ionic species
- (c) adsorption chromatography
- (d) none of the above

6-The GC trace obtained after an experiment is called a

- (a) chromatograph
- (b) chromatogram
- (c) chromatophore
- (d) graph

7-HPLC methods include

- (a) liquid/liquid (partition) chromatography
- (b) liquid/solid (adsorption) chromatography

- (c) ion exchange and size exclusion chromatography
(d) all of the above

8- Ion exchange chromatography is based on the

- (a) electrostatic attraction (b) electrical mobility of ionic species
(c) adsorption chromatography (d) partition chromatography

9-The representation of Beer Lambert's law is given as $A = abc$. If 'b' represents distance, 'c' represents concentration and 'A' represents absorption, what does 'a' represent?

- (a) Intensity (b) Transmittance
(c) Absorptivity (d) Admittance

10-Which of the following options are correct in terms of wavelength for the different types of IR spectrometer?

- (a) Near IR: 0.8 – 2.5 mm (b) Mid IR: 0.8 – 2.5 mm
(c) Far IR: 2.5 – 50 mm (d) Mid IR: 50 – 100 mm

11-An effective way of purifying liquids containing suspensions is

- (a) crystallization (b) decanting
(c) centrifuging (d) separating funnel

12-Differential centrifugation is based on the differences in _____ of biological particles of different _____.

- (a) Size, density (b) Sedimentation rate, sizes and density
(c) Size, structure. (d) Mass, size.

13-In electrophoresis, DNA will migrate towards

- (a) cathode or positive electrode (b) anode or negative electrode
(c) cathode or negative electrode (d) anode or positive electrode

14-The speed of migration of ions in an electrical field depends on

- (a) magnitude of charge and mass of molecules
(b) magnitude of charge and shape of molecules
(c) shape and size of the molecule
(d) magnitude of charge, shape and mass of molecules

13.6.1 Answer Key: 1-(b), 2-(d), 3-(c), 4-(a), 5-(c), 6-(b), 7-(d), 8-(a), 9-(c), 10-(a), 11-(c), 12-(b), 13-(d), 14-(b)

13.7 REFERENCES

- <http://www.encyclopedia.com/science/encyclopedias-almanacs-transcripts-and-maps/biochemical-analysis-techniques>
- <http://www.internetchemistry.com/biochemistry/biochemical-methods.htm>
- <http://www.namrata.co/category/biochemical-techniques/>
- <http://www.namrata.co/category/biochemical-techniques/>

13.8 SUGGESTED READINGS

- Principles and Techniques of Biochemistry and Molecular Biology by Wilson, K. & Walker, J.
- Lehninger Principles of Biochemistry 5th Edition by David L. Nelson (Author), Michael M. Cox (Author)
- Biochemistry, 4th Edition by Donald Voet, Judith G. Voet
- Biochemistry By J L Jain
- Instant Notes in Biochemistry by B.D. Hames, N.M. Hooper

13.9 TERMINAL QUESTIONS

- 1-What is chromatography?
- 2- What is the basic principle of chromatography?
- 3- What is the use of mobile and stationary phases in chromatography?
- 4- What is the difference between UV-Vis and IR spectrophotometry?
- 5-What is the principle of colorimetry?
- 6-What is centrifugation? What is the principle of centrifugation?
- 7-What are the methods used in autoradiography?
- 8- Describe the uses of electrophoresis?
- 9-What is agarose gel electrophoresis?
- 10-Describe the application and drawback of isotopic tracer technique?

LABORATORY PRACTICAL

UNIT-1 TO PERFORM ENDOSMOSIS AND EXOSMOSIS

Content

- 1.1-Objectives
- 1.2-Introduction
- 1.3-Process of Endosmosis and Ex-osmosis using potato tuber and egg osmoscope
- 1.4-Summary
- 1.5- Glossary
- 1.6-Self Assessment Question
- 1.7- References
- 1.8-Suggested Readings
- 1.9-Terminal Questions

1.1 OBJECTIVES

After reading this unit students will be able to understand-

- Process of endosmosis and ex-osmosis using potato tuber and egg osmoscope

1.2 INTRODUCTION

The movement of materials into and out of the cells in plants takes place in solution or gaseous form. Three physical processes are usually involved in it. They are diffusion, osmosis and imbibition. These movements involve movement along the concentration gradient. Hence, there is no expenditure of energy. The movement of particles or molecules from a region of higher concentration to a region of lower concentration is called diffusion. Whereas, osmosis may be considering as diffusion through a semi permeable membrane. It is defined as “a process by which molecules of a solvent tend to pass through a semi permeable membrane from a less concentrated solution into a more concentrated one.”

Demonstration of Osmosis

A thistle funnel, covered at the broad end by a differentially permeable membrane, contains a 10% sugar solution. The beaker contains a 5% sugar solution as shown in fig. 1a. The solute is unable to pass through the membrane, but the water passes freely through in both directions. The net movement of water towards the inside of the thistle funnel occurs because the thistle funnel has a lower water concentration. The level of the solution rises in the thistle funnel until hydrostatic pressure increases to the level of osmotic pressure.

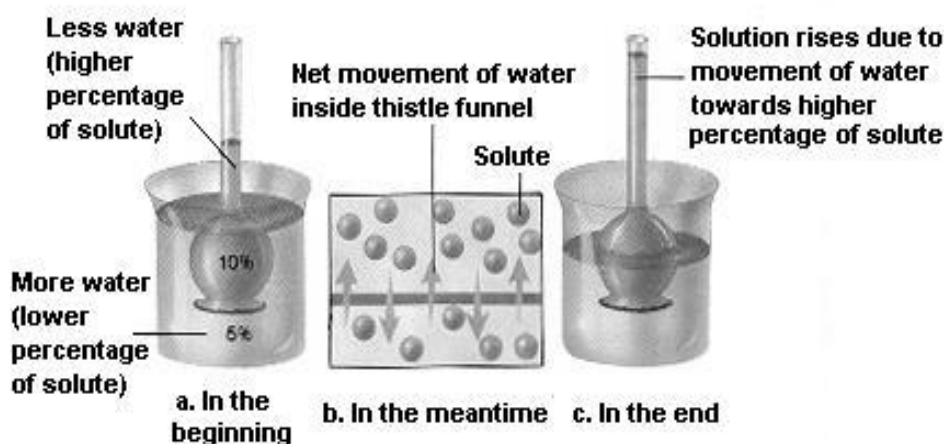


Fig.1.1- Demonstration of process of osmosis

1.3 PROCESS OF ENDOSMOSIS AND EXOSMOSIS USING POTATO TUBER AND EGG OSMOSCOPE

Introduction

If two solutions are separated by a semi permeable membrane, the membrane will be selectively permeable which will not allow passing of solute molecules whereas the solvent molecules will pass through it. According to the law of diffusion the movement of solvent molecules will be from the region of higher concentration to the lower concentration or in other word from the dilute solute to concentrated solution because the concentration of solvent will be higher in dilute solution and lower in concentrated solution.

Osmosis

It is a process involving net diffusion of water molecules from a region of higher water concentration to a region of lower water concentration through a semi permeable membrane. The osmotic movement of water from a surrounding medium into a cell is called endosmosis. Such a medium is described as hypotonic. The diffusion pressure exerted by water molecules on the semi permeable membrane is called as osmotic pressure.

Object 1: To demonstrate the phenomenon of endosmosis and exosmosis using potato tuber

Requirements: Beaker, distilled water, glucose, potato tuber, sugar, beaker, or petridish, knife, water, some sugar crystal or its concentration

Working Procedure: The osmotic entry or exit of water can be recorded by means of potato osmoscope (Fig.1.2). The potato surface serves as a semi-permeable membrane. Peel the skin of a potato and remove the inner contents to form a cup shaped hollow and place the potato in a container with water. Pour 5% sugar solution in to the potato cup up to a particular level. Pierce a pin to mark the initial level. Leave the experimental set up undisturbed for about one hour. At the end of the duration check the level of sugar solution in the potato cup. You will be able to record an increase in level caused by osmotic movement of water in to sugar solution through the tissue of potato tuber (endosmosis) which acts as semi permeable membrane. Water enters in the cavity because of osmosis, i.e. water molecules can diffuse through the semi permeable membrane from the region of higher concentration to lower concentration. In reverse case, i.e. sugar solution in the container and water in the potato cup, and mark the initial level. The movement if water would be in reverse direction and exosmosis would occur. At the end of 30 minutes note the difference. This time you will be able to record a decrease in the level of water inside the potato cup (exosmosis).

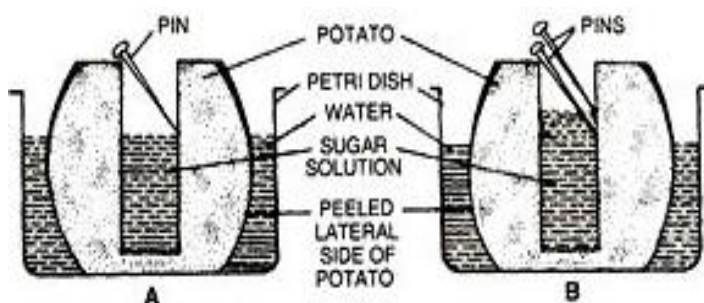


Fig.1.2: Potato osmoscope experiment to demonstrate osmosis A. Original level B. Final level

Object 2: To demonstrate phenomenon of osmosis using egg osmoscope.

Material required: Egg, HCL, beaker, graduated tube, sugar solution, water and stand

Working Procedure: The outer calcareous shell of the egg is removed by dissolving it in HCL leaving thin layer membrane as outer covering. The inner content of the shell is replaced by sugar solution through a small hole made for the purpose and graduated tube is tied in the opening. The membrane bag is hung dipped in the beaker of water with the help of stand. The level of the tube in stand is recorded (Fig.1.3).

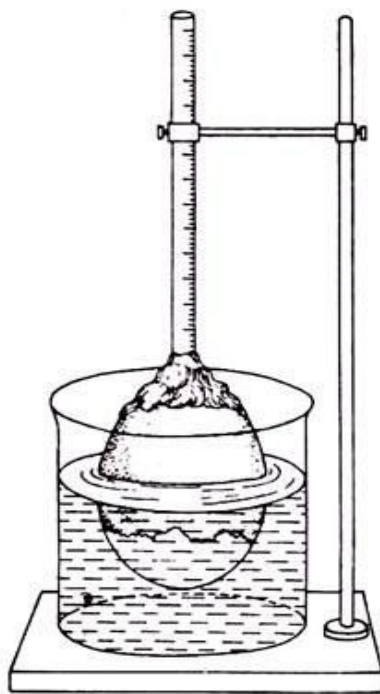


Fig. 1.3: Demonstration of osmosis using egg membrane

A rise of the level is seen after some time due to entrance of water because of diffusion of its molecules from the region of its higher concentration to the region of its lower concentration. The egg membrane acting as semi-permeable membrane permits the movement of water molecules but checks the movement of sugar molecules.

Variation: The egg membrane can be replaced by any other semi permeable membrane e.g., urinary bladder of sheep or goat.

1.4 SUMMARY

Water has many fold importance in plant body. Before taking up intake of water by plant, it is necessary to understand osmosis.

1. Molecules and ions are in constant random motion and tend to distribute themselves evenly in the space available to them. They move from a region of higher concentration to a region of lower concentration by simple diffusion along a diffusion gradient; they may also move against a diffusion gradient. Evenly distributed molecules are in a state of equilibrium. Diffusion rates are affected by temperature, molecule size and density, and other factors.
2. Osmosis is the diffusion of water through a differentially permeable membrane. It takes place in response to concentration differences of dissolved substances.
3. Osmotic pressure or potential is the pressure required to prevent osmosis from taking place. The pressure that develops in a cell as a result of water entering it is called turgor. Water moves from a region of higher water potential (osmotic potential and pressure potential combined) to a region of lower water potential when osmosis is occurring. Osmosis is the primary means by which plants obtain water from their environment.

1.5 GLOSSARY

Absorb- To suck up

Absorption- The process of **absorbing** or taking up of water and assimilating substances into cells or across the tissues and organs through diffusion or osmosis,

Diffusion- The movement of molecules or ions of a solute or solvent, be it a solid, liquid or gas from the region of its higher concentration/partial pressure / chemical potential to that of its lower concentration/ partial pressure/chemical potential.

Endosmosis- Osmosis in which fluid flows through a membrane towards a region of higher concentration

Exosmosis- The passage of a fluid through a semi permeable membrane toward a solution of lower concentration, especially the passage of water through a cell membrane into the surrounding medium.

Hypertonic solution- In a hypertonic solution the total molar concentration of all dissolved solute particles is greater than that of another solution, or greater than the concentration in a cell.

Hypotonic solution- A hypotonic solution is any solution that has a lower osmotic pressure than another solution. In the biological fields, this generally refers to a solution that has less solute and more water than another solution

Isotonic solution - An isotonic solution refers to two solutions having the same osmotic pressure across a semi permeable membrane. This state allows for the free movement of water across the membrane without changing the concentration of solutes on either side or active transport

Osmosis- Diffusion of water/ solvent molecules from a dilute solution to the concentrated solution when the two are separated by a semi permeable membrane.

1.6 SELF-ASSESSMENT QUESTIONS

1.6.1 Multiple choice questions:

1- The water readily available to plants for absorption by roots is

- | | |
|-------------------------|-----------------------|
| (a) Gravitational water | (b) Capillary water |
| (c) Rain Water | (d) Hygroscopic water |

2-The marine animals that kept in fresh water burst. It shows the process of

- | | |
|-----------------|--------------------|
| (a) Exosmosis | (b) Endosmosis |
| (c) Plasmolysis | (d) De-plasmolysis |

3-Osmosis is the diffusion of

- | | |
|-----------|----------------------|
| (a) Water | (b) Solute particles |
| (c) Gases | (d) Energy |

4-The outer solution having equal concentration as that of the cell sap is called

- | | |
|-------------------------|-----------------------|
| (a) Hypotonic solution | (b) Isotonic solution |
| (c) Hypertonic solution | (d) Neutral solution |

5-Diffusion of water through semi permeable membrane from dilute solution to concentrated solution is

- | | |
|-----------------|--------------|
| (a) Imbibition | (b) Osmosis |
| (c) Plasmolysis | (d) Necrosis |

1.6.1 Answers Key: 1-(b), 2-(b), 3-(a), 4-(b), 5-(b)

1.7 REFERENCES

- V. K., Jain (2003). Fundamental of plant physiology. S. Chand and Company, Pvt. Ltd. Ramnagar, New Delhi- 110055.
- S. N. Pandey and B K Sinha (1972). Plant Physiology. Vikas Publishing House

1.8 SUGGESTED READINGS

- Osterhout, WJV (1956). The role of water in protoplasmic permeability and in antagonism. J. Gen. Physiol. 39: 963-976
- Davson H and danielli JF (1952). Permeabilty of natural membrane. 2nd edition. Cambridge University Press. London
- Brown WMM (1967) Water and plant life. London. Hienmann

1.9 TERMINAL QUESTIONS

1.9.1. Short answer type Questions:

- 1-What is osmosis?
- 2-How is a living cell act as a osmometer?
- 3-Why is potato use for making osmometer?
- 4-What is Osmotic pressure?
- 5-Write about importance of osmosis in plant.
- 6-Calculate the osmotic potential (Ψ_s) of a 2.4 molar sucrose solution at 24°C. Show your work.

1.9.2 Long answer type Questions:

1. What are the governing factors regulating endosmosis and exosmosis?
2. Endosmosis and exosmosis do not take place in plant cell. Justify?
3. What do you understand by diffusion, osmosis and imbibition? How do these processes help plants to absorb water?
4. Discuss the relation between various osmotic quantities in plant cell under normal and stress conditions.

UNIT-2 TO DEMONSTRATE THE IMBIBITIONS, PLASMOLYSIS AND DEPLASMOLYSIS

Content

2.1-Objectives

2.2-Introduction

2.3-Process of demonstration of-

2.3.1-Imbibitions

2.3.2-Plasmolysis

2.3.3-Deplasmolysis

2.4- Effects of temperature on the permeability of plasma membrane

2.5-Summary

2.6- Glossary

2.7-Self Assessment Question

2.8- References

2.9-Suggested Readings

2.10-Terminal Questions

2.1 OBJECTIVES

After reading this unit students will be able to understand-

- Process of demonstration of Imbibitions
- Process of demonstration of plasmolysis and de-plasmolysis
- Effects of temperature on the permeability of plasma membrane

2.2 INTRODUCTION

Imbibition is the process in which water is absorbed by materials like seeds or dry wood that attract water. These materials are called **hydrophilic colloids**. When they take in water, they swell up and increase in size. This swelling creates pressure called **imbibitional pressure**, which is the maximum pressure they can build up when soaked in water.

When a plant cell is placed in a solution that has more solutes (like salt or sugar) than the liquid inside the cell, the solution is called **hypertonic**. In this case, water moves out of the cell through a process called **exosmosis**. This happens because there is more water inside the cell than outside, so water moves from the inside to the outside.

As water leaves the cell, the **cytoplasm**, **vacuole**, and **nucleus** shrink and pull away from the **cell wall**. The space between the cell wall and the shrunken parts gets filled with the outside solution. This process is called **plasmolysis**, and the cell is then called a **plasmolysed cell**.

Plasmolysis can be **reversed**. If the plasmolysed cell is placed in pure water or a solution with less solute (a **hypotonic solution**), water enters the cell again. This is called **endosmosis**. As water enters, the cell gets back to its normal shape. This reverse process is called **deplasmolysis**.

2.3 PROCESS OF DEMONSTRATION OF IMBIBITION

Introduction

The adsorption of water by hydrophilic colloids is known as imbibition. Imbibition of water increases the volume of the imbibant due to which pressure is created which is known as imbibitional pressure. Imbibitional pressure is the potential maximum pressure that an imbibant will develop if it is submerged in water. The relationship of DPD, IP (imbibitional pressure) and TP (turgor pressure) is as follows:

$$\text{DPD} = \text{IP} - \text{TP}$$

Different types of organic substances have different imbibing capacities. Proteins have a very high imbibing capacity, starch less and cellulose least. That is why proteinaceous pea seeds

swell more on imbibitions than starchy wheat seeds. The amount of water imbibed by a substance is also determined by the degree of cohesion of the molecules of the imbibing substance. Wood swells more than gelatin because there is more of cohesive attraction between wood molecules than gelatin. Increase in temperature brings about an increased imbibitions. The presence of solutes also affects rate of imbibitions. Increase in concentration of the solute decreases imbibitions. Type of a solution may also affect it. Some ions inhibit imbibitions much more than others imbibition is also affected by acidity and alkalinity. Proteins being more amphoteric in nature imbibe least in neutral medium. Towards pH1 or 14 the imbibition increases till a maximum is reached and then again falls down. During imbibition some energy is also released as heat. This is indicated by the warming of kneaded flour. The imbibitional pressure may be play important role in breaking soil profiles by germinating seeds.

Object 1: Demonstration of imbibition

Working Procedure: Dried raisins pieces are soaked in water for a few hours which swell up due to imbibition. They are measured again and an increase in the size of raisins is observed due to imbibition of water. As a result of absorption or imbibition of water, the size of the raisins increases (Fig.9.4 A & B). The difference in mass between the swollen and dry raisins gives the amount of water imbibed by the raisins.

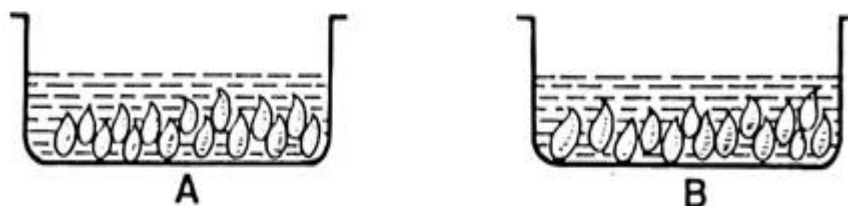


Fig.2.1: Demonstration of imbibitions of raisins

Object 2: Demonstration of imbibitional pressure

Working Procedure: A few gram (*Cicer sp.*) seeds in water are placed in a bottle and disc just above the seed level fitted through the cork of the bottle. The pointer is adjusted at zero on the scale and the apparatus is made airtight and is left for a few hours (may be up to 24 -48 hours). While making the observations, it is observed that disc is displaced above and the pointer moves down. It happens because gram seeds imbibe the water and swell considerably (Fig 2.2). The imbibition is caused due to hydrophilic colloids present in the gram seeds. The pressure which is responsible to push the disc is called imbibitional pressure.

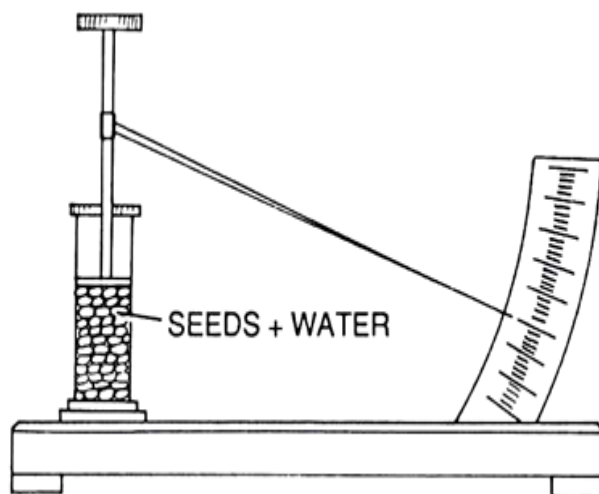


Fig.2.2: Demonstration of imbibitions pressure

2.4 PROCESS OF DEMONSTRATION OF PLASMOLYSIS AND DEPLASMOLYSIS

Introduction

When a plant cell is placed in a solution that has a higher solute concentration (hypertonic compared to the cell sap) water is lost from the cell by osmosis (exosmosis). As the concentration of water molecules in the cell is higher than the outer hypertonic solution, net movement of water molecules is from their region of higher concentration to their region of lower concentration across the selectively permeable plasma membrane. This process is called exosmosis. If exosmosis continues in a plant cell, cytoplasm along with the nucleus and vacuole shrinks to a small irregular mass due to loss of water. Eventually it pulls away from the cell wall. The space between, the cell wall and cytoplasmic mass is filled with the salt or sugar solution due to the permeability of the cell wall. This phenomenon is plasmolysis and the cell is said to be plasmolysed (Fig.2.3).

Plasmolysis is a reversible phenomenon. When the plasmolysed cell is placed in water, which is hypotonic compared to cell sap, the cell gains water due to endosmosis. As a result, the cell membrane, cytoplasm and vacuole regain their normal position. This phenomenon is called deplasmolysis. Through observation of plasmolysis and deplasmolysis, it is possible to determine the tonicity of the cell's environment as well as the rate solute molecules cross the cellular membrane.

Plasmolysis only occurs in extreme conditions and rarely happens in nature. It is induced in the laboratory by immersing cells in strong saline or sugar (sucrose) solutions to cause exosmosis, often using *Elodea* plants or onion epidermal cells, which have colored cell sap so that the process is clearly visible. Methylene blue can be used to stain plant cells. Plasmolysis is mainly known as shrinking of cell membrane in hypertonic solution and great pressure.

Plasmolysis can be of two types, either concave plasmolysis or convex plasmolysis. Convex plasmolysis is always irreversible while concave plasmolysis is usually reversible. During concave plasmolysis, the plasma membrane and the enclosed protoplast partially shrinks from the cell wall due to half-spherical, inward curving pockets forming between the plasma membrane and the cell wall. During convex plasmolysis, the plasma membrane and the enclosed protoplast shrinks completely from the cell wall, with the plasma membrane's ends in a symmetrically, spherically curved pattern.

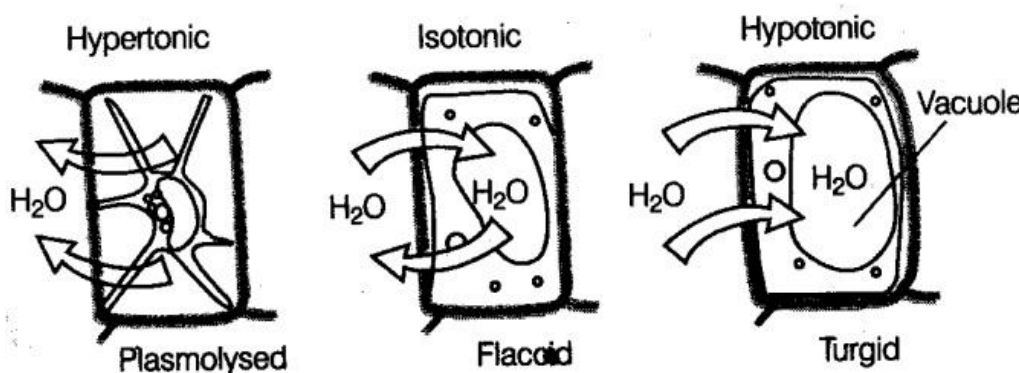


Fig.2.3: Demonstration of plasmolysis and de-plasmolysis

Object 1: To demonstrate plasmolysis

Material required: An onion, glass slide, cover slip, forceps, needle, brush, blade, pipette, petridishes, blotting paper, dropper, concentrated salt or sugar solution, water, compound microscope

Working Procedure:

1. Remove the outermost peel of the onion.
2. Cut out a small part from an inner fleshy scale leaf using the blades.
3. Pull out the transparent peel of the scale leaf using forceps.
4. Using the brush place the piece of onion peel on clean glass slide.
5. Put a drop of the concentrated salt or sugar Solution on the peel with the help of the dropper.
6. Put a clean cover slip by tilting it over the needle.
7. In this way you can prevent air bubbles form getting trapped under the cover slip.

8. Remove excessive solution with blotting paper.
9. Observe the mounted onion peel immediately under the low and high magnification of the compound microscope.
10. Observe it again under the microscope after some time. Note the shrunken cytoplasm of the cells.

Observations

After putting the drop of the hypertonic solution on the cells of the onion peel, their cell membranes withdraw from the cell walls; the enclosed cytoplasm nucleus and vacuole lose water and shrink to one side of the cell. The cell walls remain at their original positions. The space between the cell wall and the shrunken cytoplasm is filled with the salt or sugar solution.

Object 2- To demonstrate de-plasmolysis

Working Procedure- When a plasmolysed cell is placed in a hypotonic solution, (i.e., the solution having solute concentration lower than the cell sap), the water moves into the cell because of the higher concentration of water outside the cell than in the cell. The cell then swells to become turgid. It is called de-plasmolysis.

2.5 EFFECTS OF TEMPERATURE ON PERMEABILITY OF PLASMA MEMBRANE

Object 1: Effect of temperature on the permeability of the plasma-membrane

Material Required: Beet root, cork borer, distilled water, knife and water bath

Working procedure

Small equal sized pieces of beet root tissue are cut with the help of a cork-borer and thoroughly washed with distilled water. Each of these pieces is placed in a separate test tube containing water at different temperature e.g., 0°C, 10°C, 20°C, 30°C, 40°C, 60°C, 70°C, and 80°C. The experiment is kept for an hour or so and colouration of the water is observed. After sometime it is observed that the water in those test tubes which were kept at lower temperature, room temp, or slightly higher temp remain colourless while the water in test tubes kept at higher temperatures (e.g. 60⁰, 70⁰ and 80⁰ C) becomes red coloured. The low temperature water remains colourless and the intensity of red colour increases with the increase in temperature. The red colour is due to the diffusion of the anthocyanin pigments from the cell sap into the water. The higher temperature kills the cell due to which semi permeable nature of cell membrane is lost and the membrane becomes permeable.



Fig.2.4: Effect of temperature on the membrane permeability in beet roots

2.6 SUMMARY

Water has many fold importance in plant body. Before taking up intake of water by plant, it is necessary to understand imbibitions and plasmolysis.

1. Plasmolysis is the shrinkage of the cytoplasm away from the cell wall as a result of osmosis taking place when the water potential inside the cell is greater than outside.
2. Imbibition is the attraction and adhesion of water molecules to the internal surfaces of materials; it results in swelling and is the initial step in the germination of seeds.
3. Active transport is the expenditure of energy by a cell that results in molecules or ions entering or leaving the cell against a diffusion gradient.

2.7 GLOSSARY

De-plasmolysis- The entrance of water into a plasmolysed plant cell, causing the cell membrane to return to the cell wall.

Diffusion- The movement of molecules or ions of a solute or solvent, be it a solid, liquid or gas from the region of its higher concentration/partial pressure / chemical potential to that of its lower concentration/ partial pressure/chemical potential.

Hypertonic solution- In a hypertonic solution the total molar concentration of all dissolved solute particles is greater than that of another solution, or greater than the concentration in a cell.

Hypotonic solution- A hypotonic solution is any solution that has a lower osmotic pressure than another solution. In the biological fields, this generally refers to a solution that has less solute and more water than another solution

Imbibition- Phenomenon of adsorption of water or any other liquid by the solid particles of a substance without forming a solution.

Isotonic solution - An isotonic solution refers to two solutions having the same osmotic pressure across a semi permeable membrane. This state allows for the free movement of water across the membrane without changing the concentration of solutes on either side or active transport

Permeable- A material or membrane allowing liquids or gases to pass through it.

Plasmolysis- Contraction of the protoplast of a plant cell as a result of loss of water from the cell.

Selective permeable membrane-It is a type of biological or synthetic, polymeric membrane that will allow certain molecules or ions to pass through it by diffusion or occasionally by more specialized processes of facilitated diffusion, passive transport

Semi permeable: A material or membrane allowing certain substances to pass through it but not others, especially allowing the passage of a solvent but not of certain solutes.

Solution -The dispersion of a substance in molecular or ionic form throughout the medium of another is known as solution.

2.8 SELF-ASSESSMENT QUESTIONS

2.8.1 Multiple choice questions:

1-The water potential of pure water at atmospheric pressure is

- (a) -2.3 bar
- (b) +2.3 bar
- (c) 0 bar
- (d) 1 bar

2-During rainy season wooden doors are difficult to open or closure because of

- (a) Plasmolysis
- (b) Imbibition
- (c) Osmosis
- (d) Diffusion

3- Plasmolysis occurs due to

- (a) Absorption
- (b) Osmosis
- (c) Endosmosis
- (d) Exosmosis

4-The process of imbibition involve

- (a) Diffusion
- (b) Capillary action
- (c) Absorption
- (d) Both A & B

5-Which of the following conditions would cause red blood cells to burst?

- (a) pH of 7.5.
- (b) Temperature of 3°C
- (c) Being placed in distilled water
- (d) Being placed in an 11% salt solution

6-The cytoplasmic concentration of solute in a cell is 0.05%. This cell is placed in a solution that causes the cell to swell and burst. The solute concentration of this solution is

- (a) 0.005%
- (b) 0.05%
- (c) 0.5%
- (d) 5.0%

7-A cell would tend to gain water if it were moved from

- (a) Isotonic solution to a hypotonic solution
- (b) Isotonic solution to a hypertonic solution

- (c) Hypotonic solution to an isotonic solution
- (d) Hypotonic solution to a hypertonic solution

8-If the solute concentration of solution A is greater than solution B, then solution A is said to be

- (a) Isotonic to solution B
- (b) Osmotic to solution
- (c) Hypotonic to solution
- (d) Hypertonic to solution B

9-Which of the following moves material against a concentration gradient?

- (a) Osmosis
- (b) Diffusion
- (c) Active transport
- (d) Facilitated transport

2.8.2. Fill in the blank:

- 1- _____ will be zero in a fully turgid cell.
- 2- Endosmosis occurs when the plant cell is placed in _____.
- 3-The membrane which allows the movement of only water molecules to pass through it and not the solute particles _____
- 4-The membrane which allows passage of solvent as well as some selective solutes and prevents others is called _____

2.8.1 Answers Key: 1-(c), 2-(b), 3-(d), 4-(d), 5-(a), 6-(a), 7-(a), 8-(d), 9- (c)

2.8.2 Answers Key: 1- Suction pressure (DPD), 2- Hypotonic solution, 3- Semi permeable membrane, 4- Selectively permeable membrane

2.9 REFERENCES

- V. K., Jain (2003). Fundamental of plant physiology. S. Chand and Company, Pvt. Ltd. Ramnagar, New Delhi- 110055.
- S. N. Pandey and B K Sinha (1972). Plant Physiology. Vikas Publishing House

2.10 SUGGESTED READINGS

- Osterhout, WJV (1956). The role of water in protoplasmic permeability and in antagonism. J. Gen. Physiol. 39: 963-976
- Davson H and Danielli JF (1952). Permeability of natural membrane. 2nd edition. Cambridge University Press. London
- Brown WMM (1967) Water and plant life. London. Hienmann

2.11 TERMINAL QUESTIONS

2.11.1. Short answer type Questions:

- 1-Why a space is occupied between cell wall and protoplast of plasmolysed cell?
- 2- Why plasmolysed cell regain original shape when placed in water?
- 3-Why don't Plant Cells undergo Plasmolysis?
- 4-What is the process of plasmolysis?
- 5-What is the relationship of plasmolysis and hypertonic?
- 6- Write about imbibition of raisins with suitable diagram.
- 7-Write notes on Diffusion Pressure Deficit (D.P.D.)
- 8-Explain term turgor pressure.
- 9-Explain about imbibition pressure with suitable example.
- 10-Differentiate in between following:
 - (a) Plasmolysis and deplasmolysis
 - (b) Osmotic pressure and turgor pressure

2.11.2 Long answer type Questions:

- 1-Discuss about plasmolysis? How can you make use of it in determining osmotic pressure of cell shape?
- 2- How is plasmolysis related to turgor pressure?
- 3- Explain plasmolysis. What are its advantages? How would you determine O.P. of cell sap by plasmolytic method. Discuss water relations of plant cell.
- 4- What do you understand by diffusion, osmosis and imbibition? How do these processes help plants to absorb water?

UNIT-3 STUDY THE STRUCTURE AND FUNCTIONS OF STOMATA, STOMATAL FREQUENCY AND COMPARISON OF RATE OF TRANSPIRATION USING DIFFERENT METHODS OR BY USING DIFFERENT POTOMETERS

Contents

- 3.1-Objectives
- 3.2-Introduction
- 3.3-Study the structure of stomata, their opening and closing, stomatal frequency
- 3.4-Compare the Stomatal and Cuticular Transpiration of Leaf by Cobalt Chloride Paper
- 3.5-Compare the Stomatal and Cuticular Transpiration of Leaf by Using Four Leaves Methods
- 3.6- To Demonstrate Of Rate of Transpiration by Using Different Types of Potometers
- 3.7- Summary
- 3.8- Glossary
- 3.9-Self Assessment Question
- 3.10- References
- 3.11-Suggested Readings
- 3.12-Terminal Questions

3.1 OBJECTIVES

After reading this unit students will be able to:

- Study the structure of stomata, their opening and closing, stomatal frequency
- Comparison of rate of transpiration under different climatic condition using four-leaf method
- Comparison of rate of transpiration under different climatic condition using cobalt chloride paper
- Comparison of rate of transpiration under different climatic condition using different types of potometers

3.2 INTRODUCTION

Transpiration is a process of evaporation of water from the surface of the plant. This keeps the plant cool and transfers minerals and other materials to different parts of the plant. As the plant takes water from the soil, the openings absorb other minerals. For a plant to take water from the soil, water needs to evaporate from the surface of the plant. Once this happens, pressure is developed that forces the roots to absorb water from the soil and transfer it to the tips of the plant. It is through the stomata that the major work of evaporation of water is done.

Stomata were discovered by Pfeffer and name 'stomata' was given by Malpighii. Stomata cover 1-2% of leaf area. Stomata are minute pores found on the epidermis of leaves and young shoots of plants that are used to control exchange of gases and transpiration. The pore is surrounded by a pair of specialised cells called the guard cells that are responsible in regulating the size of the opening. Each guard cell is a modified epidermal cell showing a prominent nucleus, cytoplasm and plastids. The wall of the guard cell is differentially thickened. The inner wall of each guard cell facing the stoma is concave and is thick and rigid (Fig.3.1 & 3.2). The outer wall is convex and is thin and elastic. The Size and shape of stoma and guard cell vary from plant to plant. When fully open, the stomatal pore measures 3-12 in width and 10-40 in length. In many gymnosperms and xerophytic plants (plants growing in desert), the stomata are present embedded deeply in the leaves, so that they are not exposed to sunlight directly. Such deeply embedded stomata are called sunken stomata. Water is released through the stomata into the atmosphere in the form of water vapour through the process called transpiration. Besides this, the exchange of oxygen and carbon dioxide in the leaf also occurs through the stomata. Algae, fungi and submerged plants do not possess stomata.

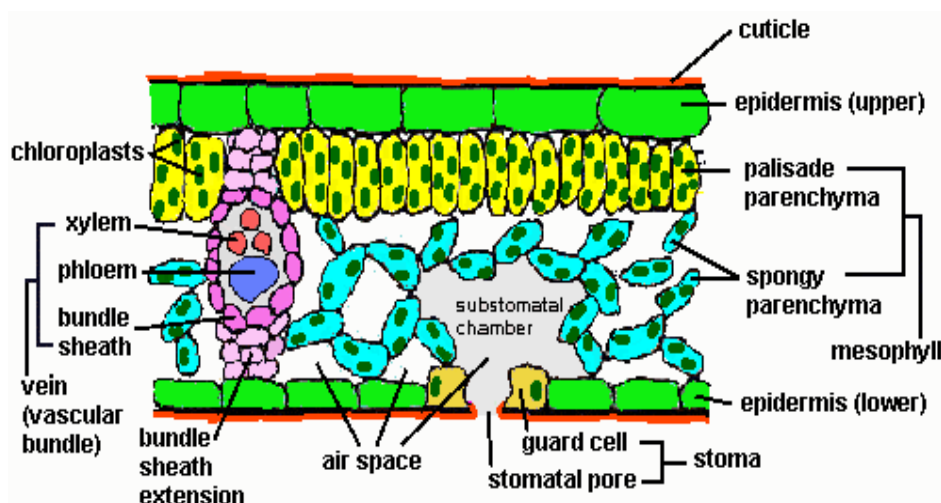


Fig.3.1: Vertical section of leaf blade showing passage of water vapour during transpiration

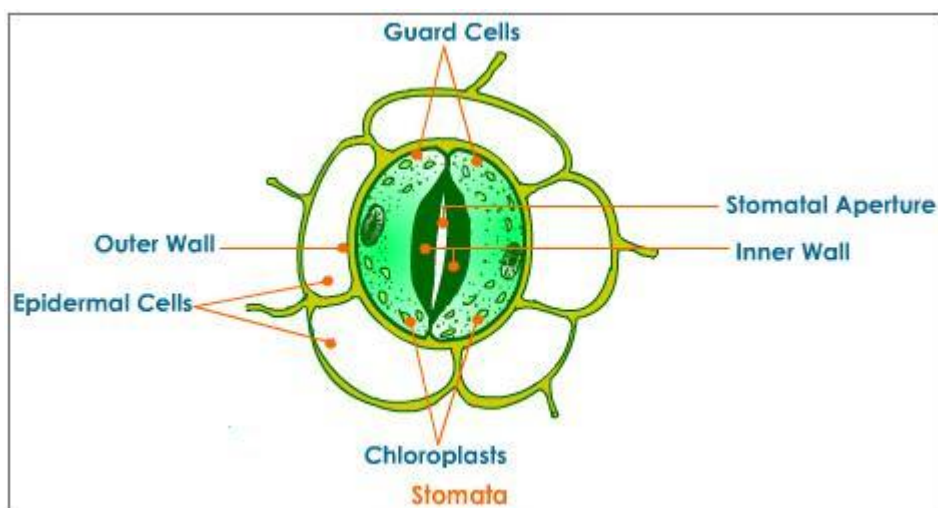


Fig.3.2: A magnified structure of stomata in lower epidermis of leaf

3.3 STUDY THE STRUCTURE OF STOMATA, THEIR OPENING AND CLOSING, STOMATAL FREQUENCY

Mechanism of Stomatal Opening and Closing

Opening and closing of stomata takes place due to changes in turgor of guard cells. Generally stomata are open during the 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 the guard cells making the guard cells fully turgid. 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 stoma to open (Fig.10.3). 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 stoma closes.

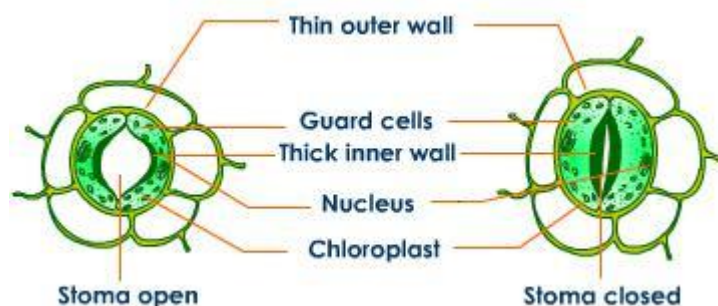


Fig.3.3: Stomatal Opening and Closing

Object-1: To study distribution of different type of stomata

Material required: : Fresh leaf of Petunia or any other herbaceous plant/fresh leaf of lily, forceps, needle, blades, watch glass, petridish, beaker, glass slide, cover slips, safranin, glycerine, water

Procedure:

1. Tear leaves with force keeping lower epidermis upwards.
2. A thin membranous lower epidermis gets separated near the broken edge. Pull this membranous part in to strip with help of finger or forceps.
3. Strip is stained with 1% aqueous solution of safranin, washed in water and then mounted in glycerine.

Observation: Distribution of stomata varies between monocots and dicots, between plant species, and between the underside and top side of the leaves on a plant. Stomata are found more on plant surfaces thriving under higher light, lower atmospheric carbon dioxide concentrations and in moist environments. Usually the lower surface of a dicot leaf has a greater number of stomata while in a monocot leaf they are more or less equal on both surfaces. In most of the floating plants, stomata are found only on the upper epidermis. Depending upon the distribution and arrangement of stomata in the leaves five categories of stomatal distribution have been recognized in plants.

(i) Apple or mulberry (hypostomatic) type: Stomata are found distributed only on the lower surface of leaves, e.g., apple, peach, mulberry, walnut, etc.

(ii) Potato type: Stomata are found distributed more on the lower surface and less on its upper surface, e.g., potato, cabbage, bean, tomato, pea, etc.

(iii) Oat (amphistomatic) type: Stomata are found distributed equally upon the two surfaces, e.g. maize, oats, grasses, etc.

(iv) Water lily (epistomatic) type: Stomata are found distributed only on the upper surface of leaf, e.g., water lily, *Nymphaea* and many aquatic plants.

(v) Potamogeton (astomatic) type: Stomata are altogether absent or if present they are vestigial. e.g., *Potamogeton* and submerged aquatics.

On the basis of number and arrangement of subsidiary cell Metacalf and Chalk recognized following types of stomata (Fig.10.4)-

1- Anomocytic type: In these stomata, accessory cells are absent. The guard cells are surrounded by ordinary epidermal cells, e.g., families Ranunculaceae, Cucurbitaceae, Papaveraceae and Malvaceae.

2- Anisocytic type: In these stomata the guard cells are surrounded by three accessory cells. Of these two are larger whereas one is smaller in size e.g., family Brassicaceae.

3- Diacytic type: In these stomata the guard cells are surrounded by two accessory cells. Their common walls are at right angle to the walls of guard cells, families Caryophyllaceae, Acanthaceae.

4- Paracytic type: In these stomata the guard cells are also surrounded by two accessory cells, but their common walls are parallel to guard cells, e.g., families Rubiaceae, Fabaceae etc.

5- Actinocytic type: These stoma surrounded by four or more subsidiary cell elongated radially to stomata. e.g., Araceae, Musaceae and Ebenaceae.

6- Cyclocytic: Stoma surrounded by four or more subsidiary cell arranged in narrow ring around the stomata. e.g., *Palmae*, *Pandanus* and *Cyclanthaceae*.

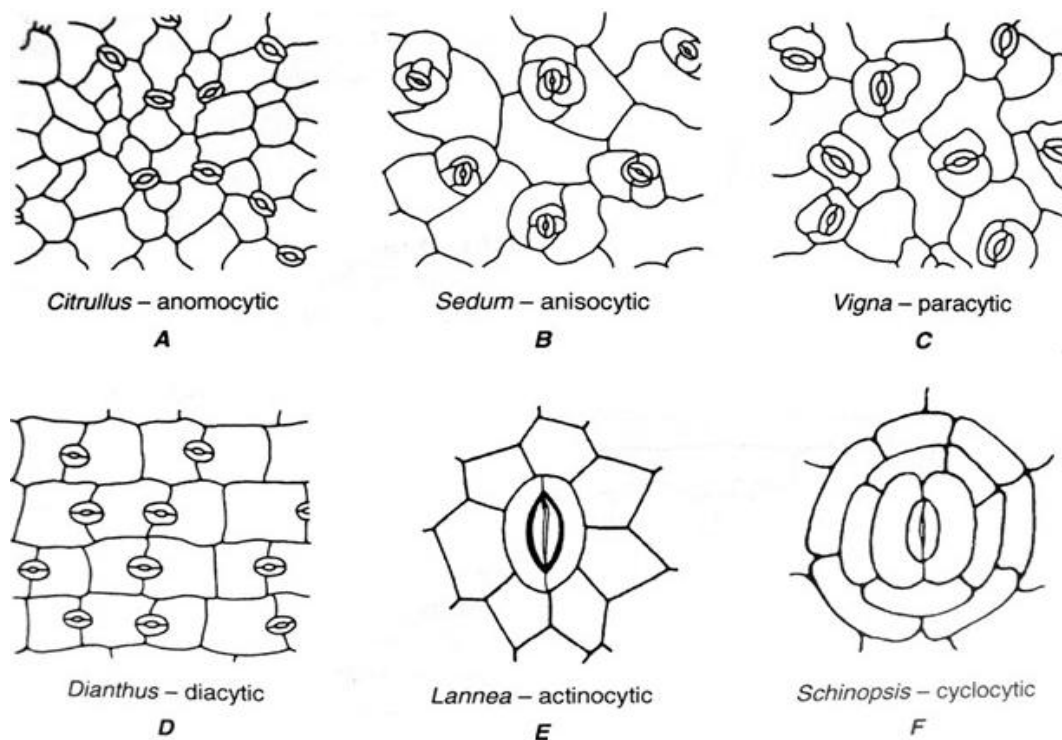


Fig.3.4: Types of stomata on the basis of their structure

On the basis of development (Pant, 1965), There are three types of stomata:

- (i) Mesogynous type:** In this type of stomata guard cells as well as subsidiary or Accessory cells both are developed from one mother cell. e. g. Rubiaceae & Brassicaceae family.
- (ii) Perigynous type:** In this type guard cells are formed from mother cell while subsidiary cells from nearby mother cells, e.g. Cucurbitaceae family.
- (iii) Mesoperigynous type:** In this type guard cells and subsidiary cells are formed from mother cell while other subsidiary cells develop independently. e.g. Ranunculaceae, Caryophyllaceae family.

Number of Stomata (Stomatal Frequency)

The number of stomata in a definite area of leaf varies from plant to plant. Xerophytes possess larger number of stomata than mesophytes. Number of stomata/sq cm. is 1000-60,000 in different plant species. The number of stomata per unit area of leaf is called Stomatal Frequency. Stomata frequency of trees and shrubs is higher than herbs. Stomata nearly occupy one to two percent of total leaf area when fully open. In isobilateral leaves (in monocots) approximately the same numbers of stomata are found on upper surface (adaxial) and lower (abaxial) surface. But

in dorsiventral leaves (in dicots) the number of stomata on the upper surface is much less in comparison to those found on the lower surface.

Calculation of Stomatal index

The distribution of stomata on the upper and lower surfaces of the leaf can be studied by removing the peels of the leaf from the upper and lower surfaces and observing the same under a microscope. The count of the number of stomata and epidermal cells in the microscopic field is taken and the stomatal index of each surface of the leaf can be calculated using the following formula:

$$\text{Stomatal index} = \frac{\text{Total no. of stomata per unit area}}{\text{No. of stomata per unit area} + \text{No. of epidermal cells per unit area}} \times 100$$

Object 2: Study the stomatal distribution on the upper and lower leaf surfaces and to calculate the stomatal index.

Material required: Fresh leaf of Petunia or any other herbaceous plant/fresh leaf of lily, forceps, needle, blades, watch glass, petridish, beaker, glass slide, cover slips, safranin, glycerine, water

Working Procedure

- (i) Pluck one fresh leaf of a lily plant.
- (ii) Take watch glasses and pour some distilled water into the watch glasses.
- (iii) Split the leaf from the lily plant obliquely.
- (iv) Take the peel from the upper and lower epidermis surface of the leaf using the forceps.
- (v) Place the peel into a separate watch glasses containing water.
- (vi) Place the peel into the other watch glass containing water.
- (vii) Using a dropper, take few drops of safranin solution and put it into the watch glasses.
- (viii) Take clean glass slides and place the leaf peel on the slides one by one, using a brush.
- (ix) Take a blade and cut a small rectangle or square piece from each peel.
- (x) Take some glycerine using a dropper and put one drop of glycerine on both slides.

- (xi) Take a cover slip and place it gently on the peel with the help of a needle.
- (xii) Take the glass slide and place it under compound microscope.
- (xiii) The distribution of stomata on the upper and lower surfaces of the leaf can be studied by removing the peels of the leaf from the upper and lower surfaces and observing the same under a microscope.

Calculation of Stomatal Index

The count of the number of stomata and epidermal cells in the microscopic field is taken and the stomatal index of each surface of the leaf can be calculated using the following formula:

Determine Stomatal index by taking the following readings		
Number of observation	Number of stomata at upper surface	Number of stomata at lower surface
1	-	-
2	-	-
3	-	-

Total no. of stomata per unit area

Stomatal index = ----- **× 100**

No. of stomata per unit area + No. of epidermal cells per unit area

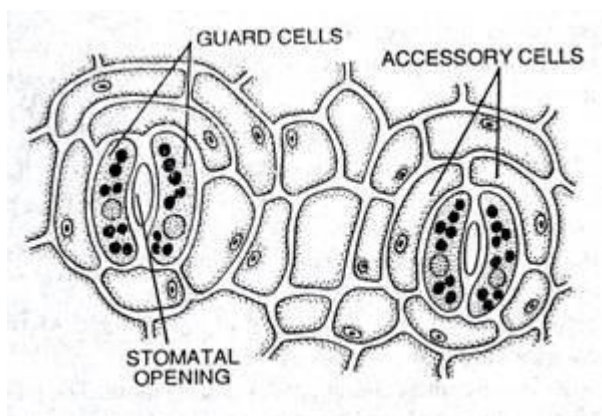


Fig.3.5 Stomata in epidermis layer (surface view)

Observe under the microscope

Count the number of stomata in the peels of both upper and lower epidermis of the leaf appearing in the microscopic field.

Precaution:

1. The curling of peel should be avoided.
2. Always use brush to transfer peel from watch glass to slides.
3. Excess of glycerine should be removed by blotting paper.

Object-3: To measure the extent of stomatal opening by Darwin's potometer

Material required: A Darwin's potometer, a potted plant, beaker, water grease, stand with clamp and base stand are required for the experiment.

Working Procedure: Darwin's potometer is a useful apparatus for following the changes in stomatal apertures, i.e., degree of opening of stomata. It essentially consists of a vertical tube, one end of which is dipped in a beaker of water. The other end is fixed to a T-tube, into one arm of which is attached a rubber tubing provided with screw-cock. A small glass chamber (potometer cup) is attached with rubber tubing to the other arm (Fig.10.6). The cup end of the T-tube is fixed air tight with the lower surface of leaf (stomata restricted only on the lower epidermis) using grease and base stand. Water is sucked up to a certain fixed level order to adjust the required level of water in the vertical tube and the screw-cock is closed and all connections made air tight. The air is sucked off through this end in order to adjust the required level of water. The whole apparatus is kept for some known time in light and in dark. The fall in the level of water is recorded under both conditions. The level of mercury falls faster in light condition. The rate of fall indicates the extent of opening of stomata which is more in light than dark.

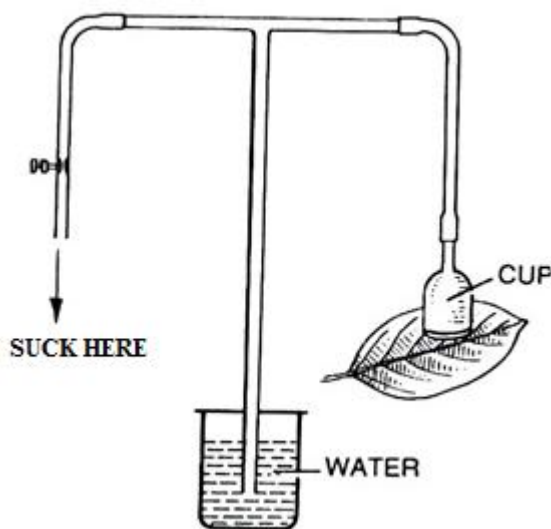


Fig.3.6: Darwin's potometer measuring the extent of stomatal opening

Observation: The rate of fall of water in the vertical tube observed provides a measure of the rate with which air passes out under suction through the stomatal openings of the leaf. If the cup is affixed to upper surface of such a leaf where there may not be any stomata, the water level in the vertical tube is maintained, but no fall in level is observed.

This certainly demonstrates that air cannot pass through the cuticle. The rate of fall of the water level and hence that of passage air is thus roughly indicative of the size of stomatal openings. If the rate of fall diminishes or becomes extremely slow, it evidently indicates that the stomata are closed or about to close.

3.4 COMPARE THE STOMATAL AND CUTICULAR TRANSPIRATION OF LEAF BY COBALT CHLORIDE PAPER

Introduction: Transpiration is the loss of water from a plant in the form of water vapour. Water is absorbed by roots from the soil and transported as a liquid to the leaves via xylem. In the leaves, small pores known as stomata allow water to escape as a vapour. Of all the water absorbed by plants, less than 5% remains in the plant for growth. The number of stomata present in two surface of leaves differ in most of the plant. In mesophytic plant more stomata are present in lower surface of leaves than upper surface of leaves. Therefore, more transpiration occurs by lower surface in comparison to upper surface of leaves.

The cobalt chloride paper is prepared by dipping and drying filter paper in 5 percent solution of cobalt chloride, which is blue when dry and turns pink when moist. Two equal small dry pieces of cobalt chloride paper (1cm^2) are placed on either side of dorsiventral leaf, opposite to one another, covered with the help of two glass slides and clipped and greased are tight. The time required for the colour change from blue to pink is recorded for both the pieces separately.

Object-1:- To compare the Stomatal and Cuticular transpiration of leaf by cobalt chloride paper.

Material Required: A potted plant of dorsiventral leaf of *Tropaeolum*, *Dolichos*, *Helianthus*, or *Tecoma* etc., cobalt chloride paper, Glass slides, Grease, Clips and stopwatch are required for the purpose of experiment.

Working Procedure: Soak filter paper in 5% solution of cobalt chloride and hang it up to dry. When dry, quickly cut into strips about $2.5\text{ cm} \times 1.25\text{ cm}$ and dry these strips again thoroughly in an oven at a low temperature. The original colour of the wet paper is pale pink, but when dried to a standard uniform shade, the colour becomes an intense blue. The uniform-sized dry strips are stored in desiccators. The treated blue dry paper is thus a moisture detector, turning pink when left in the air (the air containing moisture) or when placed in contact or near an evaporating surface. The time taken for a blue colour to disappear and attain a standard pink colour (determined by a stop watch) is a measure of the amount of evaporation as also the rates of water

loss from the surface of the leaf to which it is attached. The following tests can be conveniently performed with these dry cobalt chloride paper strips:

- (a) Select a suitable leaf attached to a potted plant. Lay a piece of strip on each surface of a leaf and immediately press them gently down between two slides, held one above another with the help of clips (Fig.10.7). Compare the respective rates of water loss from both upper and lower surfaces of a leaf and also from young and old leaves.

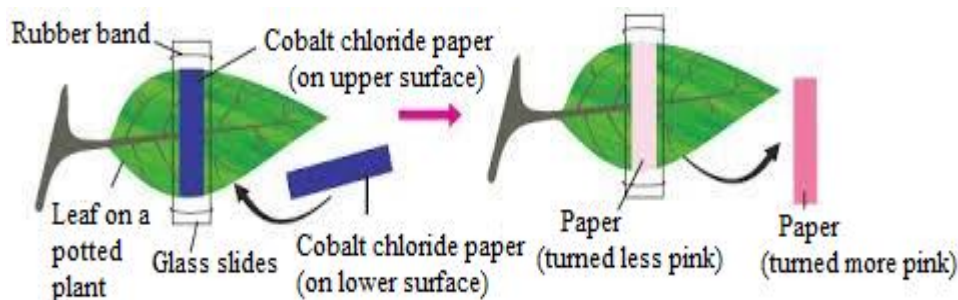


Fig.3.7: Demonstration of stomatal and cuticular transpiration

- (b) Compare the respective rates of one leaf kept in darkness for some hours (stomata closed) and the other kept in the open.
- (c) Compare the respective rates of water loss from a leaf and also from an open water-evaporating surface (physical evaporation). For open evaporating surface a petri-dish containing water can be taken and the blue paper-strip is held by suitable arrangement as close to the water surface as possible, without actually touching it.

The time in seconds for a standard colour change of cobalt chloride paper over a free water-evaporating surface (s) divided by the time for the same colour change on the leaf (E) is a measure of what is sometimes defined as transpiration index- It is convenient to multiply this index by 100 to obtain the water loss from the leaf surface as a percentage of the evaporation from a free water surface.

$$\text{Transpiration index} = S / E \times 100$$

Observations: It is observed that the paper piece on the lower side of leaf takes lesser time in changing from standard blue to standard pink as compared to the piece placed on the upper side of leaf. It is because of the faster rate of transpiration from the lower surface of leaf as compared

to the upper side of the leaf where due to absence of stomata cuticular transpiration is the only source of moisture.

Precautions:

1. Always take well watered potted plant for experiment.
2. Always handle dry cobalt chloride paper with dry hands or forceps.
3. Leaf surface should not be wet when putting cobalt chloride disc.

3.5 COMPARE THE STOMATAL AND CUTICULAR TRANSPIRATION OF LEAF BY USING FOUR LEAVES METHODS

Introduction: In principle stomatal transpiration takes place through the lower surface of the leaf than transpiration through upper surface of leaf is said to be of the cuticular type in dorsiventral leaves. Thus, if one wants to determine the relative efficiency of transpiration rate by the two surfaces, then two separate experimental sets have to be prepared using the same type of leaves. In one experimental set, the upper surface of the leaf is smeared with grease so that transpiration occurs only through the lower surface (i.e. predominantly stomatal type).

In the other set the lower surface of the leaf is smeared with grease so that transpiration occurs only through the upper surface of the leaf (i.e. predominantly cuticular type). The rate of transpiration per unit time per sq cm of leaf area is calculated separately and then compared. The experimental procedure is identical with conical flask water-oil-leaf method.

Object-1: To compare the stomatal and cuticular transpiration of leaf by using Four leaves methods.

Material Required: Four leaves, two stands, Vaseline and a string

Working Procedure: To demonstrate the transpiration from the leaf surface, four banyan leaves are taken. Both the surfaces of the A leaf, lower surface (with stomata) of B leaf, upper surface (without stomata) of C leaf are vaselined. The Vaseline is not applied on the D leaf which is used as control. Now, as shown in the figure 3.8, the leaves are hanged so that they may transpire freely.

Observation and Explanation

When the observations are taken after a day or two, they are as follows - the A leaf, which is vaselined on its both the surfaces, looks fresh and green, as no surface transpires. The B leaf is vaselined on its lower surface (with stomata), and transpiration takes place only from the upper

surface which is negligible. This leaf also remains turgid and green like the A leaf. If few stomata are present on the upper surface of the leaf, then it shrivels to some extent. The C leaf is vaselined on its upper surface, which contains less number of stomata or no stomata. The transpiration takes place from the lower stomatal surface, and the leaf shrivels to a large extent. The D leaf is not vaselined and both the surfaces transpire freely releasing much water. The leaf wilts completely in this case. This experiment proves that the rate of stomatal transpiration is faster than the cuticular transpiration.

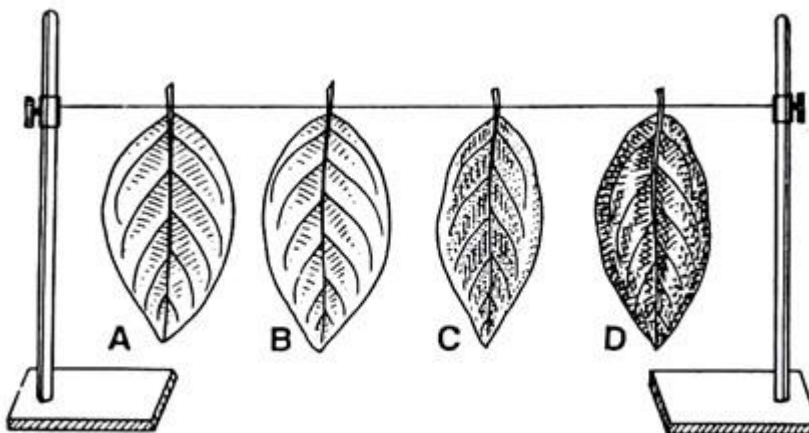


Fig.3.8 Comparison of transpiration of leaf by using Four leaves methods

3.6 TO DEMONSTRATE OF RATE OF TRANSPIRATION BY USING DIFFERENT TYPES OF POTOMETERS

Introduction: The rate of transpiration in a plant varies depending on a range of conditions, including light intensity, relative humidity, wind speeds and temperature, as well as between plant species. In this practical, students have understood the rate of water uptake by a shoot, due to transpiration, using a simple but effective potometer.

The rate of transpiration (expressed in gm per hour per square centimeter of the leaf surface) can be measured with the help of an apparatus known as potometer. Determination of the rate of transpiration by different potometers, excepting Garreau's, is an indirect one where both absorption and transpiration have been taken into consideration.

A potometer is a device used for measuring the rate at which a plant draws up water. Since the plant draws up water as it loses by transpiration, you are able to measure the rate of transpiration. Potometers are notoriously difficult to set up, because air bubbles in the xylem of the plant or in the apparatus itself will prevent the device from working properly. The basic elements of a potometer are:

- (i) A plant cutting
- (ii) A calibrated pipette to measure water loss
- (iii) A length of clear plastic tubing
- (iv) An air-tight seal between the plant and the water-filled tubing

Overview of experiment

1. Assemble potometers
2. Place each potometer at different environment condition
3. Measure water loss in every potometer for every three minutes
4. Calculate leaf surface area for each cutting

The basic design of a potometer

Potometers are designed on the principle that the rate of transpiration is nearly proportional to the rate of absorption of water by the plant. Vaseline is applied around the rubber bungs to ensure an airtight seal, thus the only water loss from the apparatus is via transpiration. The function of the reservoir is to allow the air bubble to travel back to the start of the measuring scale. As water moves up through the plant the air bubble moves along the scale giving a measure of water absorbed by the plant over time and hence the transpiration rate. The potometers described below are generally used in laboratory experiments. It consists of:

- a) **A length of capillary tube:** A bubble is introduced to the capillary. As water is taken up by the plant, the bubble moves. The distance the bubble travels in a given time is determined by the rate of transpiration by the plant.
- b) **A reservoir:** By turning the tap on the reservoir, the position of the bubble can be set at the start of the experiment. Some designs of potometer use a syringe instead of a funnel with a tap.
- c) **A tube for holding the shoot:** In the diagram the shoot is held in place by inserting a rubber bung in the tube. The hole in the bung through which the shoot passes must be thoroughly greased with petroleum jelly to keep it airtight.

Setting up

1- Cut a leafy shoot from a plant (e.g. Pelargonium) and plunge its base into water (try not to get any water on the leaves). This prevents the xylem from taking up any air.

2- Back in the laboratory, put the stem into a large sink full of water and carefully trim the shoot again, by cutting off the bottom under water with a sharp razor blade. Keep the leaves out of the water.

3- Immerse the whole of the potometer into the sink. Move it about until all the air bubbles come out.

4- Put the shoot stem into the bung, grease the joint with plenty of petroleum jelly, then put the bung into the potometer.

5- Make sure the tap is closed, and then lift the whole ensemble out of the water.

6- Leave the end of the capillary tube out of the water until an air bubble forms then put the end into a beaker of water.

Using a potometer

Allow the bubble time to round the corner and start at the beginning of the mm scale. Then time how far the bubble moves in a given period of time. Repeat under different conditions and compare. The usual conditions to try are placing the plant in a bright light, placing it by a fan, and placing it in a humid atmosphere. If the surface areas (both sides) of the leaves are measured then it is possible to compare the transpiration rates of different species of plant. A useful comparison unit could be water loss (ml)/ cm³/minute.

Limitations of a potometer

1- Introducing the air bubble is not easy.

2- The twig may not fully remain alive for a long time.

3- Any changes in the outside air temperature may affect the position of the air bubble in the capillary tube.

Object 1- To measure the rate of transpiration by using Darwin's potometer

Material required: Darwin's potometer, a plant twig cut end dipped in water, a beaker, water grease or wax and stopwatch are required for the experiment.

Working Procedure: This apparatus consists of a short glass tube from which a side tube bends upward ending in an open mouth (manometer tube) into which a plant twig is inserted through a cork. The upper open mouth of the main tube is closed by a cork. The lower end of the tube is also fitted with a cork through which passes a long graduated (in ml) capillary tube. The end of the capillary tube dips in a beaker containing water.

At the beginning of the experiment, water is filled up in all the tubes maintaining continuity. A twig (cut under water) having some leaves are inserted through the cork of the side tube. All joints should be air-tight. A small bubble is introduced through the lower end of the capillary tube. As the transpiration occurs from leaves of the twig, water is absorbed by the twig from the side tube and this produces a suction force which sucks up water from the capillary tube. As a result, air bubble within the capillary tube gradually moves upward.

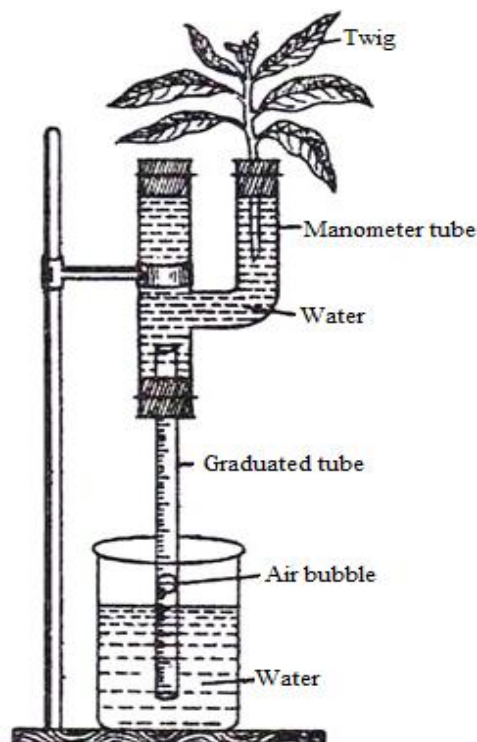


Fig.3.9: Darwin Potometer

Results: The rate of upward movement of air bubble is recorded by noting the height by which it ascends in a given time interval. The rate of transpiration is expressed as the ml of water transpired per minute per unit area of the leaves.

Object-2: To measure the rate of transpiration by using Ganong's potometer

Material required: Ganong's potometer, a plant twig cut end dipped in water, a beaker, water grease or wax and stopwatch are required for the experiment.

Working Procedure: The apparatus is filled with water using the reservoir and with the help of cork a cut stem twig immersed in the water is fixed in the side arm. The apparatus is made air tight and air bubble is introduced in the graduated arm. At this time movement of the air bubble should be towards the plant twig. The record is taken of the distance travelled by vacuole in a definite interval of time. The observation are repeated and the average relation of time to distance travelled is calculated. The total area of the leaf is measured with the help of graphic sketches and is multiplied by two due to two leaf surfaces. By using simple mathematical calculation the rate of transpiration is calculated in terms of ml/hour/unit area.

The volume of water can be calculated by formula $V = \pi r^2 l$ (V =volume; $\pi = 22/7$; r = radius of graduated tube and l = length or distance travelled by bubble.)

This experiment as well as any other similar experiment is based on the principle *that the amount of water absorbed is almost equal to that of water transpired and the difference between two is negligible*".

Modification: - The effect of different factors such as temperature, wind velocity, humidity, light, anaesthetics etc. on the rate of transpiration can be measured by using Ganong's Potometer.

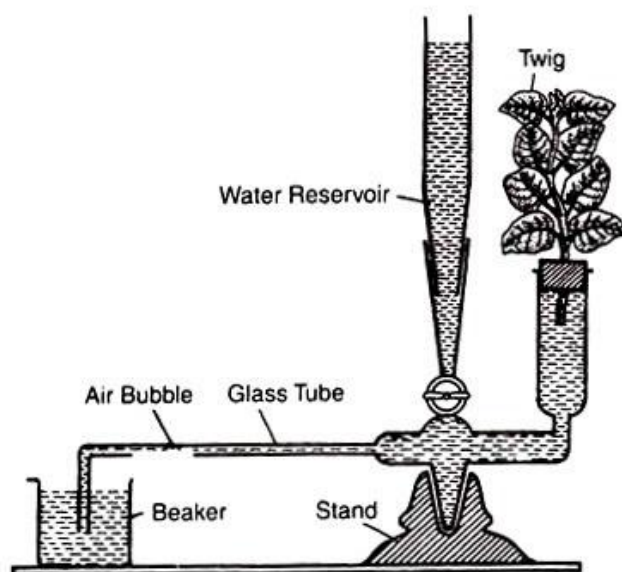


Fig.3.10: Demonstration of Ganong's photometer

Object-3- To measure the rate of transpiration by using Farmer's photometer

Description: The apparatus consists of a wide-mouthed bottle fitted with a rubber stopper having three holes. The bottle is filled with water up to the neck. In one hole a leafy twig (cut under water) is introduced in such a way that its lower cut end remains well under water. In the second hole, a water reservoir having a stopcock in its connecting tube is fitted so as to control the supply of water into the bottle. In the third hole, a narrow bent tube is fitted so that its lower end is well below the water surface of the bottle. The bent part of this tube is horizontal in position and either graduated or fitted with a centimetre scale. The outer end of the horizontal tube is again bent downward and immersed in water.

Working Procedure: At the beginning, the bottle is filled with water by opening the stopcock of the reservoir up to the mouth and also in the horizontal tube. The stopcock is then closed. An air bubble is now introduced into the outer bent end of the horizontal tube in the usual manner. As transpiration occurs from the leaves of twigs, water is absorbed from the lower cut end of the

twig and a suction force is produced which sucks water from the horizontal tube. As a result air bubble moves inward. The rate of movement of air bubble is noted, which is considered proportional to the transpiration rate.

Object-4- To measure the rate of transpiration by using Garreau's potometer

Working Procedure: This potometer is conveniently used for quantitative measurement of differential rates of transpiration from both upper and lower surfaces of a leaf. It consists of two wide-mouthed cups which are placed face to face keeping a widely expanded dorsiventral leaf; of a twig from a potted plant in between them.

Before keeping the leaf in between; the cups, some anhydrous CaCl_2 contained in two small vials are weighed and placed in both the cups. The ends of the cups are closed with corks through which two mercury manometers are connected in order to keep the vapour pressure within the cups constant. The broad rims of the cups in contact with leaf surface are made Potted air-tight by applying Vaseline carefully. A change in vapour pressure within the cups shown by the manometers is indicative of the fact that either the connections are not air-tight or that all the vapour given out by the leaf surface is not being absorbed by the CaCl_2 within the vials. The whole arrangement is clamped vertically on a stand.

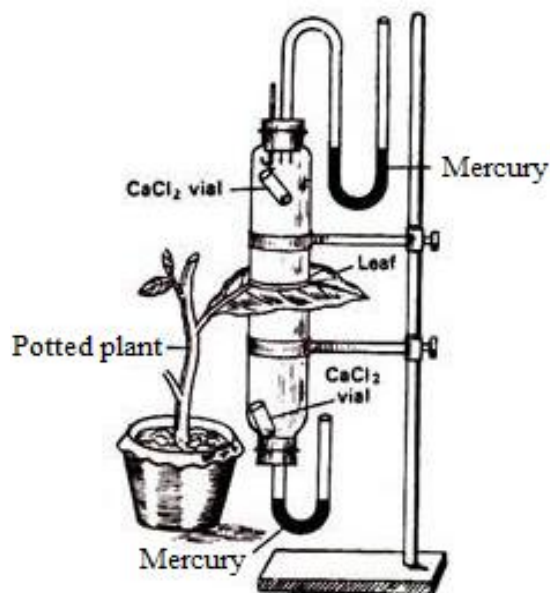


Fig.3.11 Demonstration of Garreau's potometer

Results: After a few hours, CaCl_2 vials are taken out and weighed again. The difference between the two weighing is a measure of the amount of water loss from the two leaf surfaces. As the areas are the same, this is also a quantitative measure of the differential rates of transpiration. By Garreau's potometer method, not only the rate of transpiration but a comparative assessment of the rate of transpiration of upper and lower surfaces of a leaf, i.e.,

cuticular and stomatal transpiration respectively, can be simultaneously determined. This is a very convenient and accurate method for direct and quantitative measurement of the rate of transpiration in contrast to other potometer methods.

3.4 SUMMARY

Transpiration is loss of water from plant in the form of vapour. It is a very important process keeping in view that it directly influences the water status of the plant. It also provides the prime force to pull water up in the plant body. Plants ought to have strict control over the rate of transpiration; otherwise they might land themselves in a fatal situation. Plants regulate the opening and closing of stomata temporally and according to various influencing factors at various climatic condition by controlling the water potential using various type of potometer and resulting turgor pressure in guard cells

3.5 GLOSSARY

Abscisic acid (ABA)- Plant hormone which causes stomatal closure.

Absorb- To suck up

Absorption- The process of **absorbing** or taking up of water and assimilating substances into cells or across the tissues and organs through diffusion or osmosis.

Boundary layer- A thin layer of still air above the leaf surface.

CAM Plants- Plants that take carbon dioxide during night, store it as 4 carbon compound which is decarboxylated during day and the released carbon dioxide is used for photosynthesis.

Capillary tube- Glass tubing which is very narrow inside.

Climate- the weather conditions prevailing in an area in general or over a long period.

Cuticle- In plants, the cuticle is the waxy covering on the surface of many of plant organs, e.g. leaves and young shoots. In plants, this waxy, hydrophobic covering protects the plant by minimizing water loss and curbing pathogen entry.

Cytoplasm- The cytoplasm is the material within a living cell, excluding the cell nucleus. It comprises cytosol (the gel-like substance enclosed within the cell membrane) and the organelles—the cell's internal sub-structures.

Epidermis- One or more layers of cells forming the outermost portion of the skin or integument.

Evaporation- The process in which a liquid changes state and turns into a gas.

Leaf dorsiventral- These leaves grow horizontally, so that the majority of stomata are found on the lower half so that transpiration does not increase during the day.

Lenticels- Pores on woody stems and roots for gaseous exchange.

Osmotic solutes- Osmotically active solutes in guard cells e.g. sucrose, malate.

Phloem- The vessels in plants that transport sugars.

Potometer- A potometer is a device used for measuring the rate of water uptake of a leafy plant shoot.

Relative humidity: The ratio of actual vapor pressure to saturation vapor pressure. Expressed as percentage (%).

Secondary transport: Active transport driven by the energy of proton gradient.

Solution -The dispersion of a substance in molecular or ionic form throughout the medium of another is known as solution.

Stomata frequency- Stomatal frequency can be defined as the number of stomata present per unit area of a leaf.

Stomata- Pores in the epidermis of leaves- usually on the undersides of leaves through which gaseous exchange and transpiration takes place. Singular is stoma.

Stomatal index- It is a function of both the number of stomata plus the size of the epidermal cells.

Transpiration index- Transpiration index is calculated by the relative efficiency of the rate of transpiration with that of physical evaporation.

Transpiration-The loss of water from leaves and stems of plant by evaporation. It is much faster when stomata are open than when they are closed.

Turgor pressure (hydrostatic pressure)- Turgor pressure is the force within the cell that pushes the plasma membrane against the cell wall. It is also called hydrostatic pressure, and more intricately defined as the pressure measured by a fluid, measured at a certain point within itself when at equilibrium.

Vascular bundles- Groups of xylem and phloem tissue in a plant.

Water potential- The free energy associated with water per unit volume.

Xylem vessels-Narrow, hollow, dead tubes with lignin responsible for the transport of water and minerals in plants.

Zeaxanthin- A carotenoid that acts as a blue light receptor.

3.6 SELF- ASSESSMENT QUESTIONS

3.6.1 Multiple choice questions:

1. The water readily available for plant by absorption of root

- | | |
|---------------------------|-----------------------|
| (a) A Gravitational water | (b) Hygroscopic water |
| (c) Rainy water | (d) Capillary water |

2. Loss of water from stomata of leaves

- | | |
|-------------------|-----------------|
| (a) Guttation | (b) Exudation |
| (c) Transpiration | (d) Evaporation |

3. Transpiration is very essential for plants as it

- | | |
|-------------------|--------------------|
| (a) Cools | (b) Exchange gases |
| (c) Removes water | (d) Uptake water |

4. Rate of transpiration is increased with increase in
(a) Light (b) Temperature
(c) Wind (d) All of Above
5. Water transpiration is done 90% by process of
(a) Cuticular transpiration (b) Lenticular transpiration
(c) Stomatal transpiration (d) Sweating
6. Transpiration that occurs from vital organ lenticels present on stem is termed as
(a) Cuticular transpiration (b) Stomatal transpiration
(c) Lenticular transpiration (d) Translocation
7. In transpiration, water leaves cell of plants in form of
(a) Ice (b) Water droplets
(c) Sugars (d) Dew
8. Layer that restricts evaporation in humans
(a) Hair (b) Epidermis
(c) Cuticle (d) Guard cell
9. Layer that restricts evaporation in plants
(a) Hair (b) Epidermis
(c) Cuticle (d) Guard cell

3.6.2 State true or false:

1. Low humidity in the atmosphere results in a decrease in the rate of transpiration.
2. Transpiration takes place only in the green plants.
3. The wall of a guard cell towards the stoma is thin.
4. Leaves are reduced to spines in xerophytic plants.
5. The loss of water droplets is called bleeding.
6. The pH of the guard cells increase during day time.
7. The escape of plant-sap from the cut surface is called guttation.

3.6.3 Name the following-

- 1-The respiratory openings found on stems of woody plants.
- 2-An apparatus to compare the rate of transpiration in cut shoots.
- 3- The process by which intact plants lose water in the form of droplets.
- 4- Opening on the stem through which transpiration occurs.
- 5- Opening found on the under surface of dorsiventral leaves.

- 6- The paper which is used to show loss of water through stoma of a leaf.
- 7- An instrument used for measuring the rate of transpiration.
- 8- The structure through which most of the transpiration takes place.
- 9- The process of loss of water in the form of droplets.
- 10- The structure through which guttation takes place.
- 11- The kidney-shaped cells present on stomata.
- 12- The plant having sunken stomata.
- 13- The chemical used to prevent excessive transpiration in plants.

3.6.4 Fill in the blanks:

- 1-Plants regulate opening of stomata by changing the _____ in the guard cells.
- 2- _____, _____ and _____ are the three sites of transpiration.
- 3- The air space present beneath stomata is called _____.
- 4- When relative humidity is high, transpiration rates are _____.
- 5- Excessive loss of water from plants under dry conditions can lead to _____.
- 6- Opening of stomata is stimulated by _____.
- 7- _____ present above epidermis prevents water loss.

3.6.1 Answer Key: 1. (d), 2. (d), 3.(a), 4.(d), 5.(c), 6. (c), 7.(b), 8.(b), 9.(c)

3.6.2 Answer Key: 1-F, 2-T, 3-F, 4-T, 5-F, 6-T, 7-F

3.6.3 Answer Key: 1-Lenticles, 2- Potometer, 3- Guttation, 4-Lenticles, 5-Stomata, 6- Cobalt Chloride paper, 7-Potometer, 8-Stomata, 9-Guttation, 10-Hydathode, 11-Guards cells, 12- Nerium, 13- Silicon emulsions / Phenyl mercuric acetate

3.6.4 Answer key: 1- Turgor pressure, 2- Stomata, Cuticle and lenticels, 3-Substomatal cavity, 4- Low, 5- Wilting, 6-Blue light, 7-Cuticle

3.7 REFERENCES

- Salisbury, F. B. and C. W. Ross. 1992. Plant Physiology. 4th Edition. Wadsworth Publishing Co., Belmont, CA. 682 pp.
- Taiz, L. and E. Zeiger. 2002. Plant Physiology. 3rd Edition. Sinauer Associates, Inc., Sunderland, MA. 690 pp.

3.8 SUGGESTED READINGS

- Graham, L. E., J. M. Graham, and L. W. Wilcox. 2003. Plant Biology. Prentice Hall, Pearson Education, Inc. Upper Saddle River, NJ. 497 pp.

- Nobel, P. S. 1991. Physicochemical and Environmental Plant Physiology. Academic Press, Inc., San Diego, CA. 635 pp.
- Lincoln Taiz and Zeiger Eduardo, Plant physiology, Fifth edition, Sinauer Associates, Inc, USA, 2010.
- Hopkins William G. and Hüner Norman P.A., Introduction to plant physiology, Fourth edition, John Wiley & Sons, Inc., USA, (2009).

3.9 TERMINAL QUESTIONS

3.9.1 Write Very Short answer type:

- 1- Guttation.
- 2- Significance of transpiration
- 3- Porton transport concept
- 4- Antitranspirants
- 5- Transpiration as a necessary evil

3.9.2 Write Short answer type

- 1- Write about effect of intensity of light on rate of transpiration.
- 2- Write about effect of humidity of the atmosphere on rate of transpiration.
- 3- Write about effect of temperature on rate of transpiration
- 4- Explain about any three conditions which affect transpiration.
- 5- Write a note on unique structural features of guard cells
- 6- Transpiration is less during rainy season. Comments.
- 7- Write about on significance of transpiration
- 8- Differentiate in between the following
 - (a) Transpiration and guttation
 - (b) Evaporation and transpiration
 - (c) Lenticels and stomata in old plants

3.9.3 Long answer type questions

- 1- Describe the structure of stomata and its significance.
- 2- What physical forces drive transpiration?
- 3- Explain the structure, function, their different positions and working of stomata. Discuss its role in different physiological processes.
- 4- Give a brief account of mechanism of stomata movement.
- 5- Write an essay on transpiration and its advantages to the plant.
- 6- Discuss the mechanism of transpiration in plant and state the factors affecting it? Point out the beneficial and harmful effects of the process.
- 7- Write an essay on “the Stomata and their role in transpiration.

- 8- Write explanatory note on stomata regulation of transpiration?
- 9- Explain the role of light and CO₂ in the mechanism of opening and closing of stomata?
- 10- Explain how humidity, temperature and light affect transpiration?
- 11- Describe the mechanism of opening and closing of stomata?
- 12- What are the different ways of loss of water from plants? Explain the factors affecting transpiration?
- 13- Write an account of various theories which explain mechanism of opening and closing of stomata?
- 14-What devices have plants evolved to control the rate of transpiration to prevent excessive water loss.
- 15-Highest transpiration rates are observed when there is low humidity combined with bright sunlight and moderate winds.

UNIT-4 STUDY THE EFFECT OF INTENSITY AND QUALITY OF LIGHT ON THE RATE OF PHOTOSYNTHESIS BY WILMOT'S BUBBLER AND STUDY R.Q. BY GANONG'S RESPIROMETER IN DIFFERENT SEEDS

Content

4.1-Objectives

4.2-Introduction

4.3-Study the effect of intensity and quality of light on the rate of photosynthesis by Wilmot's bubbler

4.3.1-Method to use Wilmot's bubbler

4.4- Study R.Q. by Ganong's respirometer in different seeds.

4.4.1- Procedure to use Ganong's respirometer

4.5-Summary

4.6- Glossary

4.7-Self Assessment Question

4.8- References

4.9-Suggested Readings

4.10-Terminal Questions

4.1 OBJECTIVES

After reading this unit students will be able-

- To Study the effect of intensity and quality of light on the rate of photosynthesis by Wilmot's bubbler
- To Study R.Q. by Ganong's respirometer in different seeds

4.2 INTRODUCTION

Plants need light energy to make the chemical energy needed to produce carbohydrates. Increasing the light intensity will boost the rate of photosynthesis. However, at high light intensities the rate becomes constant. Measuring the rate of oxygen evolution using a water plant is commonly used to measure the rate of photosynthesis. Oxygen is a gaseous product, so can be measured by noting volume changes of the number of bubbles evolved.

As the light intensity increases, the rate of photosynthesis increases. However, the rate will not increase beyond a certain level of light intensity. At high light intensities the rate becomes constant, even with further increases in light intensity; there are no increases in the rate. The plant is unable to harvest the light at these high intensities and the chlorophyll system can be damaged by very intense light levels.

4.3 STUDY THE EFFECT OF QUALITY AND INTENSITY OF LIGHT ON RATE OF PHOTOSYNTHESIS BY WILMOT'S BUBBLER

Object-1: To study the method to use Wilmot's bubbler

Material required: Wilmot's bubbler, water, Hydrilla (fresh plant), Vaseline, cellophane papers of red, blue and green colours, burner, thermometer, ruler, stop watch, Mercury/projector lamp e.g. 60 W.

Working Procedure: Wilmot's bubbler can easily be prepared in the laboratory. It consists of a wide mouthed bottle fitted with a cork through which is inserted a wide glass tube. The lower end of this tube is fitted with a cork with hole through which a twig of *Hydrilla* plant is tied up; its other end terminates in a narrow bent nozzle. The upper half of this tube is surrounded by another glass tube which acts as water reservoir at 25°C. This helps to maintain a constant temperature around the plant. Whole of the apparatus is filled with pond water. Care will take so that the level of water in the reservoir remains above the bent nozzle (Fig. 4.1). The apparatus will place in sun light/ source light lamp at distance from the plant for definite intervals and count the bubbles coming out. Count the number of oxygen bubbles given off by

the plant in 1 minute period. This is the rate of photosynthesis at that particular light intensity. Repeat at different light intensities by moving the lamp to different distances. When the rate of air bubbles is regular and adequate (>10 bubbles/minute), place the capillary tube/test tube over the cut end of *Hydrilla* twig and then count the number of bubbles. This should be done for 5 minutes. Repeat twice and obtain an average of the results. Record results in a table, then plot a graph of volume of oxygen/minute or number of bubble/minute against the distance between the lamp and the plant. For comparing the effects of different wavelengths of light the bubbler is wrapped in cellophane papers of different colours. Effect of different intensities of light can be compared by placing the bubbler under shade, less intense and more intense light. The intensity of the light can be measured by a lux meter.

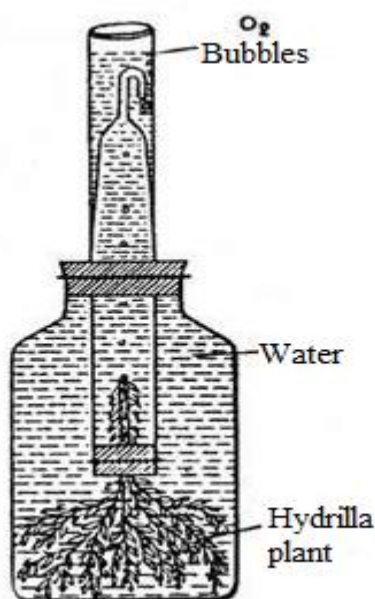


Fig.4.1: Demonstration of Wilmott's bubbler

Observation: The readings show that the rate of photosynthesis is more in sun light. Light energy absorbed by chlorophyll is converted to ATP and H^+ . At very low light levels the plant will be respiring only not photosynthesising. As light intensity increases (distance between lamp and plant decreases) the volume of oxygen (or the rate of bubble production) increases. This indicates that the rate of photosynthesis increases with light intensity. However, at sufficiently high levels of light intensity, the rate oxygen evolution remains constant. Light intensity is inversely proportional to the square of the distance, so as the distance is increased the light intensity decreases.

4.4 STUDY R.Q. BY GANONG'S RESPIROMETER IN DIFFERENT SEEDS

Introduction: Plant respiration is the chemical reaction by which plants cells stay alive.

The process of respiration is expressed as:



The rate of respiration can be measured with the help of Ganong's respirometer. It consists of a bulb like part and a bent tube. Some germinating seeds are taken in the bulb and mouth of the bent tube is kept immersed in a beaker containing caustic potash (KOH) solution. The respiroscope is fixed in a stand. Thus, the enclosed air in the flask is completely cut off from the outside atmosphere. The apparatus is left undisturbed for a few hours.

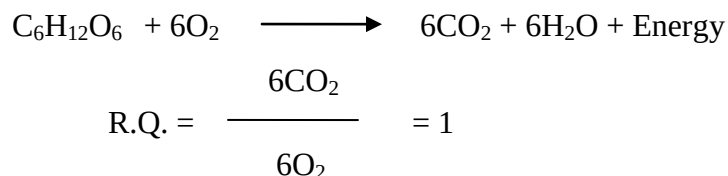
This is another aspect of respiration. ***“Respiratory quotient is the ratio of CO₂ produced to O₂ consumed while food is being metabolized.”*** The rate of respiration can be measured by the amount of CO₂ released. The rate of respiration varies in different organs and with age. In general the factors which affect respiration include internal factors such as the activity of the respiratory enzymes, the type of substrate; and external factors such as oxygen, water, temperature etc.

Respiratory substrate may be carbohydrate, protein or fats. The kind of substrate being oxidized is obtained by measuring the respiratory quotient.

$$\text{RQ} = \frac{\text{Volume of CO}_2 \text{ evolved}}{\text{Volume of O}_2 \text{ consumed}}$$

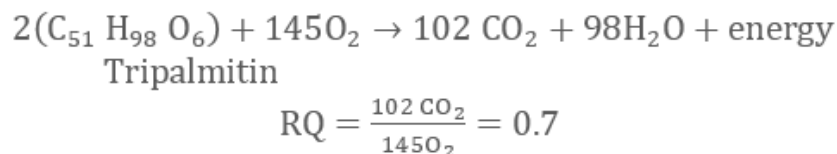
Where, RQ stands for Respiratory Quotient

RQ depends on the type of respiratory substrate used in respiration. When carbohydrate is used as substrate and is completely oxidized, RQ becomes 1. It implies equal amount of O₂ and CO₂ are consumed and evolved. This reaction is displayed in the figure below –



For carbohydrates, CO₂ /O₂ = 1 as in stem and roots.

In case, oil containing seeds e.g. mustard are used during the process of respiration, RQ becomes less than 1. Following equation shows the calculation for fatty acid and tripalmitin is used as substrate –



Object-1: Procedure to use Ganong's respirometer

Material required: Ganong's respirometer, respiratory substrate such as germinating seeds, saline water, caustic potash solution, stand, water, filter paper, beaker, balance with weighing box, caustic potash solution.

Working Procedure:

1. Pour some water in the lower end of the bulb of respirometer, and on putting a filter paper introduce some germinating seeds.
2. Now partly fill the respirometer with the saline water. The use of saline water is due to the fact that CO_2 cannot dissolve in it.

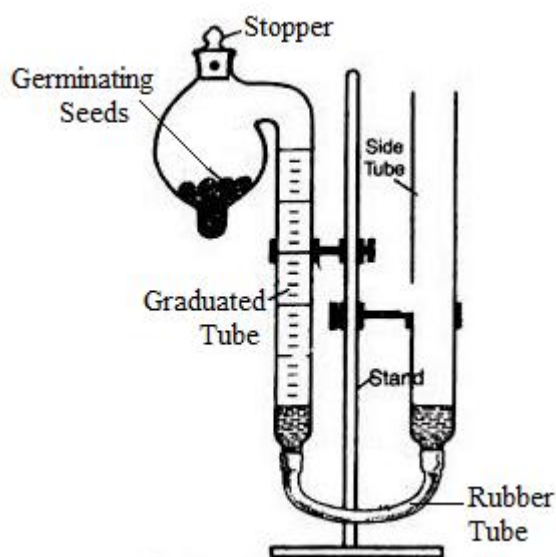


Fig.4.2: Demonstration of Ganong's respirometer

3. Twist the stopper of the bulb in such a way that the two holes come just opposite to each other. In this condition, outside air can pass into the bulbs (Fig 4.2).
4. Now adjust the level of the levelling tube and graduated tube in a way that saline is present on the same level in both the tubes.
5. Now again twist the stopper in a way that two holes are separated and the bulb is closed.

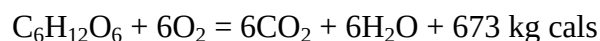
6. Note the level of the saline water and let the respiratory substrate respire for a few hours.

7. Note the final level and introduce a caustic potash crystal and note the changes.

Observations and results:

(A) If the respiratory substrate is carbohydrate (e.g., wheat, maize, oat, gram, pea, etc.):

There is no change in the level of saline water because in the carbohydrate the volume of the O₂ absorbed is equal to the volume of CO₂ liberated as shown by the following equation:



$$R.Q. = 6CO_2/6O_2 = 1/1 = 1$$

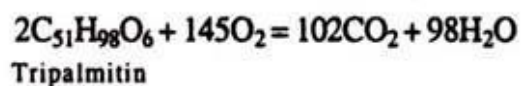
The volume of the CO₂ released can be estimated by adding crystals of KOH. The latter will absorb the CO₂ produced by the seeds resulting ultimately into the rise of the saline level. The volume of the CO₂ can be calculated by deducting the second level from the first one. Suppose it is 45 c.c.

$$\text{So, } R.Q. = \text{Vol. of } CO_2 / \text{Vol. of } O_2 = 45/45 = 1$$

(B) If the respiratory substrate is fat, (e.g., mustard or castor seeds):

In case the respiratory substrate is fat (e.g., mustard or castor seeds), less amount of CO₂ will be released than the O₂ absorbed. A vacuum will be created, and to overcome this vacuum saline level will rise in the tube. This rise will be equal to the excess amount of oxygen. Denote it as V₁.

It can be shown by the following equation:



Add crystals of KOH in saline solution. The level of saline again increases because KOH absorbs the CO₂. A vacuum is created and to overcome it the level of saline increases. Denote it as V₂.

Calculate the volume of CO₂ by V₂ (deducting the second rise from the first rise in level) and volume of O₂ by adding both the rises in the level, i.e., V₁ + V₂.

$$\text{So } R.Q. = V_2 / V_1 + V_2 = \text{Vol. of } CO_2 / \text{Vol. of } O_2 = 102/145 = 0.7$$

So R.Q. is less than one.

Discussion

During respiration, the germinating seeds absorb O₂ from the respiroscope and liberate CO₂. The caustic potash absorbs the CO₂ and a vacuum is created. So the KOH solution rises in the tube. This proves that CO₂ is liberated during respiration.

4.5 SUMMARY

As you may recall, photosynthesis occurs in plants cells that contain chloroplasts. Such cells are found in algae and in the leaves and stems of plants. Photosynthetic organisms use light energy and simple building blocks (carbon dioxide and water) to make their own food. Cellular respiration is very widespread. It is completed in mitochondria. Although the details of the two pathways are different, the overall reaction of cellular respiration is photosynthesis running in reverse. Cellular respiration and photosynthesis are interdependent. The glucose and oxygen produced by photosynthesis are used up in cellular respiration. Cellular respiration extracts the stored energy from food molecules. Energy is transferred to “charge up” ATP, and releases carbon dioxide and water as wastes product. The carbon dioxide and water are then available as raw materials for photosynthesis.

4.6 GLOSSARY

Adenosine Triphosphate (ATP) - The molecule from which cells derive energy. Comprised of an adenosine molecule bonded to three phosphates, each phosphate bond contains energy, especially the third bond. By breaking that one bond and reducing ATP to adenosine diphosphate (ADP), the cell can get the energy to carry out its various processes. **ADP** - Adenosine diphosphate, product of the Calvin cycle that is used in the light-dependent reactions.

Aerobic respiration - A metabolic process involving oxygen in the breakdown of glucose.

Carbohydrate - A molecular compound containing carbon, hydrogen, and oxygen. Subunits are sugars.

Carbon dioxide (CO₂) - a gas naturally found in the atmosphere that is a reactant for the Calvin Cycle.

Carbon fixation - ATP and NADPH are used to fix CO₂ into carbohydrates. Carbon fixation takes place in the chloroplast stroma.

Chemical equation of photosynthesis - $6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2$

Chlorophyll - primary pigment used in photosynthesis.

Chloroplast - organelle in a plant cell where photosynthesis occurs.

Diffusion: The movement of particles (molecules or ions) from an area of higher concentration to an area of lower concentration.

Dioxide: An oxide containing two atoms of oxygen in the molecule: e.g. carbon **dioxide** (CO₂).

Glucose (C₆H₁₂O₆) : It is a monosaccharide sugar that is the product of photosynthesis and it can exist as several forms.

Light: Electromagnetic radiation; the shorter the wavelength the greater amount of energy. Light supplies the energy for the light reactions of photosynthesis.

Lipid - An organic molecule that is insoluble in water. A main component of cell membranes.

Metabolism - All the reactions occurring in an organism that participate in the acquisition or conversion of energy for use in the organism.

NADPH – A high-energy electron carrier used in reduction

Oxidation - The loss of electrons

Oxygen (O₂): Colourless, odourless gas that is a product of the light-dependent reactions and is essential for respiration.

Photosynthesis - the process by which organisms convert light energy into chemical energy (glucose).

Plant respiration- is the opposite of photosynthesis, which is a biological process performed by green **plants** that creates oxygen and releases it into the air. During **respiration, plants** absorb free molecules of oxygen (O₂) and use them to create water, carbon dioxide, and energy, which help the **plant** grow.

Protein - An essential molecule found in all cells. Composed of amino acid subunits.

Reduction - the gain of electrons

Respiration - A process that occurs in cells in which cells breakdown food molecules to yield ATP. Can be either aerobic or anaerobic.

Respirometer - It is a device used to measure the rate of respiration of a living organism by measuring its rate of exchange of oxygen and/or carbon dioxide.

Respiratory quotient- **the ratio of CO₂ produced to O₂ consumed while food is being metabolized**

Stomata- Tiny holes in the epidermis (skin) of a leaf, usually on the undersides of leaves. They control water loss and gas exchange by opening and closing. Singular is stoma.

4.7 SELF-ASSESSMENT QUESTIONS

4.7.1 Multiple Choice questions:

1. Which of these is true about photosynthesis?

- | | |
|---|--|
| (a) It occurs in both animal and plants | (b) It occurs in both fungi and plants |
| (c) It is a breaking down process | (d) It is building up process |

2. R.Q. in anaerobic respiration is

- | | |
|-------|--------------|
| (a) 0 | (b) ∞ |
| (c) 1 | (d) > 1 |

3. The correct relationship of value of Respiratory Quotient is

- (a) Glucose symbol > Fats symbol > Organic acid

- (b) Glucose symbol < Fats symbol < Organic acid
(c) Fats symbol > Glucose symbol > Organic acid
(d) Fats symbol < Glucose symbol < Organic acid

4. Which of the following respiratory material may show the unit value of R.Q.

- (a) Stem of wheat (b) Leaf of barley
(c) Leaf of oat (d) All the above

5. Which knowledge is obtained by knowing the value of R.Q.?

- (a) Type of substrate (b) Number of ATP produced
(c) Type of intermediate products formed (d) None of the above

6. A mixture containing equal quantity of germinating maize and groundnut seeds are taken. The RQ of this mixture would be

- (a) One (b) Less than one
(c) More than one (d) Infinity

7. What is the value of RQ of castor seeds, if the imaginary values of Ganong's respirometer are as follows (i) First rise of saline = 10 ml (ii) Second rise of saline after adding KOH = 30 ml

- (a) 0.33 (b) 0.75
(c) 0.85 (d) 3.00

8. R.Q. is defined as

- (a) Ratio between CO₂ liberated and O₂ taken
(b) Volume of oxygen taken
(c) Volume of carbon dioxide liberated
(d) Ratio between oxygen taken and fat utilized

9. Number of CO₂ molecules used during photosynthesis.

- (a) 2 (b) 5
(c) 6 (d) 10

10. Raw materials for photosynthesis include

- (a) Light (b) Organic substances
(c) Nutrients (d) All of these

11. To form one glucose molecule (C₆H₁₂O₆), numbers of water molecules required are

- (a) Six (b) Eight

(c) Ten

(d) Twelve

12. RQ is equal to

(a) C/N

(b) N/C

(c) CO_2/O_2

(d) O_2/CO_2

13. RQ of fatty acid substrate generally

(a) 1

(b) >1

(c) <1

(d) None of the above

4.7.1 Answer Keys:

1. (d), 2.(b), 3.(d), 4.(d), 5.(a), 6.(b), 7.(b), 8. (a), 9. (c), 10. (d), 11. (d), 12. (d), 13. (c)

4.8 REFERENCES

- Taiz, L. and E. Zeiger. 2002. Plant Physiology. 3rd Edition. Sinauer Associates, Inc., Sunderland, MA. 690 pp.

4.9 SUGGESTED READINGS

- Graham, L. E., J. M. Graham, and L. W. Wilcox. 2003. Plant Biology. Prentice Hall,
- Bewley, J.D., and M. Black. 1985. Seeds: Physiology of Development and Germination. Plenum Press, New York.
- Cooper, E.L. 1997. Agriscience: Fundamentals & Applications. Delmar Publishers, Albany, New York.
- Walch, J.W. 1994. Low-Cost Biology Investigations

4.10 TERMINAL QUESTION

4.10.1 Short answer type Question:

1-What is the difference between respiration and photosynthesis?

2-What is cellular respiration?

3-What is external respiration in plants?

4-Why during day, plants give out oxygen instead of taking it for respiration?

5-How do cells obtain the energy they need to function?

6-Differentiate in between breathing and respiration.

7-Different substrate oxidizes during respiration. How does respiratory quotient (RQ) indicate which type of substrate i.e. carbohydrate, protein or fat is getting oxidize?

$\text{RQ} = \text{A/B}$

What do A or B stand?

What type of substrate have RQ of 1, >1, <1

4.10.2 Very Short answer type Question:

1. What is respiration?
2. Name a few respiratory substrates. Which of them is most commonly used?
3. Give the general equation for respiration.
4. What is respiratory quotient or R.Q?
5. What is photosynthesis?
6. What are the important events occur during photosynthesis process?
7. Mention the conditions for photosynthesis. Also mention the process involved in each of these steps.
8. How do aquatic plants get oxygen for photosynthesis?
9. How are photosynthesis and respiration related to each other?
10. How do aquatic plants get oxygen for photosynthesis?

UNIT-5 DEMONSTRATION OF COLOUR TESTS AND MICRO CHEMICAL TESTS FOR CARBOHYDRATES, PROTEINS AND LIPIDS

Content

- 5.1-Objectives
- 5.2-Introduction
- 5.3-Procedure of colour tests and micro chemical tests
 - 5.3.1-Carbohydrates
 - 5.3.2-Proteins
 - 5.3.3-Lipids
- 5.4-Summary
- 5.5- Glossary
- 5.6-Self Assessment Questions
- 5.7- References
- 5.8-Suggested Readings
- 5.9-Terminal Questions

5.1 OBJECTIVES

After reading this unit students will be able to-

- Apply knowledge of biomolecule reagents to identify types of biomolecules.
- Identify the type of biomolecule present per unknown sample.
- Interpret the results when presented with data for each of the biochemical tests.
- Design experiments to identify biomolecules using biochemical tests

5.2 INTRODUCTION

All living things are essentially composed of the same basic groups of molecules. The four types of carbon-based molecules you will perform chemical tests for in this laboratory exercise are carbohydrates (sugars and starch), lipids, amino acids, and proteins. Chemical tests are often used on molecules to distinguish one from another. The chemical interactions typically involved a color change. For example, when iodine (Lugol's solution) is added to a starch, a dark blue-black color will appear. Chemicals of this type are known as **indicators**.

5.3 PROCEDURE OF COLOUR TESTS AND MICRO CHEMICAL TESTS

5.3.1-Carbohydrates

Carbohydrates are polyhydroxy aldehydes or polyhydroxy ketones or any compound which gives these on hydrolysis. Carbohydrates can be either aldoses or ketoses depending upon whether aldehyde group (-CHO) is present or ketonic group (-C=O). The empirical formula of carbohydrates is $C_n(H_2O)_n$ or $(CH_2O)_n$ where value of n can be 3-7 for monomers. Carbohydrates are synthesized in plants by the process of photosynthesis. Carbohydrates are also called as saccharides since they are made up of sugars. There are three types of carbohydrates monosaccharide, oligosaccharide and polysaccharide. Monosaccharides are simplest carbohydrates which cannot be further hydrolyzed (broken down) into smaller molecules. Monosaccharides are crystalline and colourless and exist as solids at room temperature. Monosaccharides are extremely water soluble despite that they possess high molecular weight. Oligosaccharides are defined as sugars formed by polymerization of monosaccharide with a maximum of 9-10 monomeric units linked to one another by glycosidic bond, however most commonly occurring oligosaccharides have 2-6 monomers linked to one another. Oligosaccharides are sweet in taste, crystalline in nature and water soluble.

Oligosaccharides made up two monosaccharide units are called as disaccharides (common examples include sucrose, lactose and maltose), oligosaccharides made up three monosaccharide units are called as trisaccharides (e.g. raffinose), Oligosaccharides with four

units are called as tetrasaccharides (e.g. stachyose) and so on. Polysaccharides are polymers comprising of hundreds and thousands of monosaccharide units. There are two types of polysaccharides based upon their role or function in living organisms. Polysaccharides can be storage polysaccharides such as starch in plants and glycogen in animals. Storage polysaccharides serve as source of energy. Another type of polysaccharides are structural polysaccharides such as cellulose in plants, cellulose is the main component forming the framework of cell wall of plants; Chitin is another structural polysaccharide which is main component of cell wall of fungi.

Reducing and non reducing sugar

Sugar which have free aldehydic or ketonic group and which can reduce Benedict solution Fehling's solution are called as reducing sugar. Reducing sugar have an OH group present on carbon next to carbonyl carbon (aldehyde or ketonic). This OH group reacts with solution of Cu^{2+} (Benedict's solution / Fehling's solution) to form a coloured (red-orange) precipitate and an oxidation product. All monosaccharides (whether aldoses or ketoses) are reducing sugars. Disaccharides such as maltose and lactose are also reducing sugar. Sugar which does not possess free aldehydic or ketonic group and which does not reduce Benedict solution and Fehling's solution are called non reducing sugar. All polysaccharides

1-MOLISCH'S TEST

Molish's test is a general test performed to identify presence of any carbohydrate present in a sample.

Principle

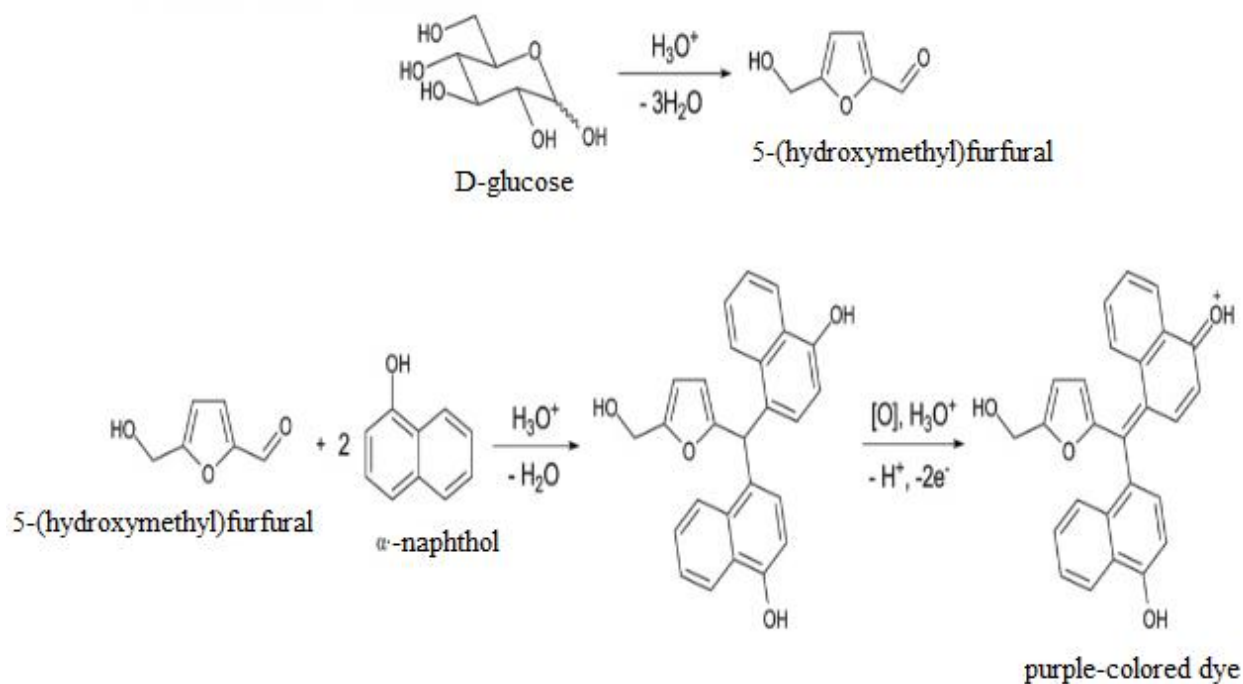
This test is based on reaction of concentrated H_2SO_4 with carbohydrates. When carbohydrate is treated with H_2SO_4 , glycosidic bond is hydrolysed and carbohydrate gets dehydrated. This dehydration leads to production of Furfural and its derivatives which react with α -naphthol to give purple colour.

Material required: α -naphthol, conc. H_2SO_4 , test tube, sample (glucose solution)

Procedure:

- Take about 2ml of aqueous solution of sample.
- Add few drops of α -naphthol reagent into it.
- Pour 1ml of conc. H_2SO_4 slowly along the side of test tube.

Observation: Formation of red violet (purple) ring at the junction of two layers shows the presence of carbohydrate.

Reaction involved:**Precautions:**

- α -naphthol should be prepared fresh each time the test is performed
- H_2SO_4 should be handled carefully.

Reagent preparation

α -naphthol (w/v) : Prepare 5% α -naphthol by dissolving 5gm α -naphthol in 100ml ethanol.

2- BENEDICT'S TEST

This test is utilized for the detection of reducing sugars. A reducing sugar is a carbohydrate possessing either a free aldehyde or free ketone functional group as part of its molecular structure. This includes all monosaccharides (e.g. glucose, fructose, galactose) and many disaccharides, including lactose and maltose. It is a commonly utilized and convenient test due to the stability of its reagent.

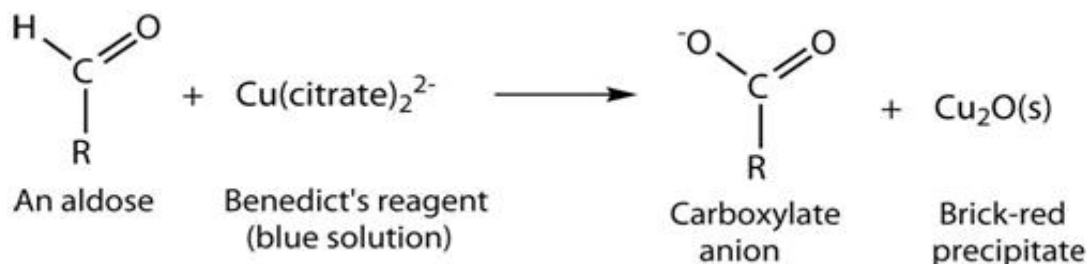
Material required: CuSO_4 , Na_2CO_3 , burner, dropper, test tube, test tube holder, sample (glucose, fructose solution)

Procedure:

- Take about 4 ml sample in a test tube.

- Add small quantity of Benedict's reagent
- Boil the sample over a burner for 2-5 minutes holding the test tube firmly with a test tube holder.
- Gently shake the test tube during heating.
- A brick red precipitate appears, indicating the presence of reducing sugar in the sample.

Reaction involved:



Conclusion: On boiling sample with the Benedict's reagent, the cupric ion present in the Benedict's reagent is reduced by the reducing agent, sugar, to form a brick red coloured precipitate of cuprous oxide.

Reagent preparation;

A. CuSO_4 solution: dissolve 17.3gm of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 100 ml distilled water and then add 100ml distilled water to the solution.

B. Benedict's reagent:

Sodium citrate	173gm
Sodium carbonate	100gm
Distilled water	600ml
Finally make the volume 800ml by addition of distilled water	

Add reagent A and B to make 1litre solution and use it as Benedict solution.

3-FEHLING'S TEST:

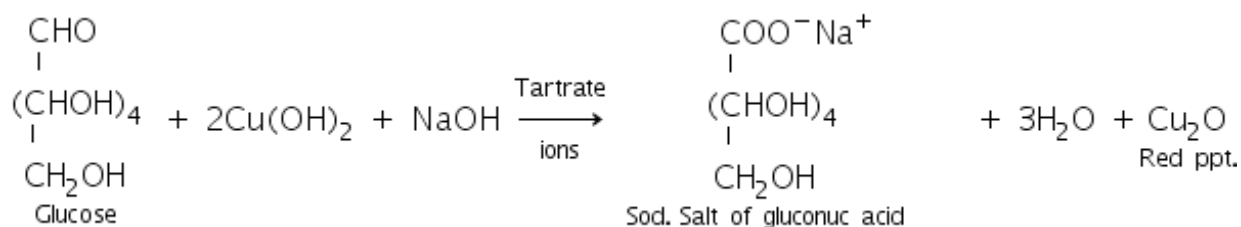
This test is utilized for detection of presence of reducing sugar in the sample.

Material required:

Potassium sodium tartrate, hydrated copper (II) sulphate, sodium hydroxide, Fehling's solution A, Fehling's solution B, test tube, test tube holder, burner, dropper, sample

Procedure:

- Take 3-4 ml sample in a test tube
- Add small quantity of Fehling's solution A.
- Then add small quantity of Fehling's solution B to the test tube containing sample.
- Boil the sample over a burner for 2 minutes, holding the test tube firmly with a test tube holder.
- A brick red precipitate appears, indicating the presence of glucose in the sample.

Reaction involved:

Conclusion: The cupric ion present in the Fehling's solution is reduced on boiling by the reducing substance, sugar, to form the brick red coloured precipitate of cuprous oxide. Formation of cuprous oxide indicates presence of reducing sugar.

Reagent preparation:

Fehling's solution I: consists of 7 g of hydrated copper(II) sulfate dissolved in 100 mL of distilled water.

Fehling's solution II: is made by dissolving 35 g of potassium sodium tartrate and 10 g of sodium hydroxide in 100 mL of distilled water.

Fehling's reagent: Equal volumes of Fehling I and Fehling II are mixed to form a deep blue solution.

Precaution:

- Fehling's solution is corrosive and toxic. Wear personal protective devices such as gloves and goggles when preparing the solution and when performing the test.
- Prepare reagent fresh.

4-ANTHRONE TEST

This is another test one for presence of carbohydrate.

Principle: Anthrone test is based on the reaction of conc. H_2SO_4 acid, which causes dehydration of carbohydrate due to hydrolysis glycosidic bond. Dehydration leads to production of furfural and its derivatives. The furfural and its derivatives formed react with anthrone and

produce green (or bluish green) colour. Polyssacharides and glycoprotein also give positive test in this reaction.

Materials: Water bath, conc. H_2SO_4 , anthrone, sample (glucose solution)

Method:

- Take 1ml of test sample in tube.
- Add 2ml of anthrone reagent. Mix .watch for colour development, it should be bluish green. If you do not see colour development, place the tubes for 10 min in boiling water bath and observe the colour.)

Observation: Addition of anthrone reagent to carbohydrate sample results in appearance of bluish – green colour.

5-IODINE TEST

Iodine has a tendency to form a coloured complex with polysaccharide. The test is utilized for identifying presence of starch and glycogen n a sample. Iodine when reacts with starch it gives blue colour and when it reacts with glycogen it gives reddish brown colour.

Material required: Iodine, potassium iodide, test tube, dropper

Procedure:

- Add about 5 drops of iodine solution to the test tube containing sample
- Blue black colour indicates the presence of starch in the sample

Reagents:

- Iodine solution: Dissolve few crystals of iodine in 20ml of 3% KI solution.
- 3% KI solution: Dissolve 3gm KI in 100ml distilled water.

6-BARFOED'S TEST

The test is performed of distinguish monosaccharides from reducing disaccharides.

Principle: Cupric acid along with acetic acid causes reduction of monosaccharides. Reduction of cupric acetate results in formation of cuprous oxide which is brick red in color. Monosacharrides upon reaction with Barfed reagent produces color within 2-3 minutes whereas disaccharides take longer time for color production. This is because disaccharides are first hydrolysed to monosaccharides and then these monosaccharides react with cupric acetate to form cuprous oxide (red ppt.).

Materials: Copper acetate, acetic acid, sample (glucose solution, fructose solution), distilled water, test tube

Procedure:

- Take 2 ml of sample to be tested in a test tube
- Add 2 ml of freshly prepared Barfoed's reagent.
- Place test tubes into a boiling water bath and heat for 3 minutes.
- Allow to cool.

Observation: Occurrence of deep blue colouration with a red precipitate settling down at the bottom or sides of test tube shows presence of reducing sugars. Appearance of a red precipitate as a thin film at the bottom of the test tube within 3-5 min. indicates presence of reducing monosaccharide. If the precipitate formation takes more time, then the sample is reducing disaccharide.

Reagents:

Barfoed's reagent

Copper acetate	13.3gm
Acetic acid	1.8gm
Make the final volume of solution up to 200ml with distilled water	

7- SELIWANOFF'S TEST

The test is utilized to differentiate between aldoses and ketoses.

Principle: This test is based upon dehydration of carbohydrates in presence of HCl. This hydration of carbohydrates occurs due to breaking of glycosidic bond and results in formation of furfural and its derivatives. Furfural and its derivatives thus formed react with resorcinol to give red color.

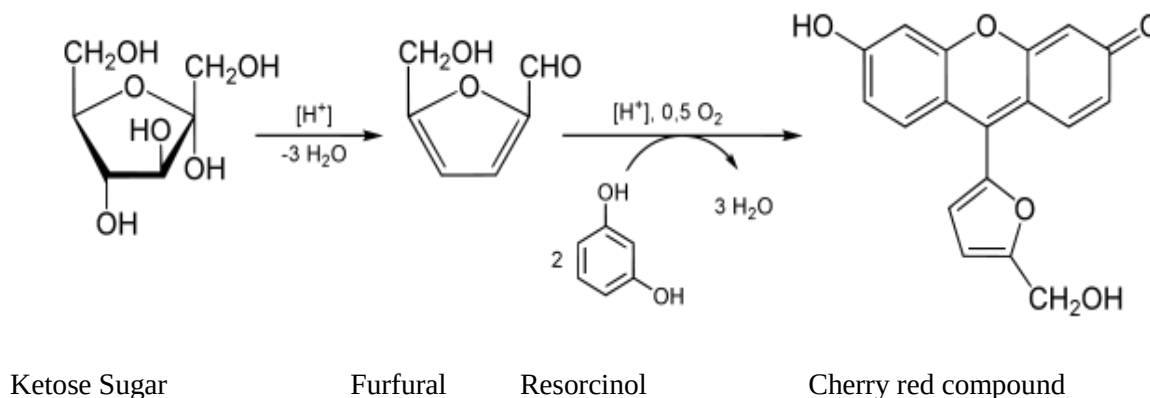
Material: Resorcinol, HCl, sample (glucose and fructose solution), test tube

Procedure

- To 3ml of Seliwanoff's reagent, add 1ml of the test solution.
- Boil in water bath for 2 minutes.
- Observe for color production.

Observation: Formation of cherry red colored precipitate in about 5 minutes shows presence of ketoses whereas appearance of faint red colour shows presence of aldoses.

Reaction involved:



Conclusion: On reaction with Seliwanoff reagent, ketoses form a cherry red condensation product within 2 minutes. Whereas Aldoses react slowly, forming the coloured condensation product.

Reagent

- **Seliwanoff's reagent:** Dissolve 50mg resorcinol in 100ml 3N HCl.
- **3N HCl:** Add 25ml HCl to 75ml distilled water.

8-BIAL'S TEST

Bial's test is utilized for detection of presence of pentose sugar in the sample. Upon heating a pentose sugar with conc. HCl, furfural is produced. Furfural condenses with orcinol in presence of ferric ions to give blue-green color.

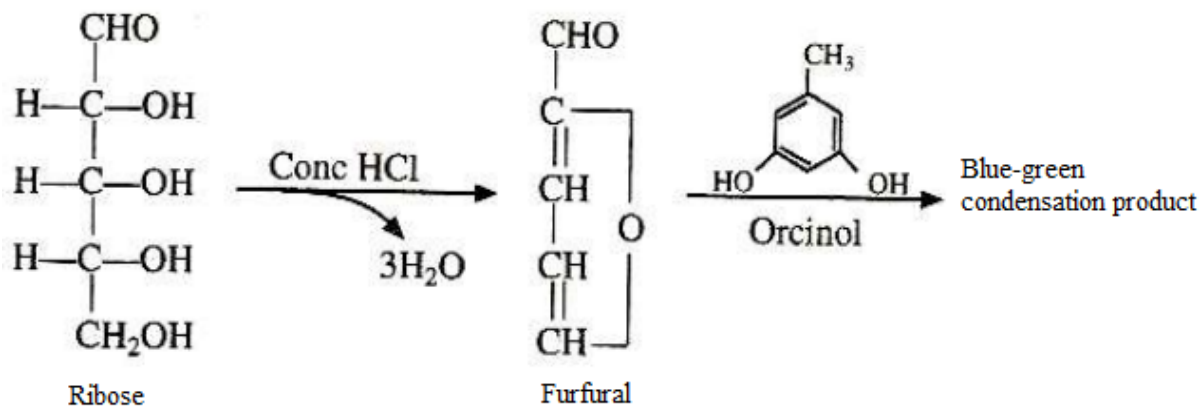
Material's required: Water bath, conc. HCl, Orcinol, Ferric chloride, distilled water, test tubes, sample (pentose sugar only).

Procedure

- Add 3ml of Bial's reagent to 0.2ml of the test solution.
- Heat the solution in a boiling water bath for 2 minutes.

Observation: Formation of blue-green product shows presence of pentoses.

Reaction involved:

**Reagents:**

1. Ferric chloride solution (10%)	
Ferric chloride	10g
Distilled water	100ml
2. Bial's Reagent	
Orcinol	1.5g
HCl	100ml
10% Ferric Chloride (FeCl ₂) solution	20-30 drops

9-OSAZONE TEST

Ketoses as well as aldoses react with phenylhydrazine to produce phenylhydrazone which further reacts with two molecules of phenylhydrazine to produce osazone. Crystals of different shapes will be shown by different osazones. Needle-shaped yellow crystals are produced by glucose, fructose and mannose, lactosazone produces mushroom shaped crystals and maltose produce flower-shaped crystals.

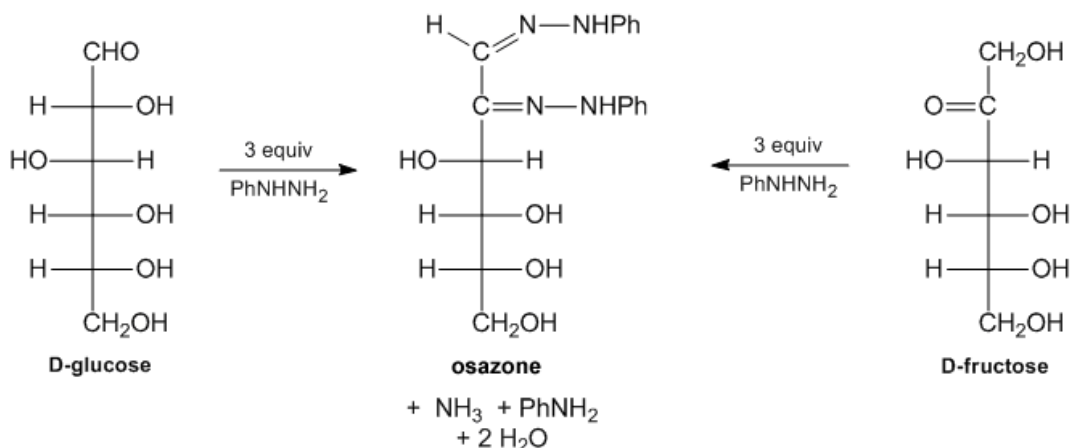
Procedure

- To 2 ml of sample, add 3ml of phenyl hydrazine hydrochloride solution and mix.
- Shake well and heat in a boiling water bath for 30 to 45 minutes.
- Take the tube out of the water bath and let it cool slowly.
- Yellow crystals of osazones will appear.
- Examine the crystals under the microscope and describe the nature of crystals.

Observation: Formation of yellow needle shaped crystals of osazone.

Conclusion: Reducing sugars forms osazone on treating with phenylhydrazine

Reaction involved:



Reagent:

Phenyl hydrazine (PhNH₂) solution:

- Phenyl hydrazine mixture is prepared by mixing equal weights of phenyl hydrazine hydrochloride and anhydrous sodium acetate.
- The mixing is to be done thoroughly in a mortar.

10-PICRIC ACID TEST

Picric acid test is done for identification of reducing sugars. Reducing sugars react with picric acid to produce red coloured product.

Principle: In this reaction when sugar solution is mixed with picric acid and boiled, it forms picramic acid. The colour of picramic acid is red.

Materials: Boiling water bath, picric acid, Na₂CO₃. Distilled water, tubes etc.

Method

- Take 1ml of test samples in tube.
- Add 1ml of saturated picric acid
- Add 0.5ml of 10% Na₂CO₃ mix.
- Boil it for 10min.
- Record colour.

Observation: Appearance of red color shows presence of reducing sugars.

5.3.2-Proteins

Proteins are a major class of biomolecule essential for survival, growth and development of all living organisms. Proteins are polymer made up of large number of amino acids (monomeric unit of proteins). Amino acids are linked to one another by formation of a peptide bond. Peptide bond is characterized as a covalent bond formed between two amino acid carboxylic COOH group of one amino acid and amino (NH₂) of adjacent amino acid are involved in the process of peptide bond formation. Addition of large number of amino acids occurs by formation of peptide leads to formation of polypeptide chain. Normally a polypeptide made up of about 20 -25 amino acids or even upto 30 amino acids is generally not considered to be a protein, they are called as peptides or oligopeptides (although many protein are peptides i.e made up of smaller number of amino acids.)

1-BIURET TEST

This is one of the most commonly utilized test to detect presence of proteins in a sample. When protein samples are treated with copper sulphate solution in presence of alkali (NaOH or KOH), protein reacts with copper (II) ions to form a violet coloured complex called biuret.

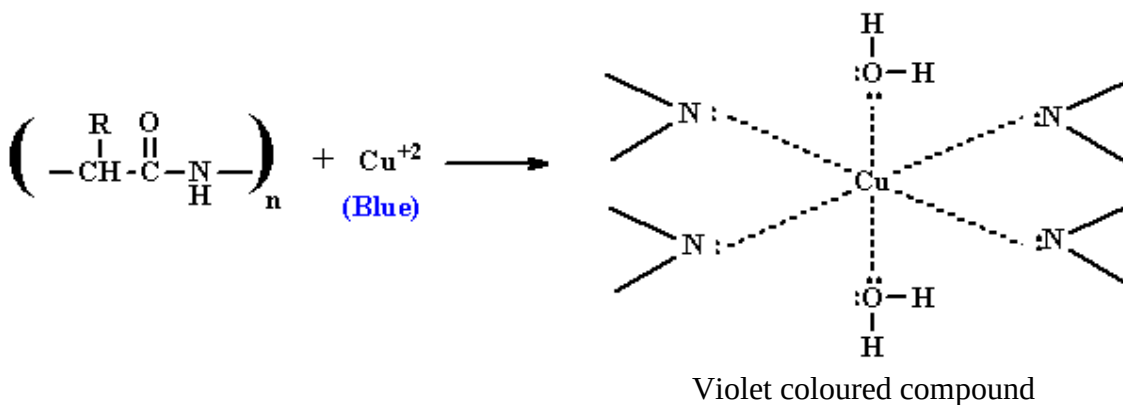
Material required: 1% CuSO₄, 40% NaOH, test tube, dropper, sample

Procedure:

- Using a dropper, take a small quantity of 40% NaOH solution.
- Add a few drops of NaOH solution to the test tube containing egg albumin.
- Using a dropper, take a small quantity of 1% CuSO₄ solution.
- Add 2-3 drops of CuSO₄ solution to the test tube containing egg albumin.
- Shake the solution to mix it well.

Observation: A violet colour appears in the test tube, which indicates the presence of proteins.

Reaction involved:



2-XANTHOPROTEIC TEST

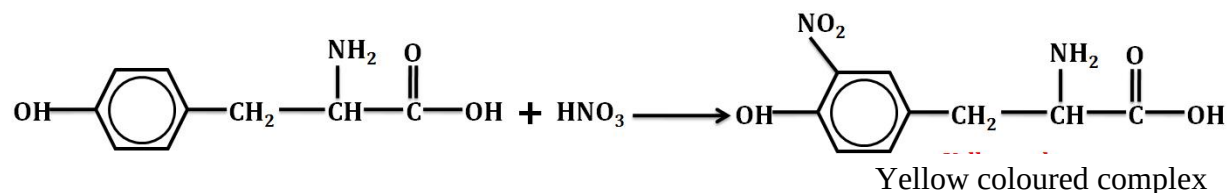
This test is done to identify presence of aromatic amino acids such as tyrosine, Phenyl alanine and tryptophan. When solution of aromatic amino acids is treated with concentrated nitric acid aromatic phenyl ring gets nitrated resulting in formation of yellow colored nitro-derivatives. At alkaline pH, yellow color changes to orange due to the ionization of the phenolic group.

Material required: Conc HNO_3 , ammonium solution, dropper, test tube, test tube holder, sample

Procedure

- Take about 2-3 ml sample of solution of any aromatic amino acid in a test tube.
- Add 5 drops of Concentrated HNO_3 to the test tube containing sample.
- Gently heat the sample for about 2 minutes.
- Yellow precipitate appears in the test tube.
- With the help of dropper, add few drops of ammonia solution to the sample.
- Shake the solution to mix it well.
- Yellow ppt. changes to orange in colour, indicating the presence of protein with aromatic amino acids.

Reaction involved:



3-MILLON'S TEST

This test is used for phenolic amino acids like Tyrosine and its derivatives. Compounds with a hydroxybenzene radical react with Million's reagent to form a red colored complex. Million's reagent is a solution of mercuric sulphate in sulphuric acid.

Materials Required: Million's reagent, dropper, test tube, sample

Procedure:

- Using a dropper, take a small quantity of Million's reagent.
- Add few drops of Million's reagent to the test tube containing egg albumin.
- Wait for some time.
- Pink colour appears in the test tube, which indicates the presence of protein.

NOTE: For all the protein test mentioned above BSA (Bovine serum albumin) can be utilized as sample.

4-NINHYDRIN TEST

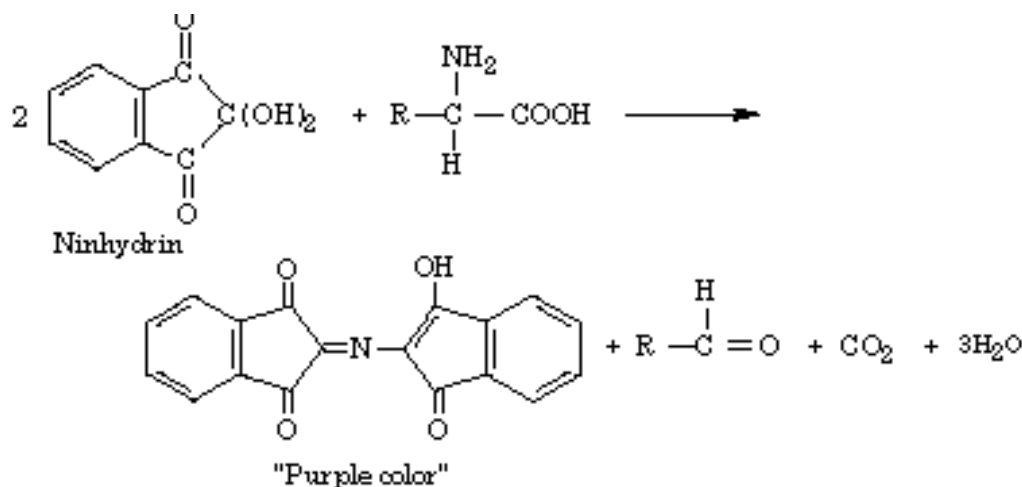
In the pH range of 4-8, all α - amino acids (A molecule containing an amino group and a carboxylic acid group that are separated by one carbon, called the α -carbon) react with ninhydrin, a powerful oxidizing agent to form a purple colored product. All primary amines and ammonia react similarly but without the liberation of carbon dioxide. The amino acids proline and hydroxyproline also react with ninhydrin, but they give a yellow colored complex instead of a purple one. Amino acids containing a secondary amine group sometimes named imino acids.

Procedure:

- Take 1ml of amino acid solution taken in a test tube.
- Add few drops of 0.5% ninhydrin reagent and vortex the contents.
- Place the test tube in a boiling water bath for 5 minutes and cool to room temperature.
- Observe for color change.

Observation: Both free and combined α -amino acids give Ninhydrin test. When free α -amino acids are present blue-purple color is produced however when amino group is secondary color produced is yellow.

Reaction involved:



Reagents: solution of amino acids or protein, 0.5% ninhydrin reagent.

5.3.3-LIPIDS

Lipids are mainly made up of a fatty acid esters and variety of alcohols lipids can be and depending upon the nature of these molecules. Lipids can be classified in the three main categories:

1. **Simple lipid:** These are made up of fatty acids esters and alcohols.

2. **Compound lipids** : These kind of biomolecule are made up of fatty acids esters ,alcohols and variety of different molecule which make their physical and chemical properties unique these are further subdivided to
3. **Derived lipids**: these lipids are formed as a result of hydrolysis of simple and compound lipids and these type of derived lipids plays crucial role in the metabolism and normal functions of biological system as hormones, lipid soluble vitamin, steroids etc.

Saturated and unsaturated fatty acids: Saturated fatty acids are solid at room temperature where unsaturated one are liquid. This is because of inefficient packaging in unsaturated fatty acid which results in decreased vander Waals interaction, which is responsible for low melting point. Whereas in saturated fatty acids due to compactness and efficient packaging they have high melting point

1-SUDAN III TEST

Material required: Sudan III solution, oil, dropper, test tube, sample

Procedure:

- Using a dropper, take a small quantity of Sudan III reagent.
- Add few drops of Sudan III reagent to the test tube containing egg albumin.
- Shake the solution to mix it well.
- Pink droplets appear indicating the presence of fat in the sample.

2-PAPER SPOT TEST

Materials Required: Peanut seeds and piece of white paper.

Procedure:

- Take a peanut seed from the watch glass.
- Crush the peanut seed and rub it on a piece of white paper.
- Paper becomes translucent at the spot, which indicates the presence of fat.

3-SOLUBILITY TEST

The test is based on the property of solubility of lipids in organic solvents and insolubility in water. Fats and oils are hydrophobic molecules *i.e.* they are non polar in nature and insoluble in water. In general lipids are molecules with large non polar extensions therefore, they are soluble in non polar solvents like benzene, ether and chloroform. There are some amphipathic lipids *i.e.* lipids whose molecules have a hydrophilic portion, (eg. phospholipids) have the property to be dragged by water. Knowledge about solubility of lipid is useful for lipid extraction from different sources.

Principle: The oil will float on water because of lesser specific gravity.

Materials: Burner, lipid samples viz. Butter, olive oil, stearic acid, glycerol, lecithin, egg yolk, corn oil, water, acetone, ethanol, chloroform, diethyl ether, test tubes etc.

Method

1. Take 5ml of distilled water and 1ml of different lipids. Shake well and note their solubility.
2. Warm each tube at 50°C for 5min and note change in solubility.
3. Take 5ml of acetone, ethanol, chloroform and diethyl ether in different tubes.
4. Add 1ml of lipid and note the solubility of each lipid.

Observation table:

Type of lipid	Different type of solvents				
	Water	Acetone	Ethanol	Chloroform	Diethyl ether
Butter					
Olive oil					
Stearic acid					
Lecithin					
Egg yolk					
Corn oil					

Results: It should be noted that the solubility of lipids from low to nil in distilled water, while solubility of lipids should be high in organic solvents.

Conclusion: Results obtained clearly demonstrated that solubility of lipids vary from one solvent to other, while lipid solubility in water is minimal, however lipids are insoluble in water.

4-TRANSPARENCY TEST

All the lipids are greasy in nature. Therefore the test may be taken as group test for lipids.

Principle: The oil does not wet the paper.

Procedure: Take 3ml of ether in a test tube and dissolve 5 drops of oil in it. Put a drop of the solution on the filter paper and let it dry. A translucent spot on the filter paper was observed and this indicates the greasy character of the lipid.

5-To Perform Qualitative Test for Fatty Acid Unsaturation

The fatty acids of vegetable oils are usually unsaturated due to presence of double bond in fatty acids chains. Unsaturation of fatty acids may vary from oil to oil, which can be estimated qualitatively. This test is helpful to compare levels of fatty acid unsaturation among different vegetable oils.

Principle: The presence of double bond in hydrocarbon chain of fatty acid represents unsaturation. These double bonds can be easily replaced by halogens viz. Iodine or bromine when it is added to lipid samples. A change in colour of bromine water or iodine solution suggests the presence of unsaturated lipids in specific sample.

Materials: Tubes, bromine , distilled water viz. Olive oil , corn oil, vegetable oil, oleic acid, stearic acid etc.

Method

1. Add 5ml of test solution and add bromine water drop by drop with a burette and mix well every time. Add bromine water in sample till there is no change in colour.
2. Note volume of bromine water added to the sample.
3. Repeat same procedure for other samples.

Conclusion: The double bond of unsaturated fatty acids are be easily replaced by halogens like bromine. Once double bond of fatty acids is replaced by bromine, even additional amount of bromine addition doesnot change its colour. This is the point which suggests the unsaturated bonds of oil are replaced by bromine.

5.4 SUMMARY

- 1-Three main types of biomolecules present in living organism are carbohydrates, proteins and lipid.
- 2-Several laboratory biochemical test can performed to identify presence or absence of different bio molecules in different samples.
- 3-Such test which are utilized to detect presence of specific bio molecules in a sample are called as qualitative test.
- 4-Molish's test and Anthrone test are done to identify presence of carbohydrates in a given sample.
- 5-Presence of starch in a sample can be detected by appearance of blue-black colour on addition of iodine to a sample.
- 6-Samples containing monosacchaarides and disaccharides can be differentiated through Barfoed's test.
- 7-Monosaccharides react within 1-2 minutes whereas disaccharides take 6-7 minutes to form coloured product in Bardood's test.
- 8-Based upon presence of aldehyde or ketonic group carbohydrates are classified as aldoses or ketoses.
- 9-Seliwanoff's test is utilized to differentiate between aldoses and ketoses as a dark red (cherry) colour in produced with ketoses whereas a pink colour is obtained with aldoses.

- 10-Reducing sugars can be differentiated from non reducing sugars through Fehling's test as only reducing sugars produce yellow colour in Fehling's test.
- 11-Reducing sugars can also be identified by Benedict's test and picric acid test.
- 12-All the lipids are greasy in nature and insoluble in water.
- 13-However, lipids are soluble organic solvent.
- 14-Lipids can be saturated or unsaturated. Unsaturated fatty acids contain presence of atleast single doublebond.
- 15-Presence of saturation and unsaturation can identified through addition of bromine water. Decolourization of bromine water shows presence of unsaturation.
- 16-Presence of proteins in a sample can be identified by Million's and Xanthoproteic test.

5.5 GLOSSARY

Aldoses: Any class of simple sugar containing an aldehyde group

Glycosidic bond: a glycosidic bond or glycosidic linkage is a type of covalent bond that joins a carbohydrate (sugar) molecule to another group, which may or may not be another carbohydrate.

Peptide bond: A **peptide bond** (amide **bond**) is a covalent chemical **bond** linking two consecutive amino acid monomers along a **peptide** or protein chain.

Saturated fatty acid: A **saturated fat** is a type of **fat** in which the **fatty acid** chains have all or predominantly single bonds

Unsaturated fatty acid: An unsaturated fat is a fat or fatty acid in which there is at least one double bond within the fatty acid chain.

Reducing sugar: A reducing sugar is any sugar that is capable of acting as a reducing agent because it has a free aldehyde group or a free ketone group.

Non-reducing sugar: Non reducing sugars do not contain free -CHO group or >C=O group on the carbon adjacent to >C=O group.

Osazones: Osazones are a class of carbohydrate derivatives found in organic chemistry formed when sugars are reacted with excess of phenylhydrazine.

5.6 SELF ASSESSMENT QUESTION

5.6.1 Multiple Choice Questions:

1- Molisch's test is utilised for identification of

- | | |
|------------------|------------|
| (a) Carbohydrate | (b) Lipids |
| (c) Protein | (d) Fat |

2- Iodine test is utilised for identification of

- | | |
|------------------|--------------|
| (a) Starch | (b) Glycogen |
| (c) Both a and b | (d) Proteins |

3-Which test can be used to distinguish between monosaccharide and reducing dissacharides

- (a) Molisch's test
- (b) Barfoed test
- (c) Anthrone test
- (d) All of above

4-Proteins can be detected to be present in a sample by

- (a) Iodine test
- (b) Molisch test
- (c) Xanthoproteic test
- (d) Benedict's test

5-Formation of pink coloured reaction product indicates presence of protein in which of the following test

- (a) Anthrone test
- (b) Biuret test
- (c) Fehling's test
- (d) Millon's test

6-Seliwanoff's reagent is prepared by dissolving

- (a) Resorcinol in HCl
- (b) Resorcinol in water
- (c) Resorcinol in H_2SO_4
- (d) Resorcinol in HNO_3

7- Barfoed's reagent is a combination of

- (a) Copper acetate and acetic acid
- (b) Water and anthrone
- (c) HCl and acetic acid
- (d) Copper sulphate and HCl

8-Benedict's test is done for identification of

- (a) Non reducing sugar
- (b) Proteins
- (c) Reducing sugar
- (d) Lipids

9- α - naphthol is prepared by dissolving

- (a) α - naphthol in water
- (b) α -naphthol in ethanol
- (c) α -naphthol in HCl
- (d) α -naphthol in H_2SO_4

10-Oil floats on water because of

- (a) Lesser weight
- (b) Lesser volume
- (c) Lesser mass
- (d) Lesser specific gravity

11-Which of the following statement is not true

- (a) All lipids are greasy in nature
- (b) Lipids are insoluble in organic solvent
- (c) Lipids can be identified by Sudan test
- (d) Lipids can made up of fatty acids esters and alcohols

12-Reducing sugar form _____ on treatment with phenylhydrazine

- (a) Furfural compound
(c) Phenyl sugar

- (b) Osazones
(d) Hydrides

13-Bial's test is utilised for differentiating

- (a) Aldoses and ketoses
(c) Protein and lipids

- (b) Aldehyde and acids
(d) Ketone and lipids

14-Anthrone reagent is prepared by dissolving anthrone in

- (a) Nitric acid
(c) Phosphoric acid

- (b) Hydrochloric acid
(d) Sulphuric acid

15-Which of the following is not a polysaccharide

- (a) Starch
(c) Sucrose

- (b) Glycogen
(d) Chitin

16-Glycosidic bond is present in

- (a) Lipids
(c) Carbohydrate

- (b) Protein
(d) All the above

17-Formation of red coloured product in picric acid test shows presence of

- (a) Reducing sugar
(c) Lipids

- (b) Non reducing sugar
(d) Proteins

18-Dehydration of carbohydrates in Molisch's test results in formation of

- (a) Furfural
(c) Oxides

- (b) Osazone
(d) α -naphthol

19-Proteins are polymers of _____ which are linked to one another by _____

- (a) Glucose, peptide bond
(c) Glucose, glycosidic linkage

- (b) Amino acid, peptide bond
(d) Amino acid, ester bond

20-Decolorization of bromine water when added to a fatty acid solution indicates presence of

- (a) Saturated fatty acid
(c) Unsaturated fatty acid

- (b) Absence of fatty acid
(d) None of the above

5.6.2 Fill in the blanks:

- _____ is a monosaccharide.
- Polysaccharides are made up of large number of _____ molecules.
- _____ are building block of proteins.
- Molisch test is used for identification of _____.

5. _____ is a polysaccharide found in cell wall of fungi.
6. Biuret test is utilised for identification of _____.
7. Lipids are made up of _____ and _____.
8. _____ is main component of plant cell wall.
9. Yellow crystals are formed in phenyl hydrazine test are called _____.
10. Anthrone test is done for identification of _____.
11. Paper spot test is utilized for identification of _____.
12. Appearance of _____ colour shows presence of starch in iodine test.
13. Benedict's test is utilised for test presence of _____.
14. Millon's test is utilised for identification of _____.
15. Reaction of furfural with _____ in Molish test give purple colour.

5.6.3 State whether following statements are true or false.

1. Monosaccharides are made up of large number of sugar subunits.
2. Amino acids are monomeric units of proteins.
3. Sucrose is made up of glucose and fructose.
4. Anthrone test is used for identification of sugar.
5. Fehling solution is mainly made up of CuSO_4 .
6. Benedict's test is used for identification of protein.
7. Presence of starch can be detected by iodine test.
8. Anthrone reagent is prepared by dissolving anthrone in HNO_3 .
9. Biuret test is done for identification of protein.
10. Decolorisation of bromine water upon addition to lipid sample show presence of unsaturated fatty acids.
11. Sudan test is done for identification of presence of lipids.

5.6.1 Answers Key: 1-(a), 2-(c), 3-(b), 4-(c), 5-(d), 6-(a), 7-(a), 8-(c), 9-(b), 10-(d), 11-(b), 12-(b), 13-(a), 14-(d), 15-(c), 16-(c), 17-(a), 18-(a), 19-(b), 20-(c).

5.6.2 Answers Key: 1- Glucose, 2-Sugar, 3-Amino acids, 4-Carbohydrates, 5-Chitin, 6-Protein, 7- Glycerol , fatty acids, 8-Cellulose, 9-Osazones, 10-Carbohydrates, 11- Lipids, 12-blue-black, 13- reducing sugar, 14- phenolic amino acids, 15- α -naphthol

5.6.3 Answers Key: 1-F, 2-T, 3-T, 4-T, 5-T, 6-F, 7-T, 8-F, 9-T, 10-T, 11-T

5.7 REFERENCES

- Experimental Biochemistry, A student Companion by Beedu Sashidhar Rao and Vijay Deshpande.
- Sadasivam, S. and Theymoli Balasubramanian (1985). Practical Manual (Undergraduate), TamilNadu Agricultural University, Coimbatore, p. 2.

- Laboratory manual for biotechnology. Ashish S Verma, Surajit Das and Anchal Singh. S. Chand publication New Delhi.

5.8 SUGGESTED READINGS

- Practical Biochemistry. RC Gupta and S Bhargava. CBS Publishers & Distributors.
- Introduction to practical biochemistry. SK Sawhney and Randhir Singh. Narosa Publishing House Pvt Ltd. NewDelhi.
- Laboratory manual and practical biochemistry. JN Pattabiraman. All India Publishers and Distributors.

5.9 TERMINAL QUESTIONS

5.9.1 Very short answer type questions:

1. Define carbohydrate?
2. Why are carbohydrate also called as saccharides?
3. Name different types of carbohydrates?
4. What is chitin?
5. Write the composition of Fehling's solution I & II?
6. Mention most commonly utilised test for identification of glucose?
7. What is benedict's reagent?
8. What is fehling's reagent?
9. How is phenyl hydrazine reagent prepared?
10. How is anthrone reagent prepared?
11. Define proteins?
12. What is peptide bond?
13. What is biuret test?
14. What are lipids?
15. What is sudan test?
16. What are osazones?

5.9.2 Short answer types questions:

1. What are carbohydrates. Classify them as aldoses and ketoses?
2. How does monosaccharides differ from oligosaccharides?
3. What are structural and storage polysaccharides?
4. Write the composition of benedict's reagent where this reagent utilised?
5. How is fehling's test done for identification of presence of sugar in given sample?
6. Describe how benedicts test is utilised for identification of sucrose.
7. What is molisch test. How it is done?

8. What is biuret test. How is it done?
9. How can xanthoproteic test be utilised for identification of proteins.
10. Differentiate between simple and compound lipids?
11. Explain how Sudan test and paper spot test are utilised for identification of lipids?
12. How does saturated fatty acids differ from unsaturated fatty acids?

5.9.3 Long answer types questions:

1. Define proteins. Mention a biochemical test utilized for identification of protein.
2. What are aldoses and ketoses. Give a biochemical test utilized for their laboratory identification.
3. Give a laboratory method to find out whether a given lipid sample contains saturated or unsaturated fatty acids.
4. What are reducing and non reducing sugars? How can they be identified to be present or absent in a given sample?



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