CROP PHYSIOLOGY

PRINCIPLES AND APPLICATIONS

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CHAPTER 1
HISTORICAL PERSPECTIVES

The meaning of Plant Physiology refers to " the science of properties and functions in normal conditions". The aim of the Plant Physiology has been descrived as early as the early 20th Century by the Russian Plant Physiologyist, V.I. Palladin as : "Which is to gain a complete and thorough knowledge of all the Phenomena occurring in plants, to analyse complex life processes. so as to interpret them in terms of simpler one and reduce them finally to the principles of physics and chemistry". Nevertheless, Noggle and fritz (1983) described the Plant Physiology as " the science concerned with processes and functions, the response of plants to changes in enviroment and the growth adn development that results from responses ". Examples of such plant responses are ion absorption, translocation, transpiration, photosynthesis, respiration, flowering etc. While , functions relate to the activity of the cell, tissues, organs or organelles and also the functions of ions, molecules and macromolecules. Comprehensive knowledge about the plant is incomplete unless we know how the processes and functions are affected by various environmental factors and the treatments and how this effect modifies the growth and development of the plant. Of course, but they are continuously influenced by many biotic and abiotic factors in the natural habitat.

For the reasons above, Plant Physiology is an interdisciplinary science, and its growth has been dependent on the growth of other related sciences. Early development was linked with the developments of disciplines like physics and chemistry and plant anatomy along with the plant morphology. With the discovery and development of other biological sciences such as cell and molecular biology and biochemistry, plant physiology has developed further. During the later part of the century, more information has been gathered about the plant physiological processes mainly due to development of these cognate sciences. Subsequently, a separate discipline of Eco-Physiology or Environmental Physiology has emerged which deals with the interaction between plant growth and the evironment. This discipline asumes greater importance in the over all development of Plant Physiology, in the years to come, mainly because of the fact that the increasing human activities are causing serious disturbances and threat to the environment. Best example is the elevated CO_2 concentration in the atmosphere largely due to the human activities.

Historical Perspectives in Crop Physiology

As mentioned earlier, the early development of Plant Physiology has been largely due to the efforts of physicists and chemists, who tried to study plant life by applying principles of physical sciences. The first ever known Plant Physiology experiment was done by the Belgium alchemist, Van Helmont (1577-1644) who attempted to use to the balance for determining the source of materials found in the plant body. He observed an increase in the weight of a potted willow plant body. He observed an increase in the weight of a potted willow plant over aperiod of five years, which was more than the loss in the weight of the soil, in which it was growing. Therefore, Van Helmont concluded that the source of nourishment other than the soil was responsible for increase in weight and that he did not think about the air. Subsequently, Joseph Priestly (1772) conducted the first experiment leading to the discovery of gaseous exchange between oants and atmosphere. Later on, Jan Ingen Housz (1786-90) demonstrated that only the green plants could purify the air and that too in sunlight only. Senebier (1782) proved that plants absorbed CO_2 from the air and Nicholas T.de Saussure (1804) showed that they evolved CO_2 during repiration.

Dutrochet (1827) demonstrated the phenomenon of osmotic pressure and later on Baranetski (1870) developed a simple osmometer to measure osmotic pressure in plants.

The differential bending of stem and root in a plant was shown by Doddart (1700, 1741). Later on, Knight (1806) demonstrated that his bending of growing plant organs was due ro gravitational pull, which was later regarded as geotropism.

Several plant physiological processes were beginning to be understood during the last few decades of nineteenth century also in the early twentieth century. Nitrogen nutrition was first investigated by Boussingant (1860-71) as plants obtained their nitrogen exclusively from the soil in both nitrate and ammonium. Kostytschew (1890) extensively demonstrated the inter-conversion of nitrogenous compounds in the soil and that the living organisms were involved in this process. By the end of first quarter of 20th century, many enzymatic reactions for nitrogen nutrition were made known. F.Knoop (1911) showed that ammonia was incorporated into glutamic acid by the activity of the enzyme glutamate dehydrogenase.

For the first time,W. Knoop (1868) employed water culture method to demonstrate essentiality of many other minerals for plant growth.

Absorption and transport of gases in plants were demonstrated by wiesner and Molisch (1890). Blackman (1895) showed that the stomata were important especially in CO_2 uptake during
photosynthesis.

Early knowledge about the plant proteins was based on the analysis of seed. Thomas B. Osborne (1909) classified proteins on the basis of their solubility. Armstrong (1910) published a detainled account of various plant carbohydrates. Dixon and Mason (1916) demonstrated the formation of sugars in photosynthesis, Which was transformed to starch later on. Studies on respiration was started as early as 1874 and the scientists like Wolkoff and Mayer (1874), Bonner and Mangen (1884) and Palladin and Kostytschew (1910) were actively involved in designing the apparatuses for the study of respiration. Some of them are still in use for laboratory demonstration of repiration. Sach (1874) demonstrated the effect of growth rate of plant or a plant organ was not uniform. He also studies the effect of temperature on plant growth.

Thus, as in most other branches of science, the growth of Plant Physiology was tremendous in 20th century leading to the bagging of prestigious Nobel Prizes especially for the photosynthetic researches.

CHAPTER 2

WATER RELATIONS

Role and significance of Water

Of all the substances necessary to plant life, water is required in the larges amount. Water is present throughout the plant body, from the soil water around the roots to the liquid-vapour interface, in the leaves. Water is the most abundant molecular species in actively growing plant cells. In most cells and tissues of higher plants, water constitutes more than 90 percent of the fresh weight. The water content of some growing cells may rise to 95 percent or more, but in dormant seeds and buds, water content may be 10 per cent or even less.

Functions of water in plants

1. Water is a major constituent of protoplasm
2. Water is the solvent in which mineral nutrients are transported from the soil solution. Also, mineral nutrients are transported from part of cell to another and from cell to cell, from tissue to tissue and organ to organ with the help of water.
3. Water is the medium in which many metabolic reactions occur
4. Water is a reactant in number of metabolic reactions
5. In photosynthesis, the hydrogen atom of water molecule is ncorporated into organic compound and oxygen atoms are released as gas.
6. Water imparts turgidity to growing cells, thus maintains their form and structure.
7. The elongation phase of cell growth depends on absorption of water
8. Gain and losss of water from the cells and tissues is reponsible for variety of movements of plant parts.
9. Water is metabolic end product during respiration.
10. Plants absorb more water and lose greater amount of water than any other substances.

Nature of Cellular Water

A large fraction of water in plant cells is free and mobile. Much of this water is present in vacuoles. The vacuolar water is similar to a dilute salt solution. But, this is subjected to a hydrostatic pressure of several bars. A small fraction of water is held firmly by the plant cell constituents by hydrogen bond forces. This adsorbed water is in the vicinity of cell membranes i.e., the membranes present in endoplasmic reticulum, mitochondria, chloroplast and also the tonoplast and plasma membrance. Further more, the interspaces within the protein and water molecules occupy lipid layers of cell membranes. This water has a very high density, much higher than that of liquid water. This fraction of cell water is almost semi-crystalline in structure.

The movement of materials into and out of the cells in plants takes place in solution or in gaseous form. Although exact process of this is not very clear, three physical processes usually involved in the movement of water. They are ***Diffusion, Osmosis and Imbibition.***

Diffusion Pressure

The diffusing particles have certain pressure called as the **Diffusion pressure,**which is directly proportional to the number as well as concentration of the diffusing particles. Therefore, the diffusion takes place always from an area of its higher diffusion pressure to an area of its lesser diffusion pressure, i.e., along a diffusion pressure gradient. The rate of diffusion increases if:

i. the diffusion pressure gradient is steeper
ii. the temperature is increased
iii. the density of the diffusing particle is lesser.
iv. the medium through which diffusion occurs is less concentrated.

Diffusion of more than one substance at the same time and place may be at different rates and in different directions but is independent of each other. A very common example of this is the gaseous exchange in plants.

Significance of diffusion

Diffusion plays important roles in the life of the plants as :

i. It is an essential step in the excahnge of gases during photosynthesis and respiration.

ii. It has an active role in stomatal transpiration involving diffusion of water vapours from the intercellular spaces into the outer atmosphere through open stomata.

iii. Its role is inevitable in the passive salt uptake in which the ions are absorbed by the process of diffusion.

B. Osmosis

It is defined as the phenomenon of movement or diffusion of water molecules from the region of its higher concentration to the region of its lower concentration through a semi-permeable membrane.

Osmotic Pressure (O.P) :

As a result of the separation of two solutions by a semi-permeable membrane, a pressure is developed in solution due to the presence of dissolved solutes in it. This is called as **Osmotic Pressure.**

Properties of Osmotic Pressure.

i The O.P is measured in terms of atmosphere.

ii O.P is directly proportional to the concentration of dissolved solutes in the solution (More concentration of a solution has higher osmotic pressure.)

iii O.P of solution is always higher than its pure solvent.

iv O.P does not increase by the addition of insoluble solutes in the solution.

Thus, during osmosis, the movement of solvent molecules takes place from the solution whose osmotic pressure is lower (i.e., less concentrated or **hypotonic**) into the solution whose osmotic pressure is higher(i.e., more concentrated or **hypertonic**)

Osmotic diffusion of solvent molecules will not take place if the two solutions separated by the semi-permeable membrane are of equal concentration or O.P.(i.e., identical or ***isotonic.***)

Factors Affecting Osmotic Pressure

i. Concentration of solution

ii Ionisation of the solute molecules

iii Temperature, and

iv. Hydration of the solut molecules.

Diffusion Pressure Deficit (DPD) :

It is a measure of the ability of the cells to absorb water and hence it is often called as ***Suction Pressure.*** The difference between the diffusion pressure of the solution and its solvent at a particular temperature under atmospheric condition is called as *Diffusion Pressure Deficit (DPD).* Diffusion pressure of a solution is always lower than its pure solvent.

If the solution is more concentrated, its DPD increases. While, the DPD decreases when the solution is diluted. In the case of plants, the cell sap is a water solution containing many organic and inorganic substances and its pure solvent is water. Therefore, the DPD of the cell sap will always be more. As a result, when the cells are placed in pure water, the water will enter into the cells due to higher DPD of the cell sap.

The DPD is also related to *Osmotic Pressure* and *Turgor Pressure* of the cell sap as well as the *Wall Pressure* (WP) as follows :

DPD (SP) = OP - WP
but, WP= TP
therefore, DPD (SP) = OP - TP
In the fully turgid cells,
OP = TP
therefore, DPD = 0

In the fully plasmolysed cells,
TP= 0
and hence, SP= OP

Entry of water into the cell :

CELL A		**CELL B**
OP = 25 atm.	⇒	OP = 30 atm.
TP = 15 atm.		TP = 10 atm.
SP = 10 atm.		SP = 20 atm.
OP = 35 atm.	⇐	OP = 40 atm.
TP = 15 atm.		TP = 20 atm.
SP = 25 atm.		SP = 20 atm.

Note : The entry of water into the cell depends only on DPD or SP and not on OP.

Cell as an Osmoticum

Root hair is the first cell, which absorbs water from the soil and acts like an osmotic system. When root hair comes in contact with soil water with higher concentration of water molecules than that of cell sap, osmosis begins to occur. The water of external envionment begins to enter inside the cell through osmosis ***(endosmosis).*** The concentration difference brings out this movement of water in which plasma membrane acts as a semi permeable membrane.

The water is absorbed by the root hair due to DPD (Diffusion Pressure 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 of cell is directly related to DPD that exists between the cell and the environment.

Therefore, it can be said that: DPD = Osmotic pressure -Turgor pressure

Water entering in to the cells influences the system in two ways.

i. It brings down concentraion of cell sap, and
ii. It stretches the elastic wall of the cell.

Thus, the entered water causes swelling of the cell and creates a pressure from inside to cause stretching of the wall, which is known as turgor pressure. Due to it, the cell increases in volume but the cell wall being elastic in nature offers resistance to this force. This resistance which works in direction opposite to turgor pressure is known as ***wall pressure.*** The wall pressure is exerted by wall in order to restore normalcy in cell size. The turgor pressure is equal and opposite to wall pressure in a fully turgid cell.

When the concentration of two solutions becomes equal, water stops to enter. At this stage, cell is fully stretched and is said to be turgid.

However, in plants, the concentrations of two solutions is never equal, but endosmosis or entrance of water never stops. In fact at this stage, the wall pressure overpowers the turgor pressure and suction pressure and thus regulates the entrance of water.

Some plants (mangrove plants) grow in saline water. The concentration of saline water is very high and it creates difficulties in absorption and the plant has to adopt accordingly. That is why, the halophytes (mangrove plants) have high osmotic pressure of the cell sap.

Thus, the cell acts as an osmoticum in which different forces such as osmotic pressure, suction pressure, DPD, turgor pressure and wall pressure are implied. These different forces work simultaneously and bring out absorption of water in a cell. The direction of different forces are given in Fig.1.

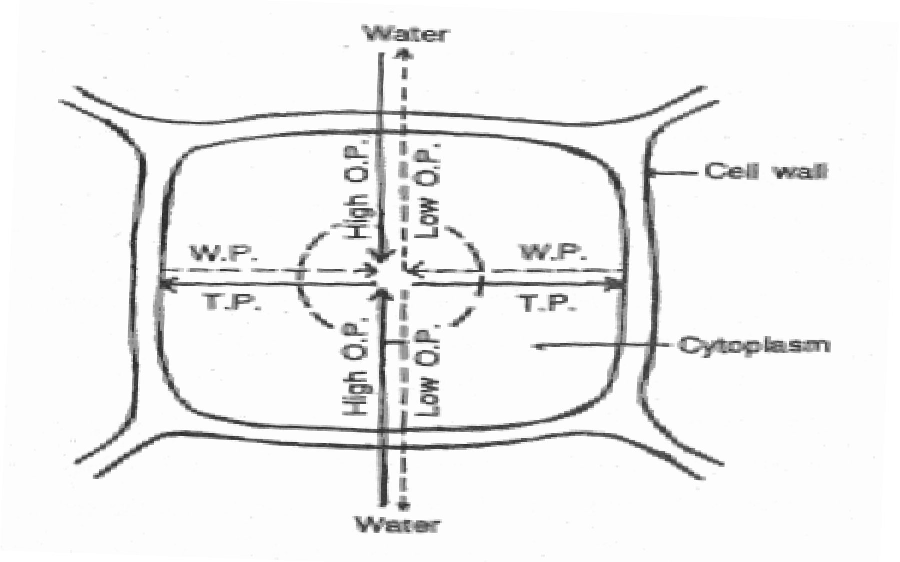


Fig.1 Different directions of the operating forces in a cell

Significance of Osmosis in Plants

The following processes of plants evidently support the importance of osmosis in the plants, which are the results of osmosis :

1. Roots from the soil absorb large quantities of water by osmosis.
2. Cell to cell movement of water and other substances dissolved in it involves osmosis.
3. Opening and closing of stomata depend upon the turgor pressure of guard cells.
4. Due to osmosis, turgidity of the cells and hence the shape or form of their organs is maintained.
5. The resistance of plants to drought and frost increases with increase in osmotic pressure of their cells.
6. Turgidity of the cells of the young seedlings allows them to come out of the soil.

Differences between Osmosis and Diffusion :

S.No	Osmosis	Diffusion
1.	It is a special type of diffusion in Which a semi-permeable membrane in between the two solution is required.	Presence of semi-permeable membrane is not required.
2.	Osmosis occurs in between two liquid media.	Diffusion may occur in any Medium, it may be between solid, liquid or gas.
3.	In osmosis, diffusion of only solvent molecules from low concentration of solution to higher concentration of solution takes place.	In diffusion, a net downward movement of a given substance from the higher concentration to lesser concentration is found.

Concept of Water Potential and Osmotic Relations of Plant Cells

It is difficult to explain accurately the movement of water in osmosis in terms of difference in concentraion. Therefore, it is always better to express in terms of *differences in free energy* between the two regions.

Free energy

It is defined as the energy available isothermally to do work. The free energy per mole of any substance is called as the chemical potential of the substance. Thus, the **chemical potential** measures the energy with which a substance will react or move.

What is Water Potential?

The difference between the free energy of water molecules in pure water and the energy of water in any other system (eg. water in solution or in a plant cell) is called as **Water Potential.** It is represented by the Greek letter, **Psi** (Ψ) which is measured in bars (a pressure unit =14.5 Ib/in 2750mm Hg or 0.987 atm.).

1 bar = 105 pascals = 100KPa = 0.1 MPa.

Water potential (Ψ) of protoplasm is equal but opposite of the DPD or SP. Since the Ψ of pure water is zero (0), the waterpotential (Ψ) of protoplasm is equal but opposite of the DPD or SP. Since Ψ of pure water is zero (0), the presence of solute particles reduces the free energy of water, thus decreases the negative value. Therefore, Ψ is always less than zero or its highest value is zero.

Components of Water Potential (Ψ)

The components of water potential are as follows :

$$\Psi w = \underset{(-)}{\Psi s} + \underset{(+)}{\Psi p} + \underset{(-)}{\Psi m}$$

Where, **Ψm** is the matric potential used for the surface such as soil particles or cell wall to which water molecules are adsorbed, **Ψs** is the solute potential, also called as osmotic potential, the amount by which water potential is reduced.

Solute Potential (Ψs)

Reduction in free energy of water in cells due to the electrostatic attraction or repulsion of dissolved salts and water molecules is called as *Solute Potential.*

Pressure Potential (ΨP)

It is due to the pressure developed with in the cells and tissues. The pressure normally exerts in the contents of plant cells. This is due to the elastic stretch of the cell wall. As a result of the inward pressure from the cell walls, a hydrostatic pressure is developed in the vacuoles. This pressure is called as the *Pressure Potential or Turgor Potential.* .

Matric Potential (γm)

The potential developed due to the binding of water molecules to protoplasmic and cell wall constituents is called as Matric Potential. It is always negligible when compared to solute or pressure potential.

Therefore, water potential becomes. **$\Psi w = \Psi s + \Psi p$**

Osmotic Potential (π)

It is nothing but the osmotic pressure but opposite in sign, i.e., π =-OP (π is characterized by negative value as p for purewater is taken as zero (0).

In *fully turgid* cell, the osmotic potential (π) and pressure potential (Ψp) are equal but opposite in sign, so that water potential (Ψ) is zero. Suppose in a cell,

Ψs is -10 bars and ΨP is, 10 bars, Ψ will be zero.

$\Psi = \Psi s + \Psi p$

Ψ = -10 bars + 10bars

Ψ = 0 bars.

And in the case of a flaccid cell, Ψ p will be zero, so

$\Psi = \Psi s + \Psi p$

Ψ = -10 bars + 10bars

Ψ = -10 bars.

In the plasmolysed cell, suppose Ψs =-10 bars and Ψp =-2 bars, then

$\Psi = \Psi s + \Psi p$

Ψ = -10 bars + (-2) bars 1

Ψ = -12 bars.

Gravitation Potential (Ψg)

In some tall trees, there is another potential due to gravity, called as ***gravitational potential*** (Ψg)

Hence, for tall trees : $\Psi w = \Psi s + \Psi p + \Psi g + \Psi m$

Relationship between water potential

The cell at 0 turgor (in flaccid condition) has a solute potential equal to its water potential. On the other hand, a cell at full turgor has a water potential of 0 bar. Its dolute potential and pressure potential at this stage are equal but opposite in sign. Under natural condition, cell is usally at a state between 0 turgor and full turgor. When the leaves are wilted, 0 turgor approaches. When the leaf reaches maximum water content, full turgor approaches.

Range of Water Potential in Leaves

Leaves of most crop plants in well-watered soils will have a water potential of **-2 to -10** bars. With decrease in water supply, the water potential becomes more negative than -10 bars. At this stage, the leaf growth rate will be affected. Leaves of crop plants may **survive for short periods** when water potential is **less than -15 bars.** They will not recover if the water potential falls below **between -20 and -30 bars.**

In contrast, the **leaves of desert plants** will survive as low water potential as **-100 bars.** The viable air dried seeds will have a water potential range of **-60 to -100 bars.**

Movement of water between cells

Water moves in and out of the cell because of the difference in water potential gradient between cells and its surrounding solution. Similarly, water can move from cell to cell by diffusing down a water potential gradient between the two cells. Thus, the direction of movement of water and force with which it moves depends on the water potential in each cell and consequently in the difference in water potential between them.

Problem :

Cell A has a of 5 bars and contains a sap with a solute (osmotic) [potential of -12 bars. Cell B has apressure potential of 3 bars and a solute potential of -6 bars. If these two cells are in contact, which way the water will move and with what force?

Cell A:

Ψ w = Ψs + Ψp
= -12 +5
= -7 bars (low water status)

Cell B:

Ψ w = Ψs + Ψp
= -6 +3
= -3 bars (high water status)

Therefore, water will move from cell B to Cell A. And the force is 4 bars (-3-(-7)). The value of is of importance beacuse it is directly proportional to the rate at which the water sill move between cells.

C. Imbibition

It is defined as the movement of water from an area of higher potential to an area of lower potential, but without the assistance of differentially permeable membrane. The adsorption of water by hydrophilic colloids is also known as imbibition. During imbibition, sone energy is also released. Solvents are usually imbibed only into the materials, with which they have affinity.

Eg. Water into proteins; Acetone into rubber

The pressure generated by imbibition is called as Imbibitional Pressure (IP). This will cause the swelling of the imbibant. The imbibitional pressure is sometimes enormous and may be playing very important roles, i.e., it helps in breaking soil profiles by germinating seeds and also for splitting of hard seed coats. Also, when a seed is placed in a rock crevice, it may split the rock due to the pressure of the imbibing water of the germinating seeds. Proteins have a very high imbibing capacity, followed by starch and cellulose. Therefore, *proteinaceous pea seeds swell more on imbibition than starchy wheat seeds.*

The relationsip between DPD, IP and TP is as follows :
DPD= IP-TP

Factors Affecting Imbibition

i. Nature of organic substances

ii. Temperature change

iii Presence of solutes

iv. Degree of cohesion of the molecules

v. Type of solution.

Significance of Imbibition

Imbibition plays a very important role in the plant life as:

1. It helps in the absorption of water by the roots of higher plants through cell walls of root hairs.
2. Imbibition of water is very essential for dry seeds before they start germination.

Plasmolysis

Under normal condition, the protoplasm is tightly pressed against the cell wall. If this plant cell or tissue is placed in ***hypertonic*** solution (whose O.P is higher than that of cell sap)the water comes out of the cell sap into the outer solution and cell becomes flaccid. This process is known as ex-osmosis and the protoplasm begins to contract from the cell wall. This is called as ***incipient plasmolysis.***

The phenomenon of shrinkage of protoplasm and its separation from the outer cell wall on account of the much-concentrated outer hypertonic solution in comparison to the cell sap is called as ***plasmolysis.*** This process separates the protoplasm from the cell wall and finally assumes a spherical form. This is due to the process of ex-osmosis. The cell or tissue is then said to be plasmolysed (Fig.2). Then, the space between the cell wall and protoplas is filled with the outer hypertonic solution.

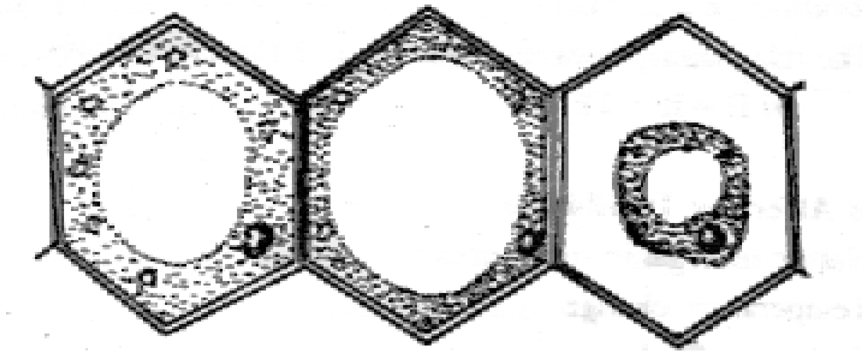


Fig.2. Stages in plasmolysis of a plant cell

Significance / Advantages of Plasmolysis

1. It indicates the semi-permeable nature of the plasma membrane
2. It is used in the determination of O.P. of the cell sap.
3. It is also used in the postt-harvest technological process of addition of sugar solution to jams and jellies to check the growth of fungi and bacteria, which become plasmolysedin the concentrated solution .

Deplasmolysis

If a plasmolysed cell or tissue is placed in water process of endosmosis takes place. Water enters into the cell sap, the cell becomes turgid, and the protoplasm again assumes its normal shape and position. This phenomenon is called as ***deplasmolysis.***

CHAPTER 3

ABSORPTION AND TRANSLOCATION OF WATER AND SOLUTES

Plants absorb water from the soil by their roots. The water is found in different forms in the soil. The chief source of water to the soil is *rain.* After the rains, a part of the water drains away. This is called as *run-away* water and it is not available to the plants. A part of the water percolates downwards through large pores between the soil particles under the influence of gravity and is known as gravitational water. It reaches to the low water table and goes beyond the root zone. Alternatively, a good amount of water is retained by the soil particles and is called as Field Capacity moisture, i.e., the water retained due to the water-holding capacity of soil particles.

This water may be present in three forms : Capillary water, Hygroscopic water and Chemically combined water. Of all these, the water available to the plants is capillary water which fills the space between the non-colloidal smaller particles of soil. Hydroscopic water is held by the soil particles of colloidal smaller particles of soil. Hygroscopic water is held by the soil particles of colloidal complex due to adhesive force. The roots can not draw this.

Field Capacity and Water Holding Capacity of Soil

It is defined as the amount of water retained by the soil after the grainage of gravitational water has become very slow. The field capacity is affected by soil profile, soil structure and temperature. For example, fine texured soil overlying a coarse texured soil will have a higher field capacity than a uniformly fine texured soil. It also depends upon the size of the soil 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 soils, the %age of moisture held in the form of film, is called Water Holding capacity of the soil.

Permanent Wilting Point or Wilting Co-efficient :

It is the percentage of soil water left after the plant growing in that soil has permanently wilted. It depends on the osmotic characteristics of the plant. Therefore, different plants if grown in the same soil will wilt at different times depending upon their osmotic potential after the water supply to the soil is stopped.

Water Absorbing Parts of Plant

Major portion of water required by the plants is absorbed by the roots. But the absorption of water by leaves and stem has also been found in a few plants. Hydrophytes absorb by general (Leaft) surface.

The uptake o f water by leaves is influenced by :

i. Structure and permeability of cuticle and epidermis
ii. Hairiness of leaf surface.
iii. Case of wetting surface.
iv. Internal environment of deficiency of water in parenchymatous cells closes to the epidermis.

Roots play the principle role in absorpiton of water. Even orchids develop modified roots for the purpose of absorbing water from the atmosphere. This type of specialized water absorbing water from the atmosphere. This type of specialized water absorbing tissue present around the cortex of the roots is called as Velamen. It consists of thin-walled parenchymatous cells and the moisture absorbed by it is transferred to the root xylem through exodermis, cortex, endodermis and the pericycle.

Mechanism of Water Absorption

In higher plants, water is absorbed through root hairs, which are in contact with soil water and from the root hair zone, a little behind the root tips. Root hairs are tubular hairs like prolongation of the cells of the epidermal layer of the roots. The walls of root hairs are permeable and consists of pectic substances and cellulose which are strongly hydrophilic (water loving) in nature. Root hairs contain vacuoles filled with cell sap.

When roots elongate, the older hairs die and new root hairs are developed so that they are in contact with fresh supplies of waterin the soil.

Mechanism or mode of water absorption by the plants

It is taking place in two ways :

1. Active Absorption of water

In this process, the *root cells* play active role in the absorption of water and metabolic energy released through respiration is consumed. Active absorption may be of two kinds.

a. Osmotic absorption : When water is absorbed from the soil into the xylem of the roots along the osmotic gradient.

b. Non-osmotic absorption : When water is absorbed against the osmotic gradient.

1a. Active Osmotic Absorption of Water

First step in the osmotic absorption of water is the ***imbibition*** of soil water by the hydrophilic cell walls of root hairs. ***Osmotic pressure*** (OP) of the *cell sap* of root hairs is usually higher than the OP of the soil water. Therefore, the *DPD* and the suction pressure in the root hairs becomes higher and water from the cell walls enters into them through *plasma membrane* (semi-permeable) by *osmotic diffusion.* As a result, the OP, suction pressure and DPD of root hairs now lower, while their *turgor pressure* is increased.

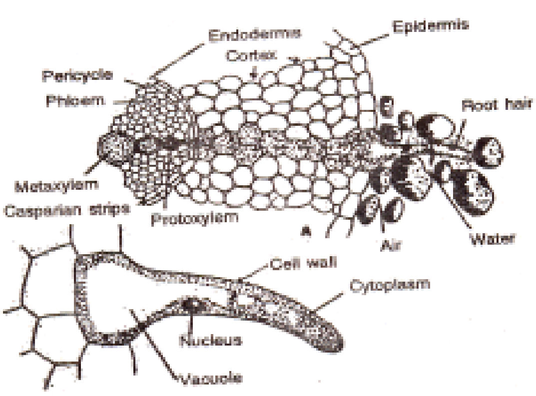


Fig.3 A part of T.S. of a Typical dicot root.

The arrow indicates the path of water

Now, the cortical cells adjacent to root hairs have higher OP, suction pressure and DPD in comparison to the root hairs. Therefore, water is drawn into the adjacent cortical cells from the roothairs by osmotic diffusion (Fig.3).

In the same way, the water reaches the innermost dortical cells and the endodermis by cell to cell osmotic diffusion gradually.

Osmotic diffusion of water into endodermis takes place through special thin walled *passage cells* because the other endodermis cells habe *casparian strips* on their walls which are impervious to water.

Water from endodermal cells is drawn into the cells of *pericycle* by osmotic diffusion which now become rigid and their suction pressure is decreased.

In the last step, water is drawn into xylem from turgid pericycle cells. It is because in the absence of turgor pressure of the xylem vessels (which are non-elastic), the suction pressure of xylem vessels becomes higher than the suction pressure of the cells of the pericycle.

When the water enters into xylem from pericycle, a pressure is developed in the xylem of roots, which can raise the water to a certain height in the xylem. This pressure is called as *Root Pressure.*

1.b Active Non- Osmotic Absorption of water

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

2. Passive Absorption of Water

It is mainly due to traspiration; the *root cells do not play active role* and they remain passive.

This theory explains that some forces are responsible for absorption of water into the roots, which are actually governed by other cells. The governing force originates in the cells of traspiring shoots rather than in root itself. These forces develop due to transpiration.

Passive absorption of water takes place when rate of transpiration is usually high. Rapid evaporation of water from the leaves during trasnpiration creates a tension in water in the xylem of the leaves. The tension is transmitted to water in xylem of roots through the xylem of stem and the water rises upward to reach the transpiring surfaces. As a result, soil warer enters into the cortical cells through root hairs to reach the xylem of the roots to maintain the regular supply of water. The force for this entry of water is created in leaves due to rapid transpiration and hence, the *root cells remain passive* during this process.

Relative Importance of Active and Passive Absorption

Many workers opined that the active absorption is the main mechanism of water absorption and gave very little importance to passive absorption.

However, according to Kramer (1969), the active absorption of warer is of negligible importance in the water economy of plants. He stated that the root pressure and the related phenomena involved in the active absorption of water is mere consequences of salt accumulation in the xylem of different kinds of roots.

Following are the reasons for treating the active absorption as unimportant :

1. Root pressure is not observed in tall plants like conifers and other gymnosperms.

2. Root pressure is not observed in fast transpiring plants.

3. Amount of water exuded by the cut end of stumps due to root pressure is not equal to that lost by transpiration.

4. The plant as a whole absorbs more water than an excised system.

5. In submerged plants, water is absorbed from the general surface, through roots are present.

It appears, therefore, that the passive absorption accounts for most of the water absorption and if active absorption exists, it co-operates with passive absorption or has no importance.

Difference between Active and Passive Water Absorption :

S.No	**Active absorption**	**Passive absorption**
1.	The active absorption of water takes place due to the activity of root and root hairs.	It occurs due to activity of upper part of the plant, such as shoot and leaves.
2.	The absorption of water occurs by the active osmotic and non-osmotic processes.	The water is absorbed due to the transpiration in aerial parts.
3.	The root hairs have high DPD as compared to soil solution, and therefore, water is taken in.	The absorption occurs due to tension created in xylem sap by transpiration pull. Thus, water is sucked by the tension.
4.	In the movement of water, living part of protoplasts (symplast) is involved.	The movement of water is through free spaces or apoplast of root and it may include cell wall and intercellular spaces (apoplast).
5.	The rate of absorption depends upon DPD or difference in osmotic concentrations between the two.	The rate of absorption depends upon transpiration rate.
6.	In non-osmotic type, respiratory enery is utilized.	Energy is never required.

Factors affecting water absorption

The plants get two types of environment for water absorption, the external and internal. The *external envionment* includes factors such as, a) available soil water, b) concentration of soil solution.
c) soil temperature and d) soil aeration. The *internal factors* include, i) traspiration, ii). absorbing root system and iii) metabolism.

I. External Environmental Factors

a. Available Soil Water

Sufficient amount of water should be present in the soil in such form, which can readily and easily be absorbed by the plants. Usually the plants absorb capillary water. Other forms of water in the soil (hygroscopic, gravitational etc.) are not easily available to plants.

b. Concentration of Soil Solution

Increased concentration of soil solution (due to the presence of more salts in the soil) results in higher OP. If the OP of soil solution becomes higher than the OP of cell sap in root cells, the water absorption will be greatly suppressed. Therefore, absorption of water is poor in alkaline soils and marshes. This is popularly known as physiological dryness.

C. Soil Temperature

Increase in soil temperature up to about 30^0C favours water absorption. At higher temperature, water absorption is decreased mainly due to the death of root cells on account of protein denaturation. At low temperature also water absorption decreases so much so that at about 10^0C , it is almost checked. This is probably because at low temperature :

1. Viscosity of water and protoplasm is increased.
2. permeability of cell membranes is decreased.
3. Metabolic activities of root cells are decreased, and
4. Growth and elongation of roots are checked.

d. Soil Aeration

Water is absorbed more efficiently in the well-aerated soil than in poorly-aerated soils, may be because of the fact that roots fail to respire anaerobically and plants die in flooded areas. The deficiency of O_2 inhibits the growth and metabolism and an accumulation of CO_2 increases the viscosity of protoplasts and decreases the permeability of cell membrane. Both these factors affect and reduce the rate of water absorption severely.

II. Internal Enivonmental Factors

i. Transpiration :

The rate of absorption of water is directly proportional to that of transpiration. A higher rate of transpiration produces a tension or pull called as **Transpiration Pull,** transmitted to roots through hydrostatic system of plants creating a favourable condition for the entry of water.

ii. Absorbing Root System

The efficiency of water absorption depends on the absorbing system or root system. The presence of more number of root hairs accounts for the increased rate of water absorption.

iii. Metabolism

The metabolism and absorption are closely related. Factors inhibiting respiration such as poor aeration, application of anaesthetics and KCN reduce the absorption rate. Thus, metabolic activities are expected to participate indirectly by forming a constantly elongated root system and always providing newer contacts with soil water.

Ascent of Sap

The water is absorbed by root hairs of the plant from where it reaches xylem via cortical cells and passage cell and through xylem, it reaches top of the plant where it is transpired by leaves and used for other metabolic activities. *Water and minerals are conducted through xylem tissue. While sieve tubes of the phloem vessels are involved in the conduct of sucrose molecules and other assimilates.*

The upward movement of water from stem base to treetop is called as **Ascent of Sap.** It may even cover a height of more than 90 metres against gravitational pull. Because of the fact that a pressure of one atmosphere can normally lift the water column only by about 10 metres, rise of water up to such heights (90m) needs some special mechanism.

Theories of Ascent of Sap

Various theories have been proposed to explain the special mechanism (s) involved in the ascent of sap. These theories can be grouped under three categories as given below:

1. Vital Theories

a. westermeir (1883) theory : He stated that the tracheids and vessels acted as water reservoirs rather than only as conducting elements.

b. Godlewski (1884) relay pump theory : Ascent of sap takes place due to pumping activity of the cells of xylem parenchyma which are living.

c. Janse (1887) theory : He supported Godlewski's theory and showed that if the lower portion of a branch is killed, the leaves above are affected within few days.

d. Sir. J.C. Bose (1923) Pulsation theory : Ascent of sap takes place due to the pulsatory activity of living cells of innermost cortical layer just outside the endodermis.

These theories are based on the fact that water movement in the xylem is taking place only with the aid of living cells.

Objections to Vital Theories

Strasburger (1891) and Overton (1911) experimentally demonstrated that the ascent sap can take place even in the case of non-living cells as in the segments of xylem in which livign cells have been killed by heat or poisons.

2. Root Pressure Theory

Although root pressure which is developed in the xylem of the roots, can rise the water to a certain height but it does not seem to be an effective force in the ascent of sap due to the following points :

i. Magnitude of root pressure is very low (about 2 atms).
ii. Ascent of sap continues even in the absence of root pressure.
iii In gymnosperms, root pressure has rarely been observed.

3. Physical Force Theories

Contrary to the above two theories, several scientists also believed that living activities or living cells are not involved in ascent of sap. It is purely a physical phenomenon. Many theories have been proposed to explain this aspect of ascent sap. Some of them are as follows.

a. Atmospheric Pressure Theory

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

There are two objections to this theory :

i. It can not act on water present in xylem of roots, and
ii. In case it is working, then also it will not be able to rise water beyond 10 metres.

b. Capillary Force Theory

In plants, xylem is placed one above the other forming a sort of continuous channel which can be compared with long capillary tubes and it was thought that water rises in capillary tuve due to capillary force ; in the same manner, ascent of sap takes place in xylem.

This theory also faces following objections :

i. A free surgace in xylem must be present to maintain capillarity.
ii. Soil water is not directly connected with the vessels, hence this theory cannot be functional in vessel bearing plants.
iii. Capillarity operates easily in plants having narrower vessels but tall plants have rarely such vessels.
iv. Capillary theory cannot operate due to presence of end walls on the conducting vessels; whereas, in plants where vessels are absent, the tracheids with end walls are present for ascent sap.
v. The magnitude of capillary force is very low

c. Imbibition Theory

As advanced by Sachs (1878, 1879), this theory assumed that ascent of sap could take place by *imbibition* through the walls of xylem. This theory is also disregarded because it is well known that *imbibitional force is* insignificant in the ascent of sap and the walls do not carry warer; it moved through the *lumen of xylem* elements and not through walls.

d. Transpiration Pull Theory or Cohesion - Tension Theory

This theory was originally proposed by Dixon and Jolly (1894) and has been supported by Curtis names, Cohesion Hypothesis, Theory of *Cohesive Force, Dixon and Jolly's Theory of Cohesion, or Transpiration Pull Theory.* It is based on the following features :

i. Cohesive and adhesive properties of water molecules to form a continuous water column in the xylem.
ii. Transpiration pull exerted on the water column

It is better explained as follows :

A. What is cohesion?

Attraction between the similar molecules is called *cohesion.* The water molecules have strong mutual attraction (cohesion) due to which they cannot be easily separated from one another. The magnitude of cohesive force of water has been measured up to 350 atm. and is much in excess of the minimum required for the ascent of sap in the tallest trees.

B. Cohesion-tension Theory

Water forms a continuous column from base of the plant to its top and remains under cohesive tension due to transpiration pull. And according to the need, water is being pulled up to the top of the tree.

C. Characteristics of cohesion-tension theory

This important and widely accepted theory has following essential features :

i. Water forms a continuous column from base of the plant to its top.
ii. Water is lost from the mesophyll cells due to transpiration because of which a pulling force is developed. It puts these cells under tension.
iii The tension may cause a break in water column but due to tensile strength or cohesive property of water molecules, the continuous water column is not broken.
iv. The tension or transpiration pull is transmitted to the root region to regulate absorption.

How water is taken up in tall trees ?

Because of the enormous atmospheric pressure (350 atmosphere) created due to the cohesive force of water molecules and due to the cohesive tension developed in the water column, water is taken up in tall trees.

Mechanism of Ascent of Sap

The loss of water from the surface of leaf mesophyll cells due to transpiration reduces the amount of water in the cells. It causes an increase in the OP of these cells. Thus, reduced water potential is developed in the mesophyll cells, i.e., the DPD increases. Water from the adjacent cells and ultimately from the conducting tissue is pulled to meet this loss of water and as a result, a pull is developed in the cells of mesophyll and xylem of the leaf. Now, 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 is beacuse of cohesive properties of continuous water column in the vessels and tracheids from leaves to roots through stem. The water column moves upward by mass flow due to transpiration pull and simultaneously the process of ascent of sap is accomplished.

Phloem Transport

As described earlier, water is absorbed and translocated through the xylem vessles. Trasport of sugars and other substances occurs through the pholem vessels and is called as **Translocation or Long-distance transport.** The translocation of solutes in plants can take place either through apoplast or symplast. Translocation of water and minerals takes place through apoplast. The transport through phloem is symplastic.

Apoplastic vs Symplastic Transport

Each plant consists of two anatomical parts : **Apoplast** and the **Symplast.** Apoplast is in fact the dead part of the plant. This part is situated outside the plasmalemma barrier and includes wall of cells. Symplastic parts are delineated by the plasmalemma and are living parts of the plant. They include cytoplasm but without vacuoles. The symplasts are interconnected through cytoplasmic connections called plasmodesmata.

Phloem Transport Mechanism

There are several hypotheses regarding the translocation of solutes in phloem. Some important theories are described below :

1. Protoplasmic Streaming Hypothesis

This is proposed by Hugo de Vries (1885) . Protoplasmic streaming or cyclosis might be a mechanism of solute translocation. The streaming depends upon the contraction of elongated protein molecules and is also linked to the utilization of ATP. It can account for the bi-directional flow of solutes.

2. Contractile Protein Hypothesis

This hypothesis is based on the contractile nature of P-proteins of sieve elements. Contraction and relaxation of these proteins helps in the translocation.

3. Mass flow Hypothesis

This was elaborated by E. Munch (1930). It is applied to the transport of solutes through phloem only. This is also called **Munch's Hypothesis.** The model described in the following Fig.

- Two vessels (A+B) made upto semi permeable membrane.
- These are inter connected to each other by capillary tube (d)
- Both vessels have sugar solutions ; but B have higher concentrated & A is less concentrated.
- Vessels (A+B) are inmarked in two reservoirs of dil.Sol. connected them capillary tube C
- Now, H2O, will flow into Vessel B, Therefore diff. in their water potential.
- This creats hydrostatic pressure, which forces sol.

According to model, the flow is the result of differential pressure between vessels A&B.

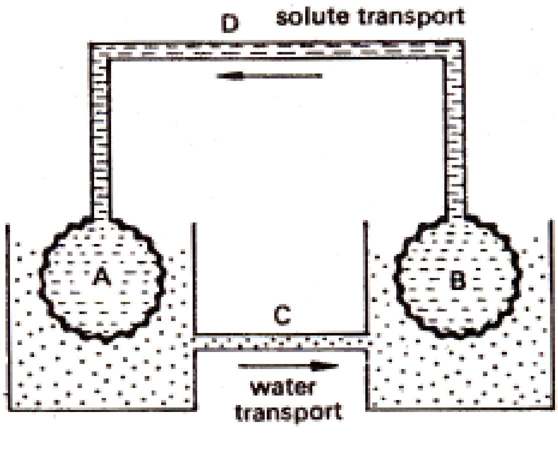


Fig.4. Model describing mass flow hypothesis

The model as applied to the plant system can be simulated as under :

- A is the sink organ (eg.root) which has low sugar concentration, because of its consumption.
- B is the source organ (e.g. leaf) which has a high sugar concentration since it is produced there.
- D is the channel for solution transport and represents phloem tubes.
- C is the channel for water return and represents xylem vessels.

Now, water will flow into the vessel B, because of differences in their water potential. This will create a hydrostatic pressure, Which will force the solution of cessel B to flow through tube D. If water returns from vessel A to B through tube C, the fluid will keep on flowing from B to A. Thus, the flow is the result of differential pressure between vessel A and B.

- from B to A through D.
- water returns from A to B through C, the fluid will keep on flowing from B to A.

CHAPTER 4
TRANSPIRATION

Although a large quantities of water is absorbed by the plants from the soil, only a small quantity alone is utilized by them. Thus, a considerable amount of water absorbed by the roots are carried to the top of the plants and lost by aerial parts in the form of water vapour or rarely in the form of liquid. About 80% of the absorbed water is lost leaving only 20% of the absorbed water for various metabolic processes.

Definition

The loss of water from the living tissues of aerial parts of the plants in the form of vapour is called as **transpiration** and that in the form of liquid is known as **guttation.**

Types of Transpiration

There are <u>three types of transpiration</u> observed in the plant system.
They are:

1. Stomatal Transpiration

Most of the transpiration takes place through stomata. Stomata are usually confined in more numbers on the lower sides of the leaves. In monocots, e.g., grasses, they are equally distributed on all sides. While in aquatic plants with floating leaves, they are present on the upper surface. The loss of water vapours through stomata amounts to about 80-90% of the total loss.

2. Cuticular Transpiration

Although cuticle is impervious to water, still some water may be lost through it. It may contribute to a maximum of about 10% of the total transpiration.

3.lenticular Transpiration

Some water may be lost by woody stems through lenticels which is called as **Lenticular Transpiration.** (Transpiration from leaves is called as foliar transpiration).

Structure of Stomata

Stomata are minute pores of elliptical shape surrounded by two specialized epidermal cells called as Guard Cells. The guard cells are bean shaped in dicots and dumble-shaped in the members of Gramineae (monocots). The inner wall of the guard cell is thick and inelastic (rigid) in nature due to deposition of a secondary layer of cellulose, while the outer wall is thin and elastic and permeable in nature. Each guard cell has a cytoplasmic lining and central vacuole containing cell sap. Its cytoplasm contains a nucleus and number of chloroplasts. which are often poorly developed and not capable of photosynthesis. The epidermal cells surrounding the guard cells are specialized, called a Subsidiary Cells, which support in the movement of guard cells and thus play important role in the opening and closing of stomata.

The number of stomata in a given area varies from plant to plant and the xerophytes possess lesser number of stomata than the mesophytes. When widely open, the stomatal pore occupies about 5% of the total leaf surface area. Under normal condition, stomata nearly occupy1-2% of the total leaf area.

The size and shape of the stomata and guard cells vary from plant to plant. For example, in Phaseolus vulgaris, the stomatal size is about 7x 3μ ; in Avena sativa -38 x 8μ and Zea mays -4 x 26μ.

Mechanism of Transpiration

The mechanism of stomatal transpiration, which takes place during the daytime, can be studied in three steps :

i. Osmotic diffusion of water in the leaf from xylem to intercellular spaces above the stomata through mesophyll cells.
ii. Opening and closing of stomata (stomatal movement), and
iii. Simple diffusion of water vapours from intercellular spaces to outer atmosphere through Stomata.

i. Osmotic diffusion

In the first stage, water gets evaporated from the surface of turgid cells and collects in the intercellular spaces increasing the water vapour pressure and lowering its DPD.

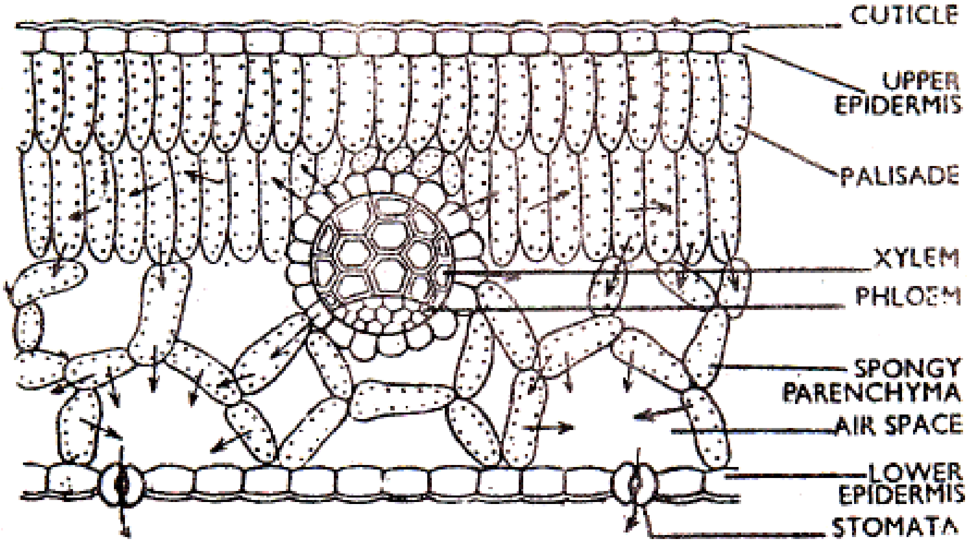


Fig.5. V.S. of dicot leaf. The arrows show the movement of water during transpiration

Inside the leaf, the mesophyll cells are in contact with xylem, and on the other hand with intercellular spaces above the stomata. When the mesophyll cells draw water from the xylem, they become turgid and their DPD and OP decrease with the result that they release water in the form of vapour into intercellular spaces close to stomata by osmotic diffusion. In turn, the OP and DPD of the mesophyll cells become higher and hence, they drawn water from xylem by osmotic diffusion (Fig.5)

ii. Mechanism of stomatal opening and closing (Steward's scheme of stomatal movements)

Microscopic examination of open and closed stomata reveal that open stomata have turgid cells while closed stomata have flaccid guard cells. Therefore, it is concluded that the opeing and closing of stomata depend upon the changes in the turgidity of their guard cells. i.e., when guard cells are turgid, pores are open but when flaccid, the pores are closed. when the turgidity increases, the outer thin walls of guard cells stretch outward causing outward stretching of their inner wall. The inner wall being inelastic (thick), becomes concave and as a result, the space surrounding the pore widens and pore opens.

Thus, in the opening and closing of stomata, **turgor mechanism** is involved. How this change in turgidity in guard cells is brought about is explained below :

During night, starch accumulates in the guard cells. The **starch is** insoluble and therefore, the **OP** of the guard cells is **not increased**. This results in the **flaccid** condition of the **guard cells** leading to the **closure of stomata** during night conditions. **Inlight,** the insoluable **starch** is **converted** into **soluble sugars** in the presence of enzyme *phosphorylase* and leads to stomatal opening as follows :

The **soluble sugars increase the OP** of the guard cells, which also results in an **increase** of their **DPD**. Due to all these changes, the **guard cells drawn water** from the **mesophyll cells** by **osmotic diffusion** and become **turgid**. The **outer thin walls** of the guard cells **expand** while the **thick inner walls are stretched.** This results in the **opeing of the stomatal pore.** High temperature and pH (about 7.0) favour conversion of starch into sugars. The increased pH is prabably due to theconsumption of CO_2 in light in photosynthesis.

In dark, the soluble sugars are converted back to starch. These **lowers** the **OP** of the guard cells and their **DPD** is also decreased with the result that water is released from the guard cells into the mesophyll cells. Due this change, the guard cells become **flaccid,** their thicker inner walls come very close to each other leading to the **closure of stomatal pore.**

Low temperature and pH (about 5.0) favour conversion of sugars into starch. The lower pH level might be due to the accumulation of CO$_2$ in dark.

With the discovery of presence of phosphorylase enzyme in the guard cells Steward (1964) proposed a modified scheme for stomatal opening. The scheme proposed by him is given in Fig.6 below :

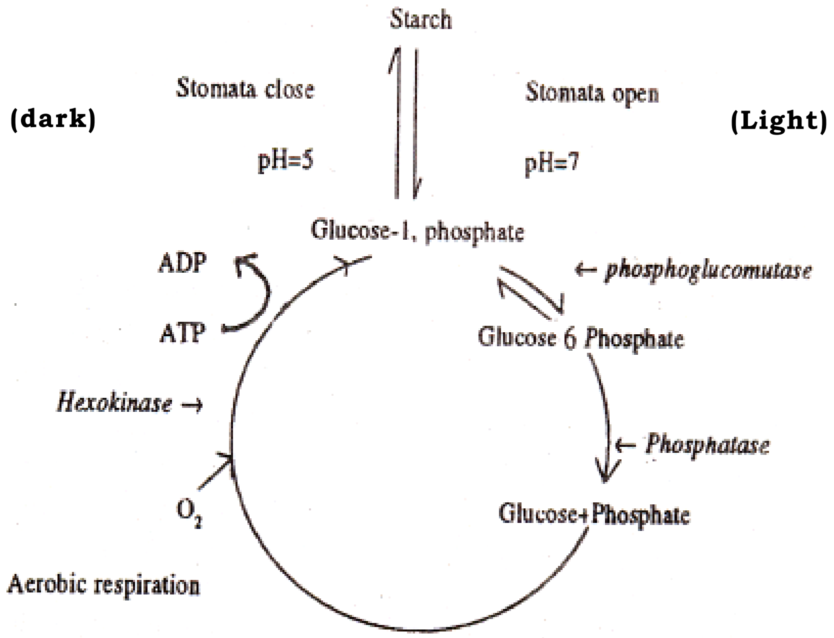


Fig.6. Steward's scheme of metabolic reations involved in opening and closing of stomata.

In his scheme, he has suggested that :

i. glucose-1-phosphate should be further converted into glucose and inorganic phosphate for the opeing of the stomata, and

ii. metabolic energy in the form of ATP will be required for the closing of stomata which probably comes through respiration.

The mechanism proposed for stomatal opeing is :

Light → high rate of photosynthesis in mesophyll cells → removal of CO2 from intercellular spaces→ increase of pH in guard cells → enzymatic conversion of starch into glucose → increase in Op of cell sap → endodermis → guard cells become turgid → stomata open.

A reverse scheme would follow during closure of the stomata.

iii. Diffusion of water vapour to atmosphere

The last step in the mechanism of transpiration is the simple diffusion of water vapours from the intercellular spaces to the outer atmosphere through open stomata. This is beacuse the intercellular spaces are more saturated with moisture in comparison to the outer atmosphere in the vicinity of the stomata.

According to this theory, *Potassium ions (K^+) have been found to play a crucial role in stomatal movements.* The opening and closing of stomata are the results of an active transport of potassium ions into the guard cells and out of them. The adjacent epidermal cell acts as ion storage cells for guard cells.

Closure mechanism involves participation of an ***inhibitor hormone, Abscissic acid*** (ABA) which functions in the presence of $CO_{2.}$ In dark, ABA inhibits potassium uptake by changing the diffusion and permeability of the guard cells. The potassium moves out of the subsidiary cells. Then, ABA induces the process of acidification in the guard cells, which results in lowering of pH. At low pH, starch is synthesised leading to closure of stomata.

Besides, there are some other theories also available to explain the mechanism of stomatal opening and closing. They are :

1. Theory of photosynthesis in guard cells (Von Mohl,1856)
2. Theory of starch ⇔ sugar interconversion (Sayre, 1926)
3. Theory of glycolate metabolism (Zelitch, 1963)
4. Theory of Proton Transport and Hormonal Regulation (Levitt, 1974):

Significance of Transpiration

It is often said that "transpirtion is a necessary evil. It is a vital and unavoidable phenomenon of plants". Therefore, the significance of transpiration can be discussed on the following aspects :

a. Transpiration is unavoidable

As long as there is a need of igress and egress of oxygen and CO_2 during metabolic processes like, photosynthesis and respiration, the transpiration is also considered unavoidable.

b. Transpiration is advageous

Transpiration is incidental due to structural arrangements of leaves. The loss of water does not serve any good purpose in plant life. Millions of plants die every year because the transpiration exceeds the rate of absorption of water through roots of plants. Besides, the transpiration also consumes lot of energy, which is not available for other metabolic activities of the plant.

c. Transpiration is advantageous

Transpiration is also regarded as boon for many reasons and its advantages to the plants are detailed below :

1. It plays an important role in translocation of food from one portion og the plant to the other.
2. It maintains an optimum temperature for the leaves.
3. It brings about the opening and closing of the stomata, which indirectly influence rate of photosynthesis and respiration.
4. It creates suction force and helps in ascent of sap.
5. It influences the absorption of water and minerals by roots.
6. It helps in evaporation of excess amount of water which otherwise would cause suffocation.

d. Transpiration is necessary

The leaves absorb an enormous amount of radiant energy. There are several ways in which the excess energy can dissipate away. Transpiration is one of the chief ways for the dissipation of excess energy, which the plant receives from the sun.

Shull (1930) estimated that approximately 0.8 cal of energy is received upon each square cm of leaf surface per minute, of which about 10% is reflected and 25% is transmitted. The remaining 65% (0.52 cal) will increase the temperature of the leaves very rapidly. If the weight of the leaf tissue is $0.02g/cm^2$ with the specific heat of 0.879, then the rise in temperature would be at 32^0C per minute.

With this rate of increase in temperature, the plant will be killed in less than two minutes, if there is no dissipation of energy. Transpiration plays a significant role here. It helps in dissipating this excess energy which will otherwise raise the temperature. Therefore, the transpiration is considered as the necessary process for the plant.

Transpiration is a necessary evil !

It is often said that transpiration is a necessary evil. It is a vital and unavoidable phenomenon of plants. The loss of water does not serve any good purpose in plant life. Besides, the transpiration also consumes energy and causes unnecessary absorption of excess water by roots. Nevertheless, the internal structure is basically meant for the exchange of gases during photosynthesis and respiration. Therefore, it cannot check the evaporation of water. Hence, many workers like Curtis (1926) have called transpiration as necessary evil !

Factors Affecting Rate of Transpiration

The factors affecting rate of transpiration can be categorized under two groups :

A. External or Environmental factors (eg. atmospheric humidity, temperature, wind velocity, light, water supply, atmospheric pressure, sprays and dusts and vital activities)

B. Internal or Structural factors (eg. stomatal apparatus and its frequency, water content of mesophyll cells and structural peculiarities of the leaf).

A. External Factors

All these factors affect the steepness of DPD gradient and thus influence rate of transpiration.

1. Atmospheric Humidity :

The rate of transpiration increases with the decrease in the humidity of the external atmosphere. because, the humidity influences the DPD gradient between the intercellular spaces and outside atmosphere.

2. Temperature

High level of temperature increases the rate of transpiration by increasing the rate of evaporation of water from cell surface and thus decreasing the humidity of external atmosphere.

3. Wind Velocity

Increase in wind velocity increases the transpiration rate by removing water vapour of the atmosphere and lowering the relative humidity. The transpiration is faster in mild wind. In the case of higher wind velocity, the transpiration rate is retarded because of guard cells becoming flaccid and stomata closed.

4. Light

Light indirectly controls rate of transpiration in two ways : firstly, by controlling the stomatal opeing and secondly, by affecting the temperature. With the increase in the light intensity, the rate of transpiration increases beacuse the stomata get opened and the temperature increases. There is a close relationship between the opening of the stomata and presence of light.

5. Atmospheric Pressure

The reduction of atmospheric pressure increases the transpiration rate by permitting more rapid diffusion of water. *The plants growing in hills will show higher rates of transpiration because of low atmospheric pressure.*

6. Water Supply

Deficiency of soil water decreases the rate of transpiration indirectly by reducing availability of water and its rate of absorption.

7. Sprays and Dusts

Sprays and dusts affect transpiration by affecting the permeability of the cuticle and temperature of leaves.

8. Vital Activities

Vital activities of the plants may also influence the transpiration rate such as heat produced from the energy provided by the respiration may increase the rate of transpiration.

B. Internal Factors

1. Stomatal Frequency

Stomatal frequency means the number of stomata per unit area of leaf surface. Salisbury used the term Stomatal Index (SI) to represent as,

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

where, SI refers to Stomatal Index, S the number of stomata per unit area and E for the epidermal cells in the same unit area.

If the stomata are open, with increased stomatal frequency, the rate of transpiration increases. It also depends on the degree of opening of stomata.

2. Structural Features of Leaf

Certain plants are adapted to reduce the rate of transpiration, eg., by reducing leaf size, the transpiration surface is reduced. Some xerophytic plants have needle-like leaves to reduce transpiration. Presence of thick deposition of cution or wax-like substances on the leaf surface also reduces transpiration.

Presence of **sunken stomata** (as in **Calotropis, Nerium** and **Cycas**) helps in reducing stomatal transpiration. These sunken stomata are found in cavities surrounded by hairs. These depressions accumulate more water vapour reducing diffusion rate of water through this pore. (Fig.7).

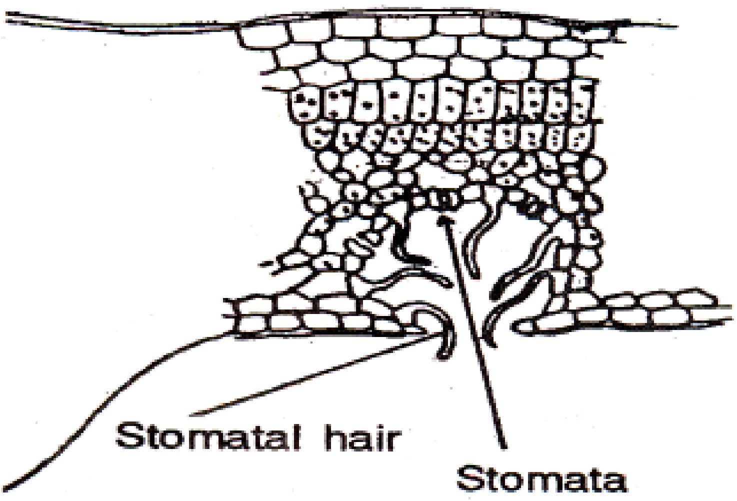


Fig.7 V.S. of leaf showing well-guarded position of sunken stomata (Nerium)

Presence of hydrophilic compounds such as gums, mucilage etc. help in retarding the rate of transpiration. Several factors such as leaf area, amount of spongy tissue, volume of intercellular spaces, orientation of leaf and extent of root system also affect transpiration rate.

ANTITRANSPIRANTS

Definition

The antitranspirants are any materials applied to the plants for the purpose of retarding transpiration. In extreme case of transpiration, about 98% of water absorbed by the plants is lost and only insignificant amount is being utilized by the plant for its own purpose. If, by any chance, this enormous loss of water can be reduced, it will be an asset to the nature and also the agriculturists to reduce the traspiration loss and increase the crop productivity in the rainfed and semi-arid regions. The antitranspiratnts are of three types.

They are discussed below :

a. Stomata closing type : (eg. Phenyl Mercuric Acetate, ABA, Atrazine, CO2 at high conc., Decenyl succinic acid, Xanthoxin)

These chemicals will induce stomatal closure and thus transpiration will be reduced. The problem of using these chemicals is the photosynthesis will also be affected because of reduced entry of CO_2.

b. Thin film forming type : (eg. Cetyl alcohol, silicone oils, low viscosity waxes, rice gruel)

These substances will form a thin film on the leaf surface. They will inhibit the loss of water vapour from the leaf. But, they will allow CO_2 to pass into the leaf.

c : Reflective Type : (eg. Kaolin, lime water spray)

The principle of using this type of chemicals is to increase the light reflection by the leaves and thus reducing the leaf temperature. Though the transpiration is reduced, CO_2 assimilation will not be affected.

Different Types of Stomata

A. Types of Stomata based on Distribution

There are about five types of stomatal distribution recognized in plants :

1. Apply or mulberry type

Stomata are found distributed only on the lower surface of the leaves. eg., apple, peach. mulberry, walnut etc. Such leaves are called as *hypostomatic type.*

2. Water lily type

Stomata are distributed only on the upper epidermis of the leaves. eg., water lily, Nymphaea and many aquatic plants. These plants are *epistomatic type..*

3. potato type

In this type, the stomata are found more on the lower surface (multistomatic) and less on the upper leaf surface (paucistomatic type). eg., Potato, cabbage, bean, tomata, pea etc. Such leaves are called as amphistomatic and anisostomatic type.

4. Out type

Stomata are equally distributed on the both lower and upper surface of the leaves. These leaves are referred as *isostomatic type.*

5. Potamogeton type

In this case, stomata are altogether absent or if present, they are vestigeal. eg., Potamogeton and other submerged aquatics.

B. Types of Stomata based on Movement

Loftfield (1856) classified three main groups of stomata in accordance with their daily movement:

1. Alfalfa Type

The stomata remain open throughout the day and closed all night, eg., peas, bean, mustard etc.

2. Potato Type

The stomata open only for a few hours in a day, eg., Allium, cabbage etc.

3. Barley Type

The stomata open only for a few hours in a day, eg., Barley and other cereals.

C. Types of Stomata based on Behavior

Considering the behavior of the stomatal movements, five categories have been recognized :

1. Photo-active movements

Light directly or indirectly controls stomatal movements. Such stomata remain open during day time and closed in nights (dark).

2. Skoto-active movements

Stomata remain closed during day time and open during night. Such cases are found in succulent plants and other CAM Plants.

3. Hydro-active movements

In some cases, stomata open due to excessive loss of water from the epidermal cells and close due to turgid conditions of epidermal cells. This is usually found during mid-day.

4. Autonomous movements

In certain cases, stomata open and close at a rate of 10-15 minutes showing diurnal or rhythmic pulsation.

5. Passive and Active movements

Opening of stomata is considered as active process and closing is the passive process and this is caused by the turgor changes in the guard cells.

GUTTATION

In some plants, watery drops ooze out from the injured margins of the leaves where the main vein ends. This is called as **guttation.** It takes place usually early in the morning when the rate of water absorption and the root pressure are higher while the transpiration is very low. The watery drops consist of water in which many organic and inorganic substances are dissolved.

The phenomenon of guttation is associated with the presence of special types of stomata at the margins of the leaves, which are called as ***water stomata or hydathodes***. Each hydathode consists of a water pore. (Fig.8), which remains permanently open. Below this, there is a small cavity followed by a loose tissue called as epithem. The epithem is in close contact with the ends of vascular elements (tracheids) of veins.

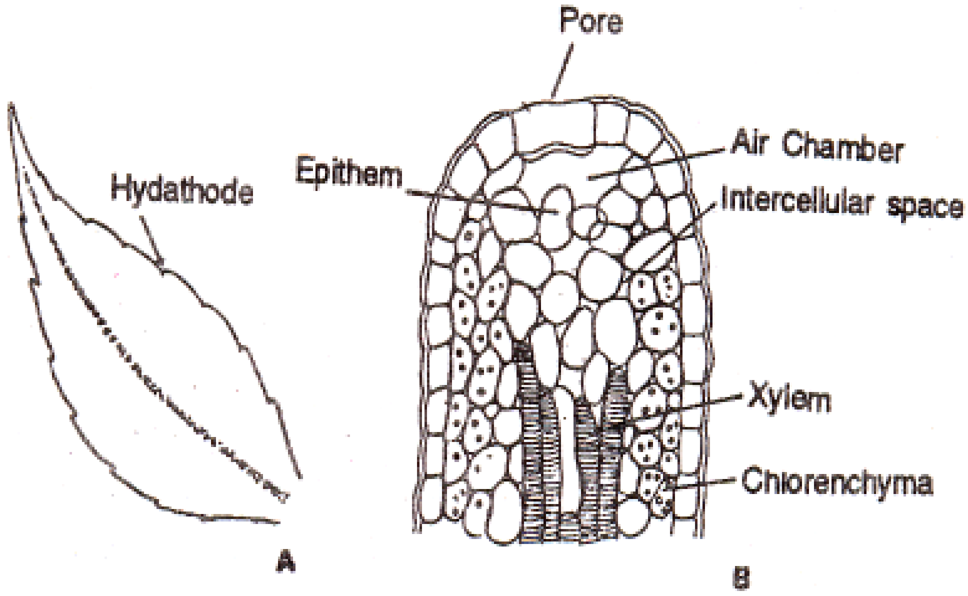


Fig.8. Hydathodes : A-Entire leaf showing position
B-V.S. of leaf through hydathode

Under higher root pressure, water is given to the epithem by the xylem of the veins. From epithem, the water is released into the cavity. When this cavity is completely filled with the watery solution, the latter begins to ooze out in the form of watery drops through the water pore.

Measurement of Transpiration

Following four methods are usually used for measuring transpiration in plants :

a. Weight method

The method consists in weighing the potted plants at regular intervals. The losss of weight over a prescribed period of time represents the weight of transpired water.

b. Cobalt Chloride method

This method is based on the fact that the dry cobalt chloride is blue in colour while the moist cobalt chloride is prink in clour. Actually, in this method, the weight of transpired water is not measured. But, it measures only the rate of transpiration.

Small piece of filter paper is soaked in3% cobalt chloride solution and thoroughly dried. A single cobalt chloride paper is clipped under a glass cover slip along with an ordinary standard pink and ordinary standard blue coloured paper on the surface of the transpiring leaf. The colour of the cobalt chloride will gradually change from blue to pink. The time taken for this change is noted. The rate of the colour change is an indication of the rate of transpiration.

c. Collecting and Weighing Transpired Water (Porometry)

This method consists in passing the air of known moisture content over a potted plant kept under a closed glass chamber through an opening. The air containing transpired water is passed out over anhydrous calcium chloride whose initial weight is already known. The increase in the weight of anhydrous calcium chloride will be equal to the weight of transpired water plus the original moisture content of the air. The weight of the original moisture content of the air being known already, the weight of the transpired water is easily calculated.

Based on this principle, the transpiration rate in crop plants is quickly measured using the modern equipment, Steady State Porometer.

d. Potometer Methods

These methods are based on the assumption that the rate of absorption of water is approximately equal to the rate of transpiration. The appratuses used for measuring the transpiration are called as Potometers. Following three types of potometers are very common :

i. Drawin's Potometer
ii. Farmer's Potometer and
iii. Ganong's Potometer

More recently, Steady State Porometer is also available in the market to measure the rate of transpiration in plants.

Differences between Transpiration and Guttaion :

S.No.	Transpiration	Guttation
1.	It occurs during day time	It usually occurs in the night
2.	The water is given out in the form of vapour	The water is given out in the form of liquid.
3.	The transpired water is pure	Guttated water contains dissolved salts and sugar.
4.	It takes place through stomata, lenticels or cuticle.	It occurs through special structure called hydathode found only on leaf tips or margin.
5.	It is a controlled process.	It is an uncontrolled process.
6.	It lowers down the temperature of the surface	It lacks such relationship.

Differences between Transpiration and Evaporation :

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

CHAPTER 5

MINERAL NUTRITION

In order to complete the life cyle normally, the living organism requires a supply of large number of substances from outside. This is called as **nutrition.** If the supply needed by the organism is both organic and inorganic, the organism is called **heterotrophic,** but if the supply needed by the organism is that of inorganic substances only, the organism is called **autotrophic or self-feeding.** All green plants synthesizing their own organic requirements are autotrophic.

Under normal conditions of growth, all green plants are autotrophic and they require from outside the supply of only inorganic substances. All the inorganic plant requirements are obtained directly or indirectly from the soil. As the sources of these inorganic requirements are minerals, the elements are known as mineral nutrients and the nutrition is called **mineral nutrition.**

Thus, the plant growth and development can proceed only when the plants are applied with the chemical elements referred as **Essential Elements.** These nutrients are absorbed by plant root from the soil.

Chemical analysis of the plant ash (the residue left after the dry matter of the plant has been burnt) has shown that plants contain about 40 different elements. Some of them are indispensable or necessary for the normal growth and development of the plants and they are called as **Essential Elements**. Rests of the elements are called as **Non-essential elements.**

It is now known that the following 15 elements are essential for majority of the plants :

C,H,O,N,P,K,Ca, S, Mg, Fe, Zn,B,Cu, Mu and Mo. Besides these, Al, Si,Cl, Na, Co and Ga may be essential for some plants.

Essential elements may be classified into three groups :

1. MAJOR ELEMENTS OR PRIMARY NUTRIENTS

The essential elements, which are required by the plants in comparatively larger amounts are called **Major Elements or Primary Nutrients.** The list includes : C,H,O,N,P and K.

2. SECONDARY ELEMENTS or NUTRIENTS

The se elements are also requited by the plant in larger quantity next to primary nutrients. Examples are : Ca, Mg and S.

3. MINOR ELEMENTS or MICRONUTRIENTS or TRACE ELEMENTS

The essential elements required in smaller amounts or traces by the plants are called as Minor or Trace Elements. They are : Fe, Mn, Cu, Zn, Mo, B and Cl. Apart from these elements, recently some more elements have also been shown to the essential for the normal growth of some plants such as Na for Atriplex, Si for rice and Cl for coconut and Al, Va and Co for ferms.

Micronutrients are usually present in the plants in different chemical forms as :

1. Inorganic ions
2. Undissociated molecules or
3. Organic complexes as chelates

Crieria of Essentiality of Elements (Arnon and Stout, 1939)

In order to show that element is truly essential, it is necessary to show not only that :

i. A deficiency of the element makes it impossible for a plant to complete its vegetative and reproductive cycle but also that

ii. It cannot be replaced by another element and that

iii. The element should also have some part to play in metabolism
The effect is not simply the result of interaction with other non-essential elements, organism etc. loutside the plant.
The effect is not simply the result of interaction with other non-essential elements, organism etc. outside the plant.

These three requirements form the criteria of essentiality of mineral elements. However, recent studies indicated that functions of *some of the elements could be partly replaced by other. (e.g. potassium by rubidium; magnesium by manganese).*

Classification of plant nutrients based on chemical behavior and physiological functions :

Nutrient element	**Uptake**	**Biochemical function]**
Its Group: C H,O,N,S	CO_2, HCO_3, H_2O, O_2 NO_3, NH_4 , N_2 ,SO_4 ,SO_3 ions from soil solution and gases from atmosphere.	Major constituent of organid material. Functional elements in enzymatic processes. Assimilation is by oxidation - reduction process.
IIts Group: P,B,Si	Phosphate, boric acid or borate, silicate from the soil solution	Esterification with native alcohol groups in plants, Phosphate esters are involved in energy transfer reaction.
IIIts Group: K, Na, Mg,	In the form of ions from the soil solution.	Maintain osmotic potential, bring about optimum conformation of an enzyme protein (enzyme activation) control membrane permeability and electron potentials.
IVth Group: Fe, Cu, Zn,	In the form of ions or chelates	Incorporated in the prosthetic group, enable electron transport by valency change.

Methods of Studying Plant Nutrients

The methods for study of mineral nutrition of plant are :
Plant analysis, Solution cultures *(Hydroponics or chemical gardening)* and Sand culture.

Hydroponics

The system of growing plants in soil-less cultures or solution cultures is known as Hydroponics. This has certain advantages over *geoponics (Soil culture or agriculture)* Such as :

a. A controlled composition of nutrient solution may be provided.
b. There is no immobilization of nutrients as there is no colloids for nutrient adsorption
c. It prevents accumulation of toxic organic decomposition products due to frequent replacement of culture solution.
d. Minimized growth of fungi and bacteria.
e. It ensures better environment for plant growth as the culture solution is kept aerated.
f. There is no weed growth.
g. Natural calamities such as flood, drought, erosion etc. can be avoided.

In India, hydroponic culture practies have been widely adopted. Tomatoes grown through this system in West bengal yielded an average of over 200 tons per acre as against 15-20 tons under ordinary soil culture. Similarly, paddy grown by this yielded 11,400 kg/ha and potatoes upto 135 tons/ha. A Hydroponic Information Centre has also been set up in Bombay (Post Box No.31) to provide detailed information on hydroponics.

Aeroponics

It is a system for growing plants with their roots supplied with moisture in the air. The rooted plants are placed in a special type of box with their shoots exposed to air and roots inside the box with computer controlled humid atmosphere. Plants like *Citrus* and olive have been successfully grown through this method.

Nutrient Availability in Soil and Mechanism of uptake by plants

Nutrient Availability in Soil Solution

Soil serves as a main source of mineral salts in which clay crystals are present in colloidal form. These crystals have a central nuclues called **micelle.**

The micelles are negatively charged and in order to maintain a balance, they attract and hold positively charged ions on the surface of the colloidal clay crystals. Thus, the balance is always maintained.

The available minerals of soil occur in ionic forms. The common cationic forms are : K,Mg,Ca, Fe,Mn,Cu,Zn and Co while anionic forms and N,P,B,S and Cl. These ions are found either in the form of loosely absorbed ions or firmly absorbed ions on the colloidal particles. The order of cation retentive capacity of colloids is as follows :

$$H^+ > Ca^{++} > Mg^{++} > K^+ > NH_4^+ > Na^+$$

The loosely absorbed ions can be easily displaced by decreasing their own concentration in the soil solution while the firmly absorbed ions can be replaced by other ions which have more affinity for the colloid or ion exchange. The ion exchange may be either cation exchange or anion exchange.

Availability of Mineral Salts

Mineral salts are found either as soluble fraction of soil solution or as adsorbed ions on the surgace of colloidal particels. It is believed that the uninterrupted supply of mineral nutrient from the absorbed fractions is possibly done by ionic exchange.

Ion Exchange :

First step in the absorption of mineral salts is the process of **Ion-Exchange** which does not require metabolic energy but greatly facilitates mineral salt absorption.

The ions adsorbed on the surgace of the wall or membranes of root cells may be exchanged with the ions of the same sign from external solution. For example, the cation K^+ of the external soil solution may be exchanged with H^+ ion adsorbed on the surface of the root cells. Similarly, an anion may be exchanged woth OH^- ion.

There are two theories proposed to explain the mechanism of ion exchange:]

a. CO_2 hypothesis (or) Carbonic Acid Exchange Theory
b. Cation Exchange hypothesis (or) Contact Exchange Theory.

a. Cos hypothesis :

According to this theory, CO_2 released by the roots during repiraion combines with water to produce carbonic acid (H_2Co_3). The carbonic acid dissociates into hydrogen ions(H+) and bicarbonate ions (HCO_3). These hydrogen ions may be exchanged for cations adsorbed on clay particles. The cations thus released into the soil solution from the clay particles may be absorbed on root cells in exchange for H^+ ions. While the dissociated bicarbonate ions release the adsorbed anions (Fig.9). Thus, both cations and anions are made available to the closeness of the roots of plants. Thus, soil solution plays an important role in carbonic acid exchange theory.

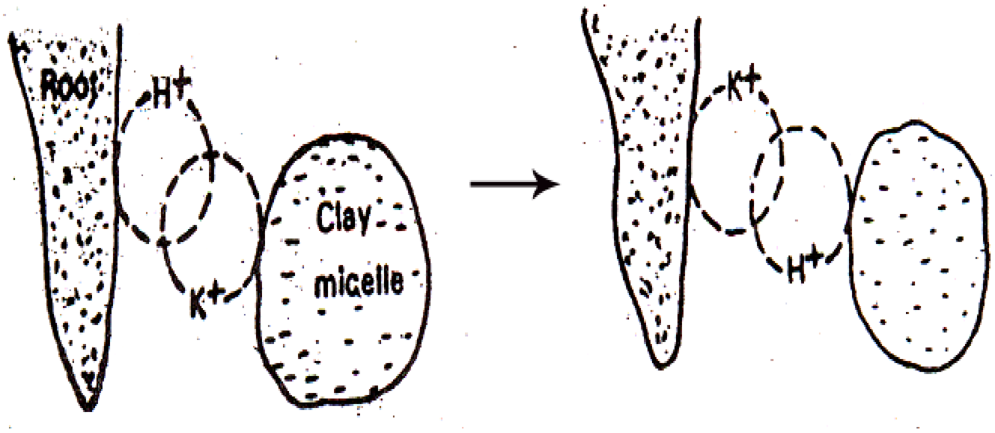
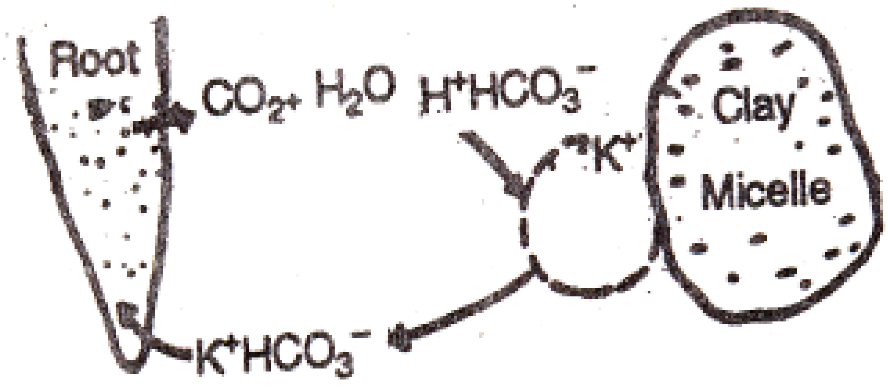


Fig.9. Figure explaining CO_2 hypothesis

b. Cation Exchange hypothesis

This theory states that the ions adsorbed on the surgace of root cells and clay particles (micelles) are not held tightly but always oscillate within small volume of space. If the roots and clay particles are in close contact with each other, the oscillation volume of ions adsorbed on root-surface may overlap volume of ions adsorbed on clay particles. (Fig.10). Then, the ions adsorbed on clay particle may be exchanged with the ions adsorbed on rootsurface **directly without first being dissolved in soil solution.**



Mechanism of Mineral uptake by Plants

Previously, it was thought that the absorption of mineral salts from the soil took place along with the absorption of water, but it is now well established that the mineral salt absorption and water absorption are two independent processes.

Mineral salts are absorbed from the soil solution in the form of ions. They are chiefly absorbed through the **meristematic regions** of the roots near tips. Plasma membrane of the root cells is not permeable to all the ions. It is **selectively permeable.** All the ions of the same salt are not absorbed at equal rate but there is **unequal absorption of ions.**

First step in the absorption of mineral salts is by Ion Exchange. Once the nutrients come and adsorb on the surface of the walls or the membranes of root cells, then the further process of the absorption of mineral salts may be of two types. They are :

1. **Passive Absorption**
2. **Active Absorption**

Various theories have been proposed to explain the mechanism of mineral salt absorption, which can be of two categories :

1. Passive Absorption of Mineral Salts

When the concentration of mineral salts is higher in the outer solution than in the cell sap of the root cells, the mineral salts are absorbed according to the concentration gradient by simple process of **diffusion.** This is called as passive absorption because it does not require expenditure of metabolic energy.

This can also be called as **Physical Absorption.** This process is not affected by temperature and metabolic inhibitors. This theory is based on the movements of ions from the region of its higher concentration to the lower concentration. Therefore, The direction of the initial uptake gets reversed if the tissues are transferred back to a low concentration.

Important theories are Mass Flow, ion exchange and Donnan equilibrium.

i. Mass Flow Theory (Bulk Flow)

According to this theory, the ions are taken up by the roots along with mass flow of water under the influence of transpiration. Therefore, transpiration effect on salt absorption is direct.

ii. Ion Exchange Theory

According to this theory, ions from the external solution in which the tissue is immersed may exchange with the ions absorbed on the surface of the cell wall or membranes of the tissue.

iii. Donnan Equilibrium

This theory explains the accumulation of ions inside the cells without involving the expenditure of the metabolic energy. According to this theory, there are certain pre-existing ions inside the cell, which cannot diffuse outside through membrane. Such ions are called as indiffusible or fixed ions. However, the membrane is permeable to both anions and cations of the outer solution (Fig.11)

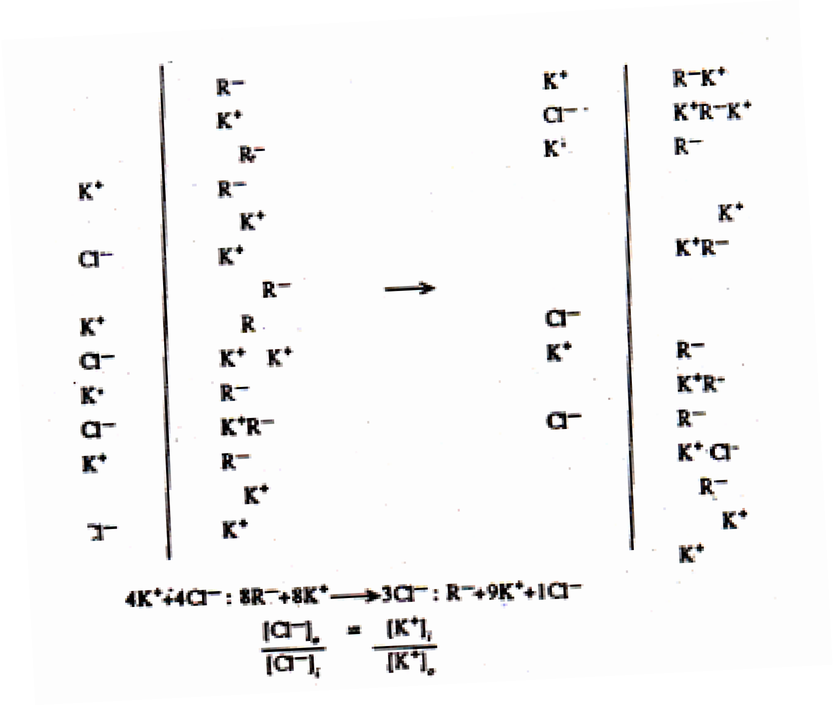


Fig .11. Figure showing Doanan Equilibrium

Suppose, there are certain fixied anions in the cell which is in contact with the outer solution containing anions and cations. Normally, equal number of anions and cations would have diffused into the cell through an electrical potential to balance each other, but to balance the fixed anions already present in the cell (pre-existing), more cations will diffuse into the cell, This equilibrium is known as **Donnan's Equilibrium.** In this particular case, there would be more accumulation of cations inside the cell.

However, if there are fixed cations (pre-existing)inside the cell, then the Donnan's equilibrium will result in more accumulation of anions inside the cell to maintain the equilibrium.

2.Active Absorptionr of Mineral Salts

This processs involved the metabolic energy for the transport of ions from soil solution to the plants. Based on the nature of participation of metabolic energy, various theories have been proposed. It includes the theories related with carrier concept such as Cytochrome Pump hypothesis, ATP theories, Protein-Lecithin as carrier theories etc.

i. Carrier Concept Theory (Honert,1973)
(for movement of both cation & anion)

According to this theory, the ion transport process is carried out by means of carriers, which may be organic molecules or vesicles. This theory explains that the plasma membrane is impermeable to free ions. The carrier combines with ions to form **carrier-ion complex,** which can move across the membrane. On the inner surface of the membrane, this complex breaks releasing ions into the cell while the carrier goes back to the outer surface to pick fresh ions (Fig.12).

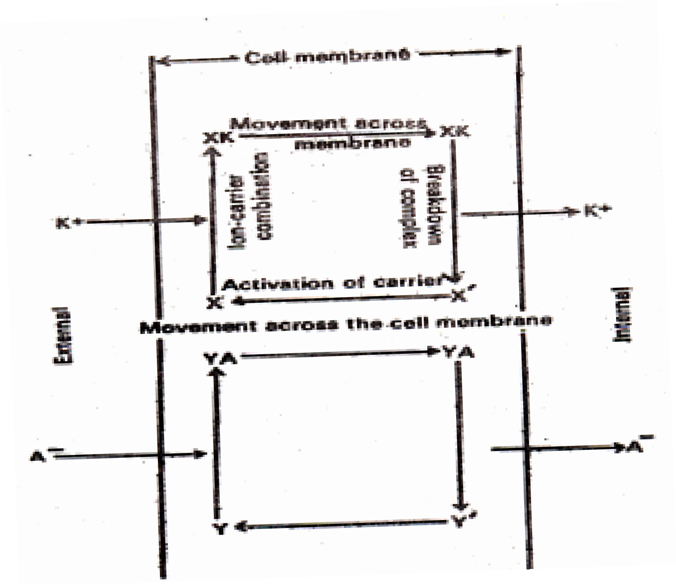


Fig.12. Explanation of carrier mechanism of ion uptake.

Here, the metabolic energy is required in the process of formation of carrier-ion complex, its transport, breakdown of complex, regeneration of carrier and movement of carrier molecules back.

ii. Protein-lecithin as Carrier (Bennet-Clark,1956)
(for uptake of both cation & anion)

It is suggested that because the cell membranes chiefly consist of phospholipids and proteins and also certain enzymes seem to be located on them, the carrier could be a protein associated with the phosphotide called as **lecithin.** This theory believes in the participation of some **amphoteric compounds** as carriers with which both cations and anions can combine.

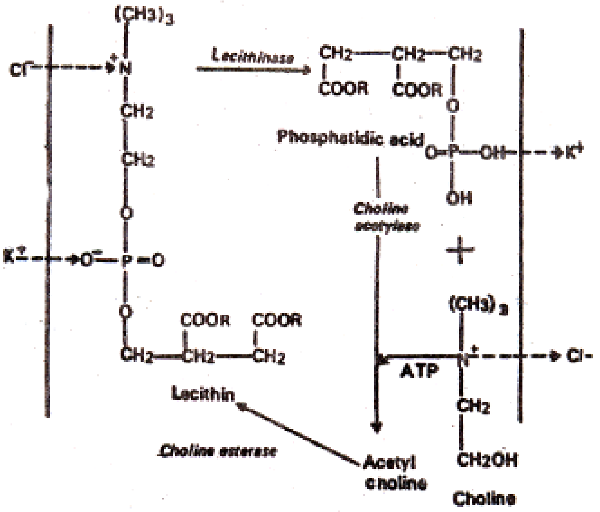


Fig:13 Diagramatic Schemes of Protein-lecithin theory

According to this theory,

1. The acidic phosphate group in the phosphatide is regarded as the active centre **binding the cation,** and the **basic choline group** (N^+) as the **anion binding centre.**

2. The **ions are liberated** on the inner surface of the membrane by decomposition of the lecithin by the enzyme **lecithinase.**

3. The regeneration of the carrier lecithin from phosphatidic acid and choline takes place in the presence of the enzymes choline acetylase **and choline esterase and ATP.** The **ATP** acts as a source of energy.

iii. Cytochrome-pump Theory
(For the movement of anions only)

Lundegardh and Burstrom (1933) claimed that a quantitative relaionship exists between anion absorption and respiration. When a plant is transferred from water to salt solution, the rate of respiration increases. They called this increase in respiration as Salt Respiration. The actual transport of anions occurs through a cytochrome system (Fig.14)

1. Dehydrogenase reactions on inner side of the membrane give rise to protons (H^+) and electrons(e_-).
2. The electron travels over the cytochrome chain towards outside the membrane, so that the Fe of the cytochrome becomes reduced (Fe^{++}). on the outer surface and oxidised (Fe^{+++}) on the inner surface.
3. On the outer surface, the reduced cytochrome is oxidised by oxygen releasing the electron (e^-) and taking an anion (A^-)
4. The electron thus released unites with H^+ and oxygen to form water.
5. The anion (A^+) travels over the cytochrome chain towards inside.
6. On the inner surface, the oxidised cytochrome becomes reduced by taking an electron produced through the dehydrogenase reactions and the anion (A^+) is released.
7. As a result of anion absorption, a cation (M^+) moves passively from outside to inside to balance the anion.

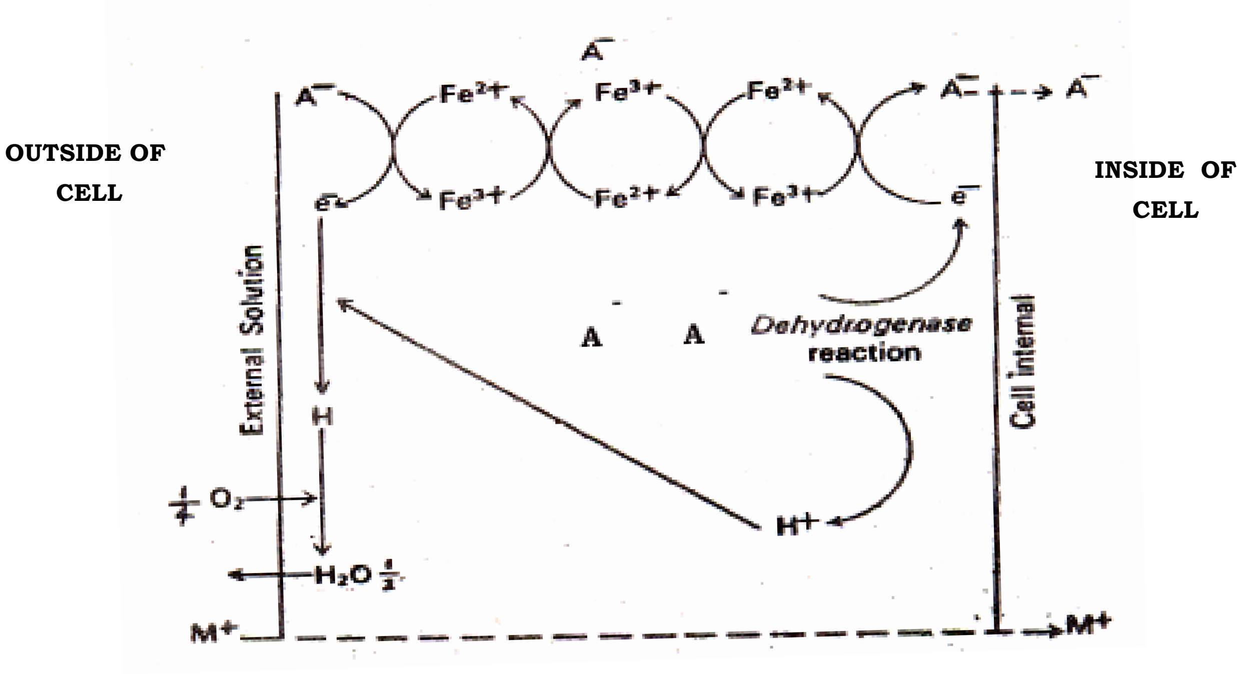


Fig.14. Diagrammatic representation of Lundegardh's cytochrome pump theory.

iv. ATP Theories

According to this theory, ion uptake into the cell is energised by ATP molecules. The energy from hydrolysis of ATP molecules can be made available to energise ion pumps through the action of enzymes.

Case I

Here, the organic compound is first phosphorylated which on dephosphorylation makes the organic compound capable to combine with cation. The cation is released when phosphorylation occurs again.

Case II

In this case, the phosphorylated organic compound combines with cation and the cations are released on hydrolysis of the complex (dephosphorylation). Thus, the role of ATP in this theory is of two kinds. i.e., by removal or addition of phosphate group.

Factors Affecting Salt Absorption

Absorption of salt by the plants is affected by several factors. Some of them are discussed below.

i Temperature

The increase in temperature increases both active and passive salt absorption processes and lowering of temperature decreases them.

ii. pH

It indirectly affects the salt absorption as the pH affects the availability of ions in the medium.

iii. Light

As opened stomata allow more transpiration and increased mass flow and photosynthesis provides energy and O_2 for salt uptake, light indirectly affects the rate of salt absorption by affecting the opening and closing of stomata and the process of photosynthesis.

iv. O_2 content

The deficiency of O_2 decreases salt uptake as the active phase of salt absorption is inhibited by the absence of O_2

v. Interaction of other ions

The absorption of one ion may be influenced by the presence of other ion. The interaction may be associated with the availability and specificity of binding sites on carriers.

vi. Growth

Different types of growth affect salt absorption in different ways, eg., growth involving increase in surface area, number of cells, synthesis of new binding sites or carriers and volumes of water uptake stimulate salt absorption. Heavily suberised root is unable to absorb salts. Vegetative growth and increased metabolic activity accompained with more water uptake enhance salt absorption.

Physiological Roles of Essential Elements

1. Nitrogen (N)

Source

Soil is the chief source of nitrogen. Plants absorb N either in the form of nitrate or ammoniacal salts. Some bacteria and heterocysts conatining blue green algae fix N of atmosphere, which can be utilized by the plants.

Physiological Roles

i. Present in the structure of the protein molecule

ii It is found in important molecules like purines, pyrimidines (which are essential in protein synthesis)etc.

iii It is also found in the porpyrines found in chlorophyll and cytochrome enzymes and hence it is essential for photosynthesis.

iv. It is participated in the co-enzymes essential to functions of many enzymes.

v. It is readily mobile within the plant tissues. When its deficiency occurs, It is transferred from older to younger tissues where it can be reutilised in growth process. As a result symptoms develop first on older leaves.

2. Phosphorus (P) Source

The plant absorb P in the form of soluble phosphates such as $H_3PO_4$and HPO_4. The absorption ability differs from plant to plant,e.g. cabbage and alfalfa can absorb phosphate from rocks whereas barley, corn and oats cannot absorb so efficiently.

Physiological Roles

i. Phosphorus content is found to be 0.2 to0.8% of the total dry weight.

ii. It is found abundantly in the growing and storage organs such as fruits and seeds.

iii. It promotes healthy root development and fruit ripening through translocation of carbohydrates.

iv. It is an essential element participating in the skeleton of plasma membrane, nucleic acids and organic molecules such as ATP (Adenosine Tri Phosphate) and other phosphorylated compounds.
v. It is also found in plants as a constituent of nucleic acids, phospholipids, the co-enzymes like NAD etc.
vii. Phospholipids along with protein may be important in cell membrane.
viii It is **readily mobile within the plant.**

3. Potassium (K)

Source

Potassium is widely distributed in soil minerals. Forms such as potash felspar, mica and glauconite are slowly converted into soluble forms by weathering processes. It is strongly fixed in soils, largely as an exchangeable base. The K is found in less available forms. Small amounts are normally present in the soil in an exchangeable form.

Physiological Role

i. It is concerned with the formation of carbohydrate and protein synthesis, photosynthesis, Transpiration regulation, enzyme action, synthesis of nucleic acids and chlorophyll, oxidative and photo phosphorylation, translocation of solutes etc.
ii. It acts as an activator of many enzymes involved in carbohydrate metabolism and protein synthesis.
iii. It is actively involved in the opening and closing of stomata.
iv. It takes an important role in drought tolerance of crops through water relations.
v. It offers resistance to pest and disease effects in crops.
vi. It is present in the soluble forms and mostly contained in the cell sap and cytoplasm.
vii. It is readily mobile within the plant tissues.
viii. It is frequently present in all parts of the plant but in fairly large proportions at growing tissues.
ix. Its function is partly replaced by rubidium.

4. Magnesium (Mg)

Source

Magnesium occurs as carbonates and held in soils as an exchangeable base. It is easily leached and for this reason may become deficient in sandy soils during wet periods. Heavy application of K fertilizers reduces its absorption.

Physiological Roles

i. It is a constituent of chlorophyll, and therefore, essential for its synthesis.
ii. It acts as a phosphorus carrier in the plant.
iii. It is essential for the synthesis of fats and metabolism of carbohydrate and phosphorus.
iv. It is required in binding two subunits of ribosomes during protein synthesis.
v. It acts as an activator for many enzymes in phosphate transfer reactions in carbohydrate metabolism and nucleic acid synthesis.
vi. It is invloved in the formation of seeds of high oil contents containing a compound called **lecithin**
vii. It is readily **mobile** within the plant tissues.
viii. Its function is partly replaced by manganese.

5. Sulphur (S)

Source

It is available to plants in the form of soluble suphates of soil.

Physiological Role

i. It is an important constituent of some amionacids (cystine, cysteine and methionine), vitamins (biotine, thiamine), coenzyme A and volatile oils.
ii. It participates in protein synthesis
iii. Sulfyhydryl groups are necessary for the activity of many enzymes
iv. Disulphide linkages help to stabilise the protein structure.
v. It adversely affects chlorophyll synthesis.
vi. Sulphur affects the nodule formation in roots of leguminous plants.
vii. Characteistic odour od Cruciferous plants (onion, garlic etc.) is due to the sulphur as constituent of volatile oils.
viii. It is known for its **uneven distribution** in various organs of plant (e.g)in a mature corn, the content observed was -40% in leaves. 23% in stem, 26% in gain and 11% in roots)
ix. It is immobile in the plant tissues. When its deficiency occurs, it is not transferred to the younger leaves but accumulated in the older leaves only. As a result, deficiency symptoms develop first on younger leaves.

6. Calcium (Ca)

source

Calcium occurs in soil with variety of minerals. The soil derived from stone or chalk rocks contains larger percentage of carbonates of lime (calcium carbonate), while sandy soils show Ca deficiency which is met by adding lime or lime stone. The presence of CO_2 dissolved in the soil water promotes solubility of carbonate of lime in soil ensuring the quick Ca absorption.

Physiological Roles

i. It is the important constituent of middle lamella in the cell wall
ii. It is essential in the formation of cell membranes.
iii. It helps to stabilise the structure of chromosomes.
iv. It is also an activator of many enzymes (ATPase, kinases, succinate dehyrogenase)
v. It provides a base for the neutralisation of organic acids.
vi. It is concerned with the growing root apices.
vii. It is essential for fact metabolism, carbohydrate metabolism and binding of nucleic acids with proteins.
viii. It is also essential in the counteraction of metal toxicity
ix. It is immobile in the plant tissues. Therefore, when needed, the element is not trans ferred to the younger leaves but accumulated in the older leaves itself. As a result, deficiency symptoms develop first on younger leaves.

Micronutrients

7. Iron (Fe)

Source

It is fairly present in the form of oxides giving red or brown colour to the soil. In well-irrigated soils, Fe is present predominantly as ferric form and in water-logged soils, ferrous compounds are formed. The availability of Fe to plants increases with acidity and is depressed by phosphates. It is **absorbed in ferric state;** but, **ferrous form is only metabolically active** for the plants.

Physiological Role

i. It is an important contituent of iron-porphyrin proteins like, cytochromes, peroxidases, catalases etc.

ii. It is essential for the synthesis of chlorophyll

iii. It acts as a catalyst and electron carrier during respiration

iv. It also acts as an activator of nitrate reductase and aconitase enzymes.

v. It is a very important constituent of ferredoxin, which plays an important role in biological nitrogen fixation and primary photochemical reaction in photosynthesis.

vi. It is immobile in the plant tissues. Its mobility is affected by several factors like presence of magnesium, potassium deficiency, high phosphorus and high light intensity.

8. Manganese (Mn)

Source

Like iron, the oxide forms of Mn are common in soil but the more highly oxidised forms (manganous dioxide) are of very low availability to plants. Its solubility increases with increased acidity and in strongly acid soils, it is frequently present in toxic concentrations. **This might be one of the chief reasons for crop failure due to soil acidity.**

Absence of organic matter and poor drainage condition of soil cause unabailability of Mn in the soil. Sometimes, Oxidising bacteria in the soils may also cause Mn unavailable over the pH range of 6.5 to 7.8.

Physiological Roles

i. It acts as an activator of some repiratory enzymes like oxidases, Peroxidases, dehyrogenases, kinases, decarboxylases etc.

ii. It is essential in the formation of chlorophyll

iii. It decrease the solubility of iron by oxidation; in certain cases, abundance of Mn leads to Fe deficiency.

iv. It is necessary for the evolution of O2 during photosynthesis.

v. It is immobile in the plant tissues. When its deficiency occurs, it is not transferred to the younger leaves but accumulated in the older leaves only. As a result, deficiency symptoms develop first onyounger leaves.

9. Copper (Cu)

Source

Copper is found in smaller quantity in soils due to the additions of growing plants and its added residue. Organic matter, soil organism and pH are the important factors affecting the availability of copper. Soils neighboring the copper deposits are normally toxic to plants.

Physicological Roles

i It acts as a catalyst and regulator

ii. It is a constituent of several oxidizing enzymes like ascorbic oxidase, lactase, tyrosinase, phenoloxidase, plastocyanin etc.

iii. It is essential for photosynthesis, respiration and to maintain carbon/ nitrogen balance.

iv. Its higher concentration is toxic to plants.

v. It is **immobile** in the plant tissues.

10. Zinc (Zn)

Source

Like copper, it is also found in soils in very small quantities and largely it results from the concentration and addition from growing plants and added residue. Its uptake is reduced by large or prolonged supply of phosphate fertilizers. It is generally found to be toxic in the neighborhood of zinc deposits.

Physiological Role

i. It is a component of enzymes like carbonic anhydrase, alcohol dehyrogenase, glutamic dehydrogenase, lactic dehydrogenase, alkaline phosphatase and carboxy pepsidase.
ii. It is essential for the evolution and utilization of CO_2 , carbohydrate and phosphorus metabolism.
iii. It is also essential for the biosynthesis of the growth hormone, Indole-3-acetic acid (IAA)and also for the synthesis of RNA.
iv. It is readily mobile within the plant tissues.
v. It is closely involved in the chlorophyll formation.

11. Molybdenum (Mo)

Source

It is found widely distributed in small amounts in soils and plants and relatively higher concentration occurs in mineral oils and coal ashes.

Physiological Roles

i It is associated with the prosthetic group of enzyme, nitrate reductase and thus involved in nitrate metabolism.
ii. It acts as an activator of some dehydrogenases and phosphatases and as cofactors in synt thesis of ascorbic acid.
iii. It is necessary in the formation of nodules in legumes for the fixation of atmospheric nitrogen.

12. Boron (B)

Source

Boron occurs in rocks and marine sediments. It is absorbed in the form of borate ions and it has some sort of antagonistic effect with other cations like, calcium, potassium and others.

Physiological Roles

i. It is necessary for the translocation of sugars within the plant system
ii. It is involved in reproduction and germination of pollens (tube)
iii. It is concerned with water reations in cells and regulates intake of water into the cell
iv. It keeps Ca in soluble form within the plant and may act as a regulator of K ratios (K/Ca etc.)
v. It is also concerned with the nitrogen metabolism and with oxidation and reducation equilibrium in cells.
vi. It is **immobile** in the plant tissues.

13. Chlorine and others

Chlorine occurs in soils as chlorides and moves freely in soil solution and form which it is] available to the plant.

Chlorine increases the water content of tobacco cells; it affects carbohydrate metabolism and speeds up photosynthesis.

Cobalt is needed by the legumious crop in the absence of nitrogen. Because, it is required by the symbiotic bacteria for fixation of atmospheric nitrogen.

Elements like, aluminum (Al), silica (Si) and selenium (Se) possess stimulating effects of certain non-essential elements by counteracting the toxicity of certain elements present in soil. Elements like, Al and Si are called as **pallast elements.** Some plants are also accumulators. They can accumulate larger quantities of some elements like Al and Si. Silica is highly essential for the paddy crop and actively involved in its metabolism.

Nutritional Disorders in Crops

Low supply or completen absence of any of the essential elements will exhibit typical symptoms, which are specific to the particular elements(s). This condition is called as **nutrient deficiency** and the symptoms as **deficiency symptoms.**

In absence or low supply of elements (deficiency), following common symptoms generally develop in the plants.

General Deficiency Symptoms

1. **Chlorosis**
   It is a physiological disease that occurs due to deficiency of mineral elements.
   (eg. N,K.Mg,Fe,Zn, Mn,S). Leaves become abnormally yellow or white due to reduction of chlorophyll contents.

2. **Mottling**
   It is a condition of plant surface marked with coloured spots due to anthocyanin pigmentation (eg. due to deficiency of N,K,Mg,P,S)

3. **Necrosis**
   It refers to the patch of dead tissues, due to deficiency of K,Mg,Zn,Ca, Mo,Mn,Fe,or B.

4. **Bronzing**
   Development of bronze/copper colour on the leaves and various parts (eg. K)

5. **Die back**
   Collapse of growing tip, affecting the youngest leaves and buds (eg. K, Ca,B or Cu)

6. **Scorching**
   Burning of tissues accompanied by light brown colour. (eg. K,Ca,B or Cu)

7. **Firing**
   Burning of tissues accompanied with dark brown or reddish brown colour (eg. Mg)

8. **Rosetting**
   Clustering of leaves due to reduced leaf size and shorter internodal distance (eg. Cu,K,Zn)

9. **Distortion of leaves**
   Irregular shaping of leaves like cupping, twisting, hooking or curling and also wavy margins.

   Cupping : B or Mo

   Twisting / Hooking : Zn/Ca/B

   Curling : Ca,B or Zn

   Head distortion : Cu (rice), Mn(Sunflower)

   Wavy : Zn

10. **Gummosis**
    Oozing out of cell sap in the form of gummous nature (eg. Cu as in coconut etc.)

Occurrence of Deficiency Symptoms

Deficiency symptoms of various nutrient elements will appear either on older or on younger leaves depending on mobility of the nutrient. Thus, the relative mibility of the nutrient influences the site of appearance of the deficiency symptoms.

Deficiency symptoms of mobile elements will appear on the older leaves because, these elements will move rapidly from older leaves to younger leaves. eg. N,P,K, Mg, Zn.

On the other hand, the deficiency symptoms of the non-mobile elements will appear on the younge leaves because of their accumulation on the older leaves due to their immobile nature. eg. Ca,B,Cu,Mn,
Feand S.

Specific deficiency symptoms of various nutrient elements and their corrective measures are given below :

1. Nitrogen (N)

i. Plant growth is stunted and poorly developed (because protein content, cell division and cell enlargement are decreased)
ii. Nitrogen deficiency causes yellowing (chlorosis) of leaves. Older leaves are affected first
iii. Flowering and fruiting are reduced
iv. Protein and starch contents are decreased
v. Prolonged dormancy and early senescence appear
vi. Root gets more lengthened as in whear
vii. Veins turn purple or red due to development of abundant anthocyanin pigment (eg. tomato, apple)
viii. The angle between stem and leaves is reduced.
ix. Plants look so sickly and conspicuously pale that the condition is called as general starvation.
x. Symptoms first occur on the older leaves due to its mobility.

Corrective Measures

For correcting N deficiency, fertilizers like ammonium suphate, calcium nitrate, urea etc. are supplied. Foliar spray of 1-2% urea is a quick method of ameliorating N deficiency.

2. Phophorus (P)

i. Young plants remain stunted with dark blue green, or some times purplish leaves.
ii. P deficiency may cause premature leaf fall
iii. Dead necrotic areas are developed on leaves and fruits.
iv. Leaves somtimes develop anthocyanin in veins and may become necrotic; leaves will be dark green in colour.
v. Cambial activity is checked
vi. Tillering of crops is reduced
vii Dormancy is prolonged
viii. P deficiency may cause premature fall of leaves
ix. Growth is retarded
x. *Sickle leaf disease* is caused in P deficiency, which is characterised by chlorosis adjacent to main veins followed by leaf asymmetry.

Corrective Measures

Spray of 2% DAP or application of Phosphatic fertilizers will correct the deficiency.

3. Potassium (K)

The deficiency symptoms vary with the degree of shortage of the element.

i. In mild deficiency cases,
   a. thin shoots may develop and
   b. there may be restricted shoot growth

ii. In acute deficiency cases,
   a. shoots may die back, eventually plant may die
   b. Plants may become stunted with numerous tillers and
   c. there may be little or no flowering

iii. Leaf will be dull or bluish green in colour.

iv. Chlorosis occurs in interveinal regions **(interveinal chlorosis)**

v. In older leaves, browning of tips (tip burns), marginal scorching (leaf scorch or development of brown spots near the margins occur.

vi. Necrotic areas develop at the tip and margins of the leaf which curve downward.

vii. In broad leaved plants, shortening of internodes and poor root system are important.

viii Two diseases are common :

a. Rosette : In beet, celery, carrot, pea, potato and cereals, bushy growth or rosette condition develops due to K deficiency.

b. Die back : In acute deficiency cases, there is a loss of apical dominance and regeneration of lateral buds, which results in bushy growth. In prolonged cases, die back of laterals is also resulted.

Corrective Measures

Supply of muriate of potash or foliar spray of 1% potassium chloride is commonly used to overcome K deficiency.

4. Magnesium (Mg)

i. Mg deficiency causes interveinal chlorosis. The older leaves are affected first and proceeds systematically towards the younger leaves.

ii. Dead necrotic spots appear on the leaves.

iii. Severely affected leaves may wither and shed or absciss without the withering stage.

iv. Defoliation is quite severe

v. Carotene content is reduced.

vi. Stem becomes yellowish-green, often hard and woody.

vii. *Sand-drown diseas* is common in tobacco due to its deficiency, which is characterised by the loss of `colour at the tips of lower leaves and between the veins (interveinal). The veins remain green but in acute cases, entire leaf becomes nearly white.

Corrective Measures

Magnesium sulphate is usually applied for redressing the deficiency. The malady can be readily corrected as foliar spray @ 2% of $MgSO_4$.

5. Calcium (Ca)

Calcium ammonium nitrate (CAN) or superphosphate or gypsum is supplied in deficient soils. In Indian soils, Ca deficiency is not a serious problem.

6. Sulphur (S)

i. Sulphur deficiency causes yellowing (Chlorosis) of leaves. Young leaves are affected first.
ii. Tips and margins of leaves roll inward.
iii. Marked decrease in leaf size, general paling with red or purple pigmentation are general symptoms.
iv. Necrosis of young leaf tips develop
v. Internodes are shortened'
vi. Apical growth is inhibited and lateral buds develop prematurely
vii. Young leaves develop orange, red or purple pigments.
viii. Leaf tips are characteristically bent downwards. The leaf margins and tips roll inwards. (eg. tomato, tobacco and tea)
x. Fruit formation is suppressed.
xi. Sclerenchyma, xylem and collenchyma formation gets increased and hence the stem becomes un usually thick due to S deficiency.
xii. **Disease :** The **Tea Yellow disease** is caused in tea plants growing in sulphur dificient soils.

Corrective Measures

Common fertilizers used for supplying nitrogen and phosphorus contain appreciable amount of sulphur sufficient to meet the crop requirement. In case of severe deficiency, gypsum is added to the soil @ 500Kg/ha.

Micronutrients

7. Iron (Fe)

i. Interveinal chlorosis of the younger leaves occurs. The veins remain green.
ii. Leaf chlorosis may produce a mottled appearance.
iii. Leaf may show complete bleaching or often becoming necrotic.
iv. In extreme conditions, scorching of leaf margins and tips may occur
v. *Lime induced chlorosis is the common disease* found in fruit trees like citrus. It is also fund in beet, spinach, brassicas and cereals. The younger leaves become white or yellowish white.

Corrective Measures

Foliar spray of 0.5% ferrous sulphate along with lime (50% requirement) will remove the deficiency in the plant and soil. Chelated iron compounds such as Fe-EDTA, give a very good response in ameliorating Fe deficiency.

8. Mangabese (Mn)

i. Deficiency causes **interveinal chlorosis** and necrotic spots of the leaf.
ii. Dead tissue spots are found scattered over the leaf.
iii. Severely affected tissues turn brown, the brown areas may also twist in the form of spirals and they may wither also.
iv. Root system is often poorly developed and badly affected and the plants may die.
v. Grain formation is also reduced and the heads may be blind (as in sulphur)
vi. Four diseases are found due to its deficiency :

a. Grey Speck also called as grey stripe, grey spot or dry spot found in oats, barely, rye and maize is the common disease of Mn deficiency. Grey spots or chlorotic spots appear on the lower half of the leaf which fuse together and form elongated brown streaks, found mostly in third or fourth leaves.

b. Pahla blight of sugarcane
Chlorotic spots develop as long streaks, commonly in young leaves. These chlorontic spots fuse together and turn red and coalesce to form long streaks from which lamina may split.

C. Marsh spot of pea
Brown , black sport or cavities develop on the internal surface of cotyledons and thus the disease appears in the seeds.

d. Speckled yellow of sugar beet
It is characterised by interveinal chlorosis in the leaves and leaf margin may curl upward over the upper surface of leaf.

Corrective Measures

Foliar spray of 0.5% manganous sulphate plus 50% lime requirement is quite effective and it should be applied in the early stage of the crop. Soil application of 15-30 kg $MnSO_4$ per ha (mixed with sand)is sufficient.

9. Copper (Cu)

i. it causes necrosis of the tip of the young leaves.

ii. Both vegetative and reproductive growth are retarded.

iii. Wilting of terminal shoots occur which is followed by frequent death

iv. Leaf colour is often faded due to reduction of carotene and other pigments.

v. Foliage shows burning of margins or chlorosis or rosetting and multiple bud formation.

vi. Gumming may also occur **(gummosis)**

vii. Younger leaves wither and show marginal chlorosis (yellowish grey) of tips.
It is called as **Yellow tip or reclamation disease.**

viii. Following two diseases are common :
*a. **Exanthema or die back of fruit tree :*** It is commonly found in citrus, plum, apple and pear. The symptoms include formation of strong water-shoots bearing large leaves, gummous tissue or the bark and longitudinal breaks. Fruits become brown, glossy and splitted. Affected shoots loose their leaves and die back and lateral shoots produce bunchy appearance.

b. Reclamation disease : It is also called as **White Tip disease** and is found in legumes, cereals, oats and beet. The tips of leaves become chlorotic followed by a failure of the plants to set seed.

Corrective Measures

Foliar spray of 0.5% of $CuSO_4$ is recommended.

10. Zinc (Zn)

i. Older leaves show chlorosis which starts from tips and the margins

ii. Leaves become leathery

iii. Plants show rosetting due to shortening of internodes and premature shedding.

iv. Whitening of upper leaves in monocots and chlorosis of lower leaves in dicots are often found.

v. Leaf margins distorted, become twisted or wavy which later curl and look sickle shaped *(Sickle leaf)*

vi. Seed production and fruits size is greatly reduced.

vii. The following diseases are commonly notice :
a. Khaira of paddy : The entire older leaves show rusty brown appearance (due to chlorosis) and ultimately die.
b. White bud (tip) of maize : Unfolded newer leaves are often pale yellow to while.
There is appearance of light yellow streaks between the veins of older leaves followed by white necrotic spots.
c. Rosette of fruit trees : It is also called as little leaf disease. Yellow mottling of leaves, reduction of leaf size with rosette appearance (due to reduced internodal distance) and die back of the affected branches are symptoms of the disease.

d. Frenching of citrus : Initially, yellow spots develop between the veins. Leaves become progressively smaller and develop chlorophyll at the basal end of mid rib.

Corrective Measures

Foliar spray of 0.5% ZnSO4 twice at 7-10 days interval during early stages of growth will alleviate the problem. Also, soil application of 25 kg $ZnSO_4$ per ha is also found beneficial.

11. Molybdenum (Mo)

i. Deficiency causes chlorotic interveinal mottling of the older leaves.
ii. Leaves often show light yellow chlorosis and leaf blades fail to expand.
iii. In acute deficiency cases, necrosis of leaf tissues occurs.
iv. Flower formation is inhibited.
v. Failure of grain formation occurs (as in oats)
vi. Its deficiency causes two diseases :

a. Whiptail of Cauliflower and Brassica : The symptoms begin as appearance of translucent areas near the midrib which become ivory tinted or necrotic. The leaf margins become ragged with upward curling. Before the death of the growing point, the leaf elongates and lamina remains suppressed thus gives a typical whip tail condition.

b. Scald of legumes : The leaf shows paling, wilting, marginal rolling or scorching.

Corrective Measures

The Mo deficiency is commonly found in cauliflower, legumes, oats and other brassicas which can be corrected by soil application of 0.5 to1.0 Kg/ha sodium or ammonium molybdate or by its foliar spray@ 0.01-0.02% conc.

12. Boron (B)

i. It causes death of shoot tip
ii. Flower formation is suppressed.
iii. Root growth is stunted.
iv. Leaves become coppery in texture.
v. Plants become dwarf, stunted with apical meristem blacken and die followed by general breakdown of meristematic tissue.
vi. Terminal leaves become necrotic and shed prematurely
vii. Leaves show symptoms like distortion such as cupping and curling, appearance of white stripe, scorching, pimpling, splitted midrib and reduced growth.
viii. Stem shows symptoms like die-back of apex, abnormal tillering, appearance of various forms of deformities such as curling and brittle lesions, pimpling etc.
ix. Fruits are severely deformed and develop **typical cracking or splitting.**
x. Following *diseases* are commonly found due to B deficiency :
    a. Heart rot of sugar beet and marigold
    b. Canker and internal black spot of garden pea
    c. Browning of cauliflower
    d. Top sickness of tobacco
    f. Hard fruit of citrus.

Corrective Measures

Foliar spray of 0.2% borax acid will be effective for quick recovery. Liming of soil should be strictly avoided when boron-containing fertilizers are applied.

PHYSIOLOGICAL DISORDERS IN CROPS

Following physiological disorders are often observed in different crops. Symptoms of various disorders, causal factors and remedial measures for redressing different disorders are briefly discussed belwo.

A. MANGO

1. Softnose fruits
2. Malformation of inflorescence
3. Black tip of Fruits.

B. BANANA

1. Seediness **(Kottaivazhai)** in fruits
2. Goose flesh of fruits
3. Yellow pulp of fruits
4. Degrain of bunch
5. Finger drop

C. GRAPES

Coulure in grapes

D. COCONUT

Crown chocking

E. GUAVA

Fatio disease

F. CITRUS

1. Die-Back
2. June Drop
3. Fruit Cracking

G. PINE APPLE

Crook-Neck Disease

H. APPLE

1. Intenal Bark Necrosis (=Apple Measles)
2. Bitter Pit

Detailed description of various physiological disorders and their corrective measures are described in the following pages :

A. MANGO

1. Soft Nose Fruits

This disorder is caused due to **excess N** application of trees. In acid sandy soil, the incidence is about 7% in trees receiving lower N levels and increases to 78% on trees receiving 10 times more N fertilizer.

Remedy

Disorder reduced by maintaining **higher Ca level** in leaf (2.5%) by applying either **Calcium Nitrate or Gypsum or Lime Stone.**

2. Malformation in Inflorescence:

Causes :

Malformation disorder increased in trees receiving either P and K or P alone.

Remedy

Application of N,P and K in the ration of 9:3:3 reduces the incidence of floral malformation.

Remedy

Application of N,P and K in the ration of 9:3:3 reduces the incidence of floral malformation.

3. Black Tip of Fruits

In India, black tip or mango necrosis is common.

Symtoms

Yellow spots first appear at the distal end of fruits, later on turning to brown and finally black. Disorder usually occurs in orchards near brick kilns. There is astrong interaction between boron and fumes of brick kiln causing the dirorder.

Remedy

Corrected by foliar spray of 0.3% Borax

B. Banana

1. Seediness in Fruits

Causes

It is a developmental disorder and a predisposition to seediness. It is locally called as **"Kottaivazhai ".** The disorder is predominant in Poovan variety of banana.

Characterization of Disorder

Presence of distinctly conical and ill-filled fruits with a prominent central core having under-developed seedy structures rendering the fruits inedible. Characteristic features of the disorder are : Potential to reduce production by 10 to 25%

Genetically inherited.
Bunches held at an angle above horizontal position.
Peduncle shoed irregular curvatures.
Pollen is infertile, shriveled, shrunken & broken.
Pericarp much smaller than normal fruit.

Remedy

GA (50 ppm)+2,4-D (20ppm) sprayed immediately after last hand is opened reduces the incidence of the disorder.

2. Goose Flesh of Fruits

This physiological disorder affects banana peel and often observed in ripe fruits.
Affected fruits are wilted and shrivel in appearance and peels turns brown when fruits handled.

Causes

Disorder occurs more frequently during **dry winter months** when **atmospheric humidity is relatively low.** Therefore, moisture content of peel plays a vital role in causing the disorder. It is caused by disruption of subepidermal cellular structure due to partial dehydration of peel during ripening. Fruit ripening is characterized by pronounced conversion of starch to soluble carbohydrates. The reaction increases osmotic pressure considerably to draw moisture from peel to pulp.

3. Yellow Pulp of Fruits

Disorder is characterized by premature and abnormal development of immature fruits. Excess of K in relation of N is attributed to the disorder.

4. Degrain of Bunch

Symptom

It is a drooping of ripe banana fruits from bunch due to rotting of pedicels. Long stalks show increassaed incidence than shorter ones.

Remedy

Avoiding excess does of N would reduce the incidence.

5. Finger Drop

Related climatic conditions favoring excessive photosynthetic activity in post-flowering.

Remedy

Regulated N supply and extra does of K corrects the disorder.

C. GRAPES

Coulure Disorder : (French word-meaning shelling)

Physiological Disorder referring to pre-mature drop of bud, flower and/or berries Prominent in seedless grape varieties (Thompson seedless, Pusa seedless, Beauty seedless).

Factors Causing Coulure

1. Imbalance C/N ratio

The disorder is often associated with low C/N ratio and a ratio of less than6.0 causes the disorder.

2. Plant Growth Regulators

Growth regulators like Auxins, Cytokinins, CCC possess beneficial effect in reducing disorder.

3. Development Process and Nutrition

Causes : Some of the developmental processes such as poor pollination, improper fertilization, poor pollen germination, low fertility or ovule abortion.

4. Nutient deficiencies

Boron deficiency results in poor set and that of **Zinc deficiency** causes shattering of flowers.

5. Climatic Factors.

Temperature

A temperature level of 15.5^{0}C affects pollen germination, pollen tube growth leading to poor fruit set and causes flower abscission.

Lowlight :

Lowlight intensity results in poor carbohydrate synthesis through reduced rate of photosynthesis thus causing berry abscission (shading by excessive foliage or cloudy weather).

Rainfall

Heavy rainfall at full bloom dilute or washes away stigmatic fluid and results in poor pollen germination (areas with abundant rainfall often have coulure disorder in vine yard).

Relative Hudimity

High relative humidity is found to **decrease** coulure disorder

Water Stress

Persistent water stress at flowering hastens abscission of flowers. The water sress at first four weeks following bloom has marked decreasing effect on fruit set (effect seen through reduction in photosynthesis and cytokinins in xylem and increased ABA in leaves and stems).

Remedy

1. Optimum and timely operations like irrigation and nutrient application.
2. Application of FYM and mineral fertilizer combination (INM)
3. Judicious adoption of orchard management practices like training, girdling vine etc.
4. Spraying NAA, IBA etc. to prevent pre-harvest berry drop.
5. Spray of growth retardant like CCC to increase fruit set by arresting excessive vegetative growth.
6. Spray of sodium borate @ 0.01% or boric acid @0.2% or $ZnSO_4$ @ 0.5%

D. COCONUT

Crown Chocking

Malady was first observed in 1964 in Assam and then in West Bengal. Young palms of 5 to 10 years are mostly affected.

Symptoms

Typical symptom will be the emergence of shorter leaves with deformed and crinkled leaflets, associated with severe tip necrosis. Deformed leaflets fail to unfurl giving a chocked appearance to frond. In young palms, peripheral leaves crown around bud and prevent normal young palms, peripheral leaves crown around bud and prevent normal unfurling of flag leaf. In acute cases, necrosis of primordial tissues leads to death of crown(not suddenly).Affected palms loose vitality slowly and succumbs finally within 3-4 years.

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Tissue Testing

Leaf and soil analysis showed increased calcium content in affected palms while boron is reduced.

Remedy

Application of borax @ 50-100g/palm to basin

E. GUAVA

Fatio Disease

Symptoms

Affected leaves showed red spots and subsequently dried up. Branches develop cracks and finally die back.

Causes

Deficiencies of organic matter, N,Zn, and B are found responsible for causing the disorder.

F. CITRUS

1. Die-Back

Nature of the disorder is physiological since no organism found assoicated with it.

Symtoms

Whole plantation, individual trees or trees in patches are affected. Symptoms first appear when terminal leaves and shoots turn yellow different from mottling or yellowing of senescing leaves. Symptoms appear all at once in new shoots and fresh flushes. Tree may be apparently in perfect health; but, all of a sudden pale tender leaves but not maturing. Variegated leaves (with regular stripes of green and yellow alternating) are observed. The roots are shriveled and reduced innumber with appearance of die-back symptom. Disintegration rotting of internal structures of brancehes and roots is often seen. With symptoms rapid progressing, trees die in about 4 years.

Causes

Soil Factors

1. Soil containing excessive lime
2. Low soil organic matter
3. Poor soil aeration
4. Waterlogged soil conditions

Plant Factors

1. Growing dense cover crop]
2. Allowing too many young lateral shoots to develop

Climatic Factors

1. Cloudy and damp weather
2. Heavy rains at intervals

Remedy

1. Draining excess stagnating water by opening deep trench
2. Subsequent application of manures and fertilizers as recommended.
3. Application of ammonium sulphate (@ 3Kg/tree)at intervals of 3 months.
4. Application of FYM (50kg/tree)

2. June Drop in Citrus

Disorder of abnormal drop of flowers or fruits in orange groves in certain seasons. June Drop applied to heavy dropping period characteristic in American orchards.

Causes

1. Insufficient supply of N or moisture in soil
2. High wind velocity.
3. Low temperature.
4. Sudden variations in wealther
5. Excessive wate stagnation
6. Deep ploughing/ other tillage practices causing root damage at blooming or early fruit setting.
7. Pest and disease incidences.

Remedy

Foliar spray of 2,4-D @ 5-25 ppm alleviates the dirorder.

3. Fruit Cracking

Effect of irregular physiological changes in plant tissues caused by irregular watering and manuring. It is most prevalent in Mosambi varieties. Though considerable damage is caused by this trouble, severity is limited.

G. PINEAPPLE :

Crook-Neck Disease

It is caused by physiological disturbance to the plant due to Zn and Cu deficiencies.

Causes

Deficiency of copper has unfavourable influence on Zn absorption even with adequate soil Zn level.

Remedy

Foilar spray with 1% $ZnSO_4$ and spraying soil around the plant with 1.5-2.0% $CuSO_4$ avoiding trees would correct the disorder.

H. APPLE :

1. Internal Bark Necrosis (or Apple Measles or Rough Bark Disease).

Symtpoms : Elevation on epidermis, pimply lesions with inner bark necrosis, stunted growth, die-back of shoots and interveinal chlorosis of lower leaves and the symptoms of the disorder.

Causes

Increasing levels of K

Low Ca

Toxic levels of Mn

Lower conc. of B and P

Remedy

Regulated supply of K, DAP spray and application of recommended level of gypsum correct the disorder.

2. Bitter Pit

Causes

1. Warm and summer weather increases incidence
2. Improper supply of N
3. Low fruit Ca level
4. High K and Mg supply
5. High ratio of (K+Mg/Ca)

Remedy

Gypsum application, uniform supply of N in ammoniacal form during spring & nitrate form in summer and autumn.

Foliar Nutrition

Foliar nutrition is a useful method of applying supplemental macronutrients during citical growth periods when is it not possible or impractical to apply fertilizers to soil because of unseasonal periods of dry weather as in the case of monsoon season..

Usefulness of Foliar Nutrion

1. It ofers a remedy in which the time lag between soil application of fertilizers and plant absorption may be too long to satify the need of the plant.
2. It also serves as a means of applying macronutrients to crops when their soil application may not be vert effective or not feasible.

Limitations of Foliar Nutrition

1. Effectiveness of foliar nutrition depends on the ability of nutrients first to penetrate through the cuticle and the outer cell walls of epidermal cells.
2. Nutrients absorbed by leaves may be lost by leaching through rain, snow, dew, mist and fog.

CHAPTER 6

PHOTO-PHYSIOLOGICAL PROCESSES

The term photo-physiology refers to the study of the **physiological reactions as influenced by the light.** Light influences various physiological processes directly or indirectly like photosynthesis, flowering phenomena(photoperiodism), phototrophism, chlorophyll synthesis, seed germination, protoplasmic streaming etc.

Visible Spectrum of Light

- Sun is the ultimate and huge source of light energy. The earth intercepts very little amount of this energy.

- The so-called light is the visible part of the spectrum of electromagnetic radiation.
  ` It ranges from 380 to 760.

- A white light consists of violet (390-430nm), blue (430-470nm), blue green (470-500nm), green(500-560nm), yellow(560-600nm), orange (600-650nm), and red(650-76nm).

- But, there are several physiological processes, which are affected by 280 to 800 nm of which, the wavelength of light ranging from 400t o 700 nm is very efficient in increasing the rate of photosynthesis. This range of wavelength (400-700nm) of the visible spectrum of light is called as **Photosynthetically Active Radiation (PAR)**

- Light travels in the form of a stream of discrete particles calle **Photos,** which contain a packet of ` **energy** called as **Quantum.**

- It is considered that the longer wavelength of light will possess lesser energy while the shorter wave length will have greater energy level.

A Summary of light affected processes are given below :

S.No.	**Wavelength (nm)**	**Band**	**Process**
1.	280nm and below	xand γ	This region is lethal and is absorbed by nucleic acid cosmic rays.
2.	280 to 400	UV rays	It induces rosette growth and thick leaves in plants.
3.	400to 510	Blue	It actively influences photosynthesis and accelerates phototropism. It is blue light and is absorbed by carotenoids, flavoproteins, phytochrome etc.
4.	510to 610	Green	In this region, least amount of photosynthesis and (yellow) morphogenesis are found
5.	610to700	Orange & Red	This is the most active zone of light useful to plants inwhich the highest rate & amount of photosynthesis is observed. Pollen and seed germination and flowering are remarkably influenced. Pr form of phytochrome

			absorbs this light.
6.	700 to 800	Infra-red	This range of light is utilized by bacterio-chloro phyll to carry on bacterial photo-synthesis. Pfr form of phyto chrome absorbs this light. Elongation of internodes occurs.

Absorption and Utilization of Light Energy by Photosynthetic Pigments :

- Chief source of light energy for photosynthesis is sun

- The **earth receives only about 40%** (or about 5x1020 K.cal.) of the total solar energy. The rest is either **absorbed** by the atmosphere or **is scattered** into space.

- All the incident light energy falling on green parts of the plants is not absorbed and utilized by the pigments. Some of the incident light is **reflected,** some is **transmitted** through them while only a small portion is absorbed by the pigments.

- Photosynthetic pigments absorb light energy only in the visible part of the spectrum. However, certain photosynthetic bacteria use infrared light of comparatively shorter wavelengths.

- Only about 1% of the total solar energy received by the earth is absorbed by the pigments and is utilized in photosynthesis.

- There is very weak absorption and strong relection of light by the pigments in green part of the spectrum and **hence, the chloroplasts appear green in green plants.**

- All the pigments except shlorophyll a are called as accessory pigments. All the light energy absorbed by accessory pigments is tranferred to chlorophyll a molecule, which alone can take part in primary photo chemical reaction in photosynthesis. Chlorophyll a molecule also absorbs light energy directly.

Excited States of Atoms or Molecules, Fluorescence and Phosphorescence

Light rays consist if tiny particles called **photons.** The energy carried by a photon is called as **quantum.** Light rays of shorter wavelengths contain more energy per photon of light than the light rays of longer wavelengths. for example, one photon of blue light contains about 70K.cal. of energy while one photon of red light contains about 40k.cal. of energy only.

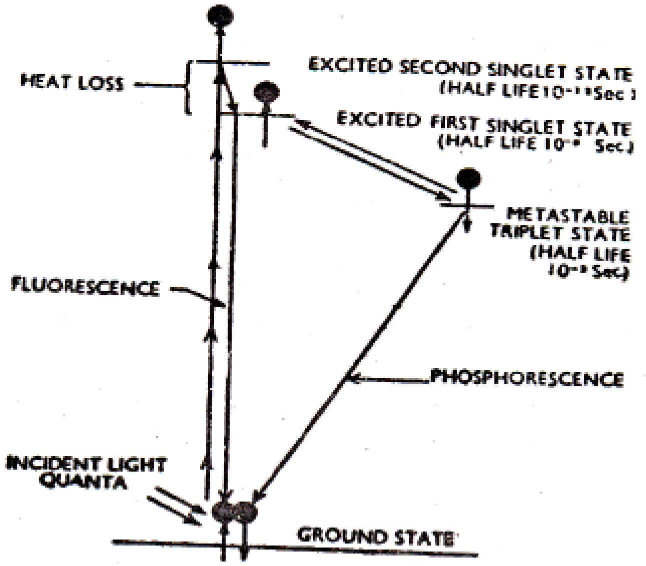


Fig-15 Diagram showing excitation of molecules of fluorescence or phosphorescence

The normal state of the molecules or atoms is called as ***ground state or singlet state***. When an electron of a molecule or atom absorbs a quantum of light, it is raised to a higher energy level, which is called as excited second singlet state. This is unstable and has a half-life of 10-^{12} seconds.

* The electron comes to the next higher energy level by the loss of some of its extra energy in the form of **heat.** This is called as excited **first singlet state** and is also unstable with a half-life of 10^{-9} second.

* From the first singlet state, the excited electron may return to the ground state in two ways.

a. either losing its remaining extra energy in the form heat, or

b. by losing extra energy in the form of radiant energy. The latter process is called as Fluorescenc(Fig.15). The substances, which show this property of **fluorescence,** emit florescent light only during the period they are exposed to incident light. Secondly, the fluorescent light is of higher wavelength than the incident light.

* The excited molecule or the atom may also lose its electronic excitation energy by internal conversion and comes to another excited state called as triplet state which is metastable with the half-life of 10^{-3} second.

* : From the triplet state, the excited molecule or the atom may return to the ground state in three ways

a. by losing its remaining extra energy in the form of heat.

b. by losing the extra energy in the form of radiant energy. This latter process is called as Phosphorescence. (Fig.15). The substances, which show this property of phosphorescence, emit phospho rescent light even after the incident radiant light is cut-off. Secondly, the phosphorescent light is of higher wavelength than the incicent light and fluorescent light.

c. the electron carrying the extra energy may be expelled from the molecule and is consumed in some further chemical reaction and a fresh normal electron returns to the molecule, or the extra energy is utilized in the reaction and the same electron which has now become excited and returns to the molecule. This is what exactly happens with the excited Triplet State of chlorophyll a molecule, which takes part inthe primary photochemical reaction in photosynthesis.

Significance of the Phenomenon of Fluorescence in Photosynthesis.

The phenomenon of fluorescence greatly helps in the understanding of the transfer of light energy in between the photosynthetic pigments. Some photosynthetic pigments absorb light rays of shorter wavelengths (with more energy) and while the others absorb longer wavelengths (with lesser energy). The pigments, which absorb light rays of shorter wavelengths, will emit fluorescent light (of higher wavelengths) which in turn will be absorbed by other pigments, which otherwise normally absorb light rays of higher wave lengths.

Quantum Requirement and Quantum Yield

The number of photons (or quanta) required to release one molecule of oxygen in photosynthesis is called as **Quantum Requirement (QR).** A minimum quantum requirement of 8-10 is required for the photosynthesis. On the other hand, the number of O2 molecule released per photon of light in photosynthesis is known as **Quantum Yield (QY).** The QY is **always less than one.** It is well known that the rate of photosynthesis is maximum in red light followed by blue band of the visible spectrum. Each photon of red light is known to contain about 40 k.cal of energy.

CHAPTER 7
PHOTOSYNTHESIS

Definition

The photosynthesis can be defined as the formation of carbon containing compounds from carbon dioxide and water with the help of green pigments and light while oxygen is liberated as the by-proudct.

The simples equation for photosynthesis can be :

$$6CO_2 + 6H_2O \xrightarrow[\text{Green Pigments}]{\text{Light}} C_2H1_2O_2 + 6O_2$$

Ruben and Kamen (1941) demonstrated that the **source of liberated O_2 is the water,** through their experiment with oxygen isotope, ^{18} O as giben belwo:

$$2H_2^{18}O + CO_2 \xrightarrow[\text{Light}]{\text{Green cells}} \underset{\text{Carbohydrates}}{(CH_2O) + H_2O + {}^{18}O_2}$$

Requirements of Photosynthesis

Thus, the **inputs or components** of photosynethesis will be :

1. Carbon di oxide
2. Water
3. Light
4. Green pigments in chloroplasts of cells

Output of Photosynthesis

1. Carbohydrates
2. Oxygen molecules

Process of Photosynthesis

Photosynthesis is an oxidation-reduction process in which water is oxidized and CO_2 is reduced to carbohydrate and water; and O_2 molecules are by-products. The reduction of CO_2 to carbohydrate level needs **assimilatory powers such as ATP and NADPH $_2$**. Reduction of CO_2 occurs in dark **(light-independent)** but the prouction of assimilatory powers takes place in the presence of light and the process is light-dependent.

Thus, **Photosynthesis** consists of **two phases :**

1. Light dependent phast (light reaction or Hill reaction) and

2. Light-independent phase (dark reaction or Blackman’s reaction).

Photosynthetic Apparatus

Simplest type of photosynthetic apparatus is observed in prokaryotic cells such as bacteria and blue-green algae in which they are represented by isolated and freely lying photosynthetic lamellae. In other algae, there is no distinction of grana and stroma regions. In these organisms, the photosynthetic lamellae are found closely arranged running parallel to each other; and carotenoid pigments dominate in the photosynthetic apparatus. These are called as Chromatophores. The pigments are evenly distributed in chromatophores and are primitive in nature.

In higher plants, well-developed photosynthetic apparatus is found which is commonly called as **Chloroplasts.** A choroplast is an advanced, well-organized and complicated photosynthetic apparatus.

Structure of Chloroplast

Each chloroplast is surrounded by a *double membrane* system. The external surface of the outer membrane is smooth whereas innner membrane is thrown into lamellated structure showing two distinct regions, called **Stroma and Grana Lamellae.**

Stroma

Stroma is the **main site** for the **dark reaction** of photosynthesis. It forms the matrix (ground substance) of the chloroplast. In the matrix, the lamellae are loosely arranged. The lamellae found in the stroma are called as **Stroma Lamellae.** Besides these structures, ribosomes and osmophilic granules are also found in the stroma.

Besides photosynthesis, lipid, protein and nucleic acid metabolisms are also found to take place in the stroma region of chloroplast.

Grana

Granum (= singular) is the most but complicated portion of chloroplast. Light reaction of photosynthesis takes place only in the grana region of chloroplasts. About 40-60 grana are present in each chloroplast.

In granum region, the lamellae are compactly arranged just like a stack of coins arranged one above the other back to back.

Grana lamellae form sac-like structures called **Thylakoids.** In higher plants, these thylakoids are closely packed together. Park and Beggins (1964,1967) observed some projections known as Thylakoid Bodies. On these thylakoid bodies, some specialised smaller and rounded particles are found which are named as **Quantasomes.** These quantasomes are known to contain chlorophyll molecules (**200-300** chlorophylls per quantasome) arranged in monolayer (single layer). Later on, these Quantasomes are referred as Photosynthetic Units.

Photosynthetic Pigments

There are **three types** of photosynthetic pigments, viz.,

1. Chlorophylls
2. Carotenoids
3. Phycobillins

Properties of photosynthetic pigments

* Chlorophylls and carotenoids are insoluble in water and can be extracted only with organic solvents like acetone, petroleum ether etc.
* Carotenoids include carotenes and xanthophylls (or carotenols)
* Different pigments absorb light of different wavelengths and show characteristic absorption peaks both *in vivo* and *in vitro* (refer Table below)
* They show fluorescence
* Chlorophylls and carotenoids are insoluble in water and can be extracted only with organic solvents.
* Phycobillins are soluble in water.

Distribution of Photosynthetic Pigments in Plant Kingdom

S.No.		**Pigment**	**Distribution in Plant Kingdom**
o1.	Chlorophylls :	Chlorophyll a	All photosynthesizing plants except bacteria.
		Chlorophyll b	Higher plants and green algae
		Chlorophyll c	Diatoms and brown algae.
		Chlorophyll d	In some red algae
		Chlorophyll e	*In Tribonema* and Zoospores of *Vaucheria*
		*Bacterio*Chlorophyll a	Purple and green bacteria
		*Bacterio*Chlorophyll b	In a strain of purple bacterium *Rhodopseudomonas, chlorobium*

		Chlorophyll (Bacterioviridin). Green baceria.
02.	<u>Carotenoids :</u> Carotenes	Mostly in algae and higher plants.
	Xanthophylls (Carotenols)	Mostly in algae and higher plants.
03.	Phycobillins : Phycoerythrins	In blue-green and red algae.
	Phycocyanins `	-do-
	Allophycocyanin	-do-

Structure of photosynthetic Pigments

Chlorophylls :

* They are **magnesium porphyrin** compounds

* The prophyrin ring consists of four **pyrrol rings** joined together by **CH** bridges.

* A long chain of C atoms called as **Phytol chain** is attached to the porphyrin ring.

* Molecular formulae :

Chlorophyll a : $C_{55} H_{72} O_5 N_4 Mg$

Chlorophyll b : $C_{55} H_{70} O_6 N_4 Mg$

* Both the chlorophylls (***a*** and ***b***) consist of :

Mg-Porphyrin head which is **hydrophilic** in nature and a

Phytol tail possessing **lipophilic** properly.

Difference between two chlorophylls

In chlorophyll b there is -CHO (aldehyde) group, instead of a _CH_3 (acetyl) group in chlorophyll a molecule.

Synthesis of Chlorophyll

* Chlorophyll is formed from protochlorophyll in the presence of light with the loss of two hydrogen atoms as follows :

$$\text{Protochlorophyll} \xrightarrow[-2H]{} \text{Chlorophyll}$$

Location of Chlorophyll Pigments in Chloroplast

The photosynthetic pigments are located in **grana portions of the chloroplasts** of higher plants. Chlorophyll molecules **form a monomolecular layer** between the alternative protein and lipid layers in grana lamellae (thylakoids). The **hydrophilic heads** of the chlorophyll molecules remain **embedded in the protein layer while the lipophylic phytol tails in the lipid layer.**

Absorption Spectra of chlorophylls

The chlorophyll pigments chiefly absorb in the violet-blue and red parts of the spectrum. The absorprtion band shown by the chlorophylls in violet-blue region is also called as **Soret band.**

In ether solution, chlorophyll a shows a maximum absorption peak at 663 nm in the red region and at 435nm in the violet region-while chlorophyll b has prominent absorption peak at 645nm and 453nm. The other absorption band for chlorophylla ***a*** could be 615, 578,533 and 410nm, and of chlorophyll ***b*** 595, 549 and 430nm.

Absorption Peaks of Different Chlorophylls

Type of Chlorophylls	**in vivo(nm)**	**in vivo(nm)**
Chlorophylls a	435,670,680 (several forms)	410,660
Chlorophylls b	480,650	452,642
Chlorophylls c	645	445,625
Chlorophylls d	740	450,690

in vivo : Within the living organisms or plants

in vitro : In glass vessels. This is ususally applied to biological processes when they are experimentally made to occur in isolation from the whole organisms.

* Carotenoids include carotenes and xanthophylls. The latter are also called as **carotenols**

* Different pigments absorb light of different wavelengths and show characteristic absorption peaks.

Photosynthetic Units

The photosynthetic units are defined as the **smalles group of chlorophyll pigment molecules, which are necessary for the photochemical reaction during photosynthesis.** They absorb and migrate the quantum of light to the trapping centre where they help in the release of electron.

Emerson and Arnold (1932) found that about **2500 chlorophyll molecules** are necessary for **fixing up one molecule of CO2 or for releasing one molecule of O2; they labelled this number as Chlorophyll Unit.** Subsequently, the name has been changed to **Photosynthetic Unit.**

Since the reduction or **fixation of one molecule of CO_2 requires about 10 quanta of light,** it is assumed that **10 flashes of light are required to yield one molecule of O_2** or for reduction of one CO_2 molecule. Therefore, each unit would now contain only 1/10 of 2500, i.e., 250 chlorophyll molecules. It is the number 200-300 chlorophyll molecules per photosynthetic unit, which is widely accepted and is now considered as Physiological Unit of Function.

Later, Park and Beggins (1964) found these photosynthetic units as distinct granular structures in the grana region of chloroplast and they named these structures as **Quantasomes, which are considered as the morphological expression of the physiological photosynthetic units.**

Quantasomes measure 180° A x 160° A and are 100 ° A thick. Park and Biggins (1964) revealed that **one quantasome contains about 230 chlorophyll molecules.** This is very close to the number of chlorophyll (200-300) contained in the **physiological photosynthetic units.**

MECHANISM OF PHOTOSYNTHESIS

The process of Photosynthesis is a complicated oxidation-reduction process ultimately resulting in the oxidation of water and reduction of CO_2 to carbohydrates.

The mechanism of photosynthesis can be studied with the following two processes :

I. *Primary Photochemical Reaction or Light reaction or Hill's reaction, and*
II. *Dark reaction or Blackman's reaction or Path of carbon in photosynthesis*

Importance of two processes photosynthesis

In the primary photochemical reaction, **assimilatory powers (NADPH2 + ATP)** are **generated** and **O_2 is released.** These assimilatory powers are utilized in the dark reaction during which process CO_2 is reduced to carbohydrates.

I. PIRMARY PHOTOCHEMICAL REACTION (OR) LIGHT REACTION (OR) HILL'S REACTION *(Activities found in **thylakoids or grana**)*

In 1937, Robert Hill demonstrated that isolated chloroplasts evolved O_2 when they were illuminated in the presence of suitable electron acceptor, such as ferricyanide. The ferricyanide is reduced to ferrocyanide by photolysis of water. This reaction is now called as **Hill Reaction.**

Photolysis of water or Hill Reaction (oxidation of water)

Splitting of water molecule into OH^- and H^+ ions in the presence of light is called as Photolysis of water. The OH^- ions unite to form water molecules again and release O^2 and electrons (e-). While, the H^+ is used in the formation of energy-rich molecules, NADPH2 during light reaction. It explains that **water is used as a source of electrons for CO_2 fixation and O_2 is evolved as a by-product.**

It is believed that photolysis of water invloves a strong oxidant, designated as **"Z".**

Primary photochemical reaction is **faster** than the dark reaction. It takes place only in the presence of light in the grana portions of the chloroplasts. It is complex process with several important events.

Primaty Photochemical Reactions can be studied under the following steps :

A. Red drop, Emerson Enhancement Effect and Two Pigment Systems :
i. Red Drop and Emerson Enhancement Effect (EEE)
ii. Two Pigment Systems.
B. Production of Assimilatory Powers
i. Electron Transport System in Photosynthesis or Reduction of NADP
ii. Photophosphorylation

They are <u>three kinds of Photophosphorylation</u>, viz.,

- i. *Cyclic Phtophosphorylation*
- ii. *Non-cyclic Photosphorylatioh, and*
- iii *Pseudocyclic Photophosphorylation*

C. Energy relationships and efficiency of photosynthesis
D. Interrelationship between light and dark reactions

A. Red drop, Emerson's Enhancement Effect (EEE) and Two Pigment Systems

Emerson and Lewis (1943) observed a sharp decrease in the quantum yield of photosynthesis at the wavelengths longer than 680nm. Beacuse this drecrease takes place in the red spectrum of the light, they called this drop as **Red Drop.**

However, Emerson and Chalmers (1951) found that this inefficient wavelength of 680 nm could be made to be fully efficient if supplemented with the light of shorter wavelengths. They also observed that the quantum yield from the combined beams of light (red and far red of shorter and longer wavelengths respectively) was found to be greater than the sum effect of both beams when used separately. This enhancement of rate of photosynthesis is called as **Emerson's Enhancement Effect.**

ii. Two Pigment Systems

With the discovery of Red Drop and Emerson Enhancement Effect, it is concluded that at least two pigment systems are involved in photosynthesis. These two pigment systems are known as **Photosytem I (PS I)** and **Photosytem II (PS II).** The wavelengths of light longer than 680 nm influence only PS I. While, wave lengths of light shorter than 680nm influence both the PS I and PS II.

Components of Photosystem I :

PS I complex consists of :

- * ~ 200 chlorophylls
- * ~ 50 carotenoids
- * one molecule of P 700
- * one cytochrome f(cyt.f)
- * cyt.b 563
- * **FRS** (Ferredoxin Reducing Substance)
- * one or two membrane bound ferredoxin molecules etc.
- * It is also rich in chl a, iron and copper
- * PS I controls the process of **producing a stron reductant to reduce NADP and $NADPH_2$, besides producing ATP molecules.**

Components of Photosystem II :

- * ~ 200 chlorophylls
- * ~ 50 carotenoids
- * a molecule of P 680
- * a primary electron acceptor Q
- * a Plastoquinone
- * four plastoquinone equivalents
- * four Mn molecules bound to one or more proteins
- * two cyt.b 559
- * one cyt.b6 and
- * Chloride
- * PS II is concerned with the **generation of strong oxidant and weak reductant** coupled with the **release of O_2.**

B. Production of Assimilatory Powers

Arnon (1956) used the term **assimilatory powers to refer ATP** (Adenosine TRi-Phosphate) **and NADPH2** (reduced Nicotinamide Adenine Dinucleotide Phosphate) molecules.

The process of reduction of NADP into NADPH2 may be denoted as **Electron Transport System (ETS)** in photosynthesis or Reduction of NADP. The process of formation ATP from ADP and inorganic phosphate (Pi) in light reaction of photosynthesis is called as **Photosynthetic Phosphorylation or Photophorylation.**

i. Electron Transport System (ETS) or Reduction of NADP

The ETS involves transport of electron from water and NADP to form NADPH2 . The H+ released during the process of photolysis of water reduces NADP to $NADPH_2$ during the process of non-cyclic photophosphorylation (see Fig.16).

When chl a molecule receives a photon of light, it becomes excited and expels and extra energy along with an electron in both the pigment systems (PS T & II). This electron, after travelling through a number of **electron carriers,** is either cycled back or consumed in reducing NADP (Nicotinamide Adenine Dinucleotide Phosphate) to $NADPH_2$. The extra light energy carried by the electron is utilized in the formation of ATP molecules at certain places during its transport.

ii. Photophosphorylation

Arnon has contributed a lot in the understanding of the electron transport and phosphorylation in chloroplasts.

They are of two types

1. Cyclic photophosphorylation
2. Non-cyclic photophosphorylation.

1. Cyclic Photophosphorylation

This is a pathway of ATP formation during light reaction of photosynthesis. **It involves only PS I and a wavelength of light greater than 680 nm.**

- * In cyclic photophosphorylation, activation of PS I by wave length greater than 680 nm (P700) causes electron to flow from P700 to ferredoxin to cyt-b6 which in turn passes back the electrons to P700 via cyt-f and plastocyanin.

* The synthesis of ATP in this electron transport is possible at two places, i.e., between ferredoxin and cyt-b6 and cyt-f, by means of phosphorylation of one ADP molecule to form one ATP molecule in each place.

* During cyclic photophosphorylation, **2ATP molecules are produced per electron transfer.** Since the **electron** which was **ejected from P700** in the above electron transport system is **cycled back,** the process is called as **Cyclic Electron Transport** and the accompanying phosphorylation as the **Cyclic Photophosphorylation** (Fig.16).

Significance of Cyclic Photophosphorylation

1. When cyclic photophosphorylation alone operates, the CO_2 **assimilation drops down** because of the shortage of reduced NADP ($NADPH_2$).
2. It generates **only ATP** molecules and as such **can not drive dark reactions** of photosythesis.
3. It is an **important system** in providing **ATP for synthetic processes** (other than photosynthesis)like synthesis of protein, lipids, nucleic acids and pigments within chloroplasts.

Limitations of Cyclic Photophosphorylation

1. This system operates if the activity of the PSII is blocked.
2. Under these conditions :

a. only PS I remains active
b. Photolysis of water does not take place
c. blockage of non-cylic ATP formation causes a drop in CO2 assimilation in dark reaction and, therefore,
d. there is a shortage of reduced NADP .(i.e., $NADPH_2$)

2. Non-cyclic Photophosphorylation

It occurs in green plants and **involves both PS I and PSII**

* During this process, the electron is excited by the absorption of a photon (quantum) of light by P700 form of chlorophyll ***a*** molecules in PS I.
* FRS traps this excited electron.
* The electron is now transferred to a **non-heme iron protein called ferredoxin (Fd)**.
* From Fd, the electron is transferred to NADP via the intermediate **protein electron carrier, ferredoxin-NADP-reductase** so that NADP is reduced to NADPH2 in the presence of H+ released from the reactions of photolysis of water.

Fig.17. Schematic representation of light induced electron transport in photosynthesis showing non-cyclic photo phosphorylation. Two pigment system, viz., PS PS II, Photolysis of water molecule and generation of assimilatory powers and I are indicated. (PQ: Plastoquinone; PC: Plastocyanin: FRS: Ferredoxin Reducing Substances and Fd; Ferredoxin).

* Now, when a photon (quantum) of light is absorbed by P 680 form of chlorophyll 'a" molecule in PSII, it gets excited and an electron is ejected from it so that an **electron deficiency or a 'hole" is left behind** in the P680 molecule.
* A compound of unknown identity traps this ejected electron as PQ (sometimes called as Q because it causes quenching of the characteristic **fluorescence** of chlorophyll **a in PS II**)
* From PQ the electron passes downhill along a series of compounds or **intermediate electron carriers** and is ultimately received by PS I where it **fills the "hole".**
* The series of compounds **(or electron carriers)** consists of :
    - i. Plastoquinone (PQ)
    - ii Cytochrome-b6
    - iii Cytochrome-f, a **copper containing Protein,** and
    - iv. Plastocyanin (PC)

*` During this electron transport, at one place, between cyt-b and cyt-f, phosphorylation of one molecule of ADP to form ATP molecule takes place(photophosphorylation).

* In the above scheme of Non-cyclic photophosphorylation, the electron ejected from PS II did not return toits place of origin; instead, it was taken by PSI. Similarly, the electron ejected from PS I did not cycle back and was consumed in reducing NADP. Therefore, this electron transport is called as Non-cyclic Photophos phorylation (Fig.17). However, Non-cyclic Photophosphorylation is inhibited by the chemicals like, CMU (3-(4'-dichlorophenyl)-1, 1-dimethyl urea)). This entire process is called as Hill Reaction or Light Reaction.With the production of ATP and NADPH2, the plant is now ready to reduce CO2 to form the carbohydrates. This is also called as 'Z' Scheme of Photophosphorylation.

Significance of Hill Reaction or Light Reaction

1. Light energy has been converted into chemical energy during primary photochemical reaction and is trapped in ATP and NADPH2 molecules.
2. This chemical energy is finally stored in carbohydrate molecules when ATP and $NADPH_2$ (assimilatory powers) are utilized in dark reaction of photosynthesis in reducing CO_2 to carbohydrates.

Differences between Cyclic and Non-cyclic photophosphorylation :

S.No.	**Cyclic photophosphorylation**	**Non-cyclic Photophosphorylation**
1.	Associated with PS I	Associated with both PS I and PSII
2.	Electron expelled from chl molecule is cycled back.	Electron expelled from chl molecule is not cycled back. Its loss is compensated by electron coming from photolysis of water.
3.	Photolysis of water and O2 evoluation do not take place	Photolysis of water and O2 evolution take place.
4.	Phosphorylation takes place at two places	Phosphorylation takes place only at one place.
5.	Two ATP molecules are produced.	one ATP and one $NADPH_2$ are produced.
6.	NADP is not reduced	NADP is reduced to $NADPH_2$ and used in assimilation of CO_2.
7.	This system is found dominantly in photosynthetic bacteria.	This is dominant in green plants
8.	The process is not inhibited by DCMU/CMU	The process is stopped by use of DCMU or CMU.

3. Pseudocyclic Photophosphorylation

Amon et al.(1954) demonstrated yet another kind of phosphorylation. They observed that:

1. In the absence of CO_2 and NADP, it can produce ATP from ADP and PI in the presence of FMN (Flavine Mono Nucleotide) or vitamin K and O_2.
2. The process is very simple.
3. It requires no chemical change except for the formation of ATP and water.
4. This is also called as **Oxygen dependent FMN catalysed photophosphorylation or pseudocyclic photophosphorylation** involving reduction of FMN with the production of O_2.

II. **PATH OF CARBON IN PHOTOSYNTHESIS (OR) DARK REACTION (OR) BLACKMAN'S REACTION** ***(Activities found in stroma).***

The dark reaction of photosynthesis is purely enzymatic and slower than the primary photochemical reaction. It takes place in stroma portion of chloroplast and is independent of light, i.e., it can occur either in presence or absence of light. Main credit for investigating the sequences of dark reaction in photosynthesis goes to Melvin Calvin who was awarded Nobel Prize in 1961. A.A.Benson, J. Bassham and other co-workers have also contributed a lot.

By employing labelled 14C in photosynthesis, Calvin and his co-workers were able to formulate the complete metabolic path of carbon assimilation in the form of a cycle, which is called as Calvin Cycle. They observed that carbon reduction is a cyclic process and the first stable product is the 3-C compound, i.e., 3-phosphoglyceric acid. The first acceptor of labelled CO2 was found to be 5-carbon compound (ribulose 1,5-diphosphate; RuDP). The entire process of photosynthesis runs in a cyclic fashion, which includes formation of hexose or starch and regeneration of RuDP.

Different Pathway of Photosynthesis or Alternate Choices of Pathways of Dark Reactions of Photosynthesis.

They are more than one pathway of dark reactions of CO_2fixation leading to the synthesis of carbohydrate. Following are the three types of the pathways, which are well eatablished now.

1. Calvin cycle (C3 plants)

2. Hatch and Slack cycle (C4 plants)
3. CAM cycle (CAM plants)

1. C3 CYCLE (or) CALVINCYCLE

This dark reaction process of photosynthesis has been named variously such as -Calvin Cycle, Bassham and Calvin Cycle, Bassham and Calvin Cycle, Blackman reaction, Carbon assimilation, Path of carbon in photosynthesis, Reductive Pentose Phosphate Cycle.

Calvin cycle consists of two important steps :

i. Synthesis of carbohydrate
ii. Regeneration of RuDP

Sequences of reaction taking place in Calvin (C_3) cycle are furnished below :

i. Synthesis of Carbohydrate

1. CO_2 is first accepted by RuDP and forms an unstable 6-carbon compound from which two molecules of phosphoglyceric acid (PGA) are formed. The reaction is regulated by enzyme called carboxydismutase or RuDP carboxylase **(Rubisco)**.
2. The phosphoglyceraldehyde molecule is converted into dihydorxyacetone phosphate in presence of enzyme triose phosphate isomerase.
3. The phosphoglyceraldehyde molecule is converted into dihydorxyacetone phosphate in presence of enzyme triose phosphate isomerase.
4. Phosphoglyderaldehyde and dihydorxyacetone phosphate (one molecule each) unite to form fructose-1,6-diphosphate with the regulation of aldolase enzyme.
5. From fructose-1,6-diphosphate, different types of compounds are synthesized converted into glucose or starch.

In this Calvin Cycle, only one molecule of CO_2 is utilized at a time; therefore, the cycle has to run for 6 times totally, for carbohydrate synthesis using CO_2, and H_2O. Hence, regeneration of RuDP is essential to carry on the processes of photosynthesis.

ii. Regeneration of Ribulose diphosphate

Regeneration of ribulose diphosphate is essential to carry on the process of photosynthesis. The sequential reactions leading to the regeneration of RuDP would be as follows:

1. The so formed fructose-6-phosphate and phosphoglyceral dehyde combine and break into4-carbon compound (erythrose-4-phosphate) and 5-carbon compound (xylulose-5-phosphate) in presence of enzyme transketolase.
2. Erythrose-4-phosphate combines with a molecule of dihydorxyacetone phosphate to form sedoheptulose-17-diphosphate in presence of enzyme aldolase.
3. From sedoheptulose-1,7-diphosphate, one phosphate is removed in presence of enzyme phosphatase to form sedoheptulose-7- diphosphate.
4. Sedoheptulose-7-diphosphate and phosphoglyceraldehyde combine in presence of transketolase and produce one molecule each of xylulose-5-phosphate and ribose-5-phosphate.
5. Both these compounds convert into ribulose-5-phosphate in presence of enzyme phosphopentose isomerase. Thus, ribulose-5-phosphate is formed.
6. Ribulose-5-phosphate is converted into ribulose-1,5-diphosphate mediated by phosphopentokinase utilising ATP molecule coming from photophosphorylation. The ATP is converted into ADP. Schematic representation of the Calvin (C_3) cycle is shown in Fig.18.

Fig.18. Simplified schematic representation of Calvin (C_3) cycle

Three Phases of Calvin Cycle

It is evident that Calvin Cycle consists of the following three phases, viz.,

i. *Carboxylative phase* (Reaction No.1 of Carbohydrate synthesis)
ii. *Reductive phase* (Reaction Nos.2-5 of Carbohydrate synthesis)
iii. *Regenerative phase* (Reaction Nos.1-6 of Regeneration of RuDP)

Enzymes involved in Calvin Cycle

In the first carboxylative phase, only one enzyme, viz., *carboxydismutase* is invloved which catalyses the carboxylation of ribulose 1,5-diphosphate to form PGA.

In the second reductive phase, three enzymes are involved for reducing PGA to triose phosphate and they are :

i. Phosphoglyceryl kinase
ii. Triose phosphate dehydrogenase, and
iii. Triose phosphte isomerase.

In the regenerative phase, seven enzymes participate and they are :

i. Aldolase
ii. Phosphatase
iii. Transketolase
iv. Transaldolase
v. Phosphoribose isomerase
vi. Phosphopentose epimerase, and
vii. Phosphorubulokinase.

Why named as C_3 cycle or C_3 Plant ?

Because **first visible product** of Calvin Cycle is **3-Phosphoglyceric acid (PGA),** Whichis **a 3-carbon compound,** Calvin cycle is popularly known as **C_3 Cycle.** The plants, which possess C_3 pathway or Calvin Cycle are called as **C_3 plants.** Most of the higher plants possess C_3 pathway for the fixatuib of CO_2 in photosynthesis, *Examples are* : rice, wheat, barley, oatt, rye, most of the pulses and oilseed crops etc.

2. CYCLE (or) HATCH AND SLACK CYCLE

For many years, the Calvin cycle as described earlier was thought to be the only photosynthetic reaction operating in higher plants. But, Kortschak etal. (1965) reported that 4-carbon compound, dicarboylic acids, malate and aspartate were the major labelled proudcts when sugarcane leaves were to photosynthesize for shorter periods using $^{14}CO_2$. Later on, Hatch and Slack (1966) of Australia also confirmed this in several plants. They have proposed an alternate pathway, which is called as **C_4 Cycle** or **Hatch-Slack Pathway or Di-carboxylic Acid Cylce** (C_4), because 4-carbon containing di carboxylic acids are the earliest products after carbosylation in this pathway.

Examples of C4 Plants

Examples of C4 plants are : sugarcane, maize, sorghum, pearl millet, Amarantus, *panicum maximum, Chloris, Atriplex, Digitaria and Cyperus.*The C_4 cycke has also been reported in some members of the families, *Cyperaceae, and certain dicots belonging to Amaranthaceae, Chenopodiaceae, Compositae, Euphorbiaceae, Portulacaceae.*

Structural Peculiarities of C_4 Plants:

1. Presence of bundle sheath cells containing chloroplasts.
2. Bundle sheath cells are radially arranged around a vascular bundle.
3. Bundle sheath cells lack grana in their chloroplasts.
4. Bundle sheath cells are arranged in one or more layers consisting of large thick-walled cylindrical cells, around vascular bundle, a characteristic feature of C_4 plants.
5. Bundle sheath cells remain surrounded by one or more wreath like layers of mesophyll cells. This anatomical arrangement is called as Kranz type (Kranz=wreath, a German term). (Kranz: Wreath of mesophyll cells around bundle sheath cells)
6. Mesophyll cells have well-developed grana.
   (Thus, C_4 pathway constitutes an example of ***dimorphism of chloroplasts)***
   But, some C_4 plants (bermuda grass)have grana in chloroplasts of bundle sheath cells).
7. The ratio of PS I : PS II activity is three times higher than the bundle sheath cells.
8. The mesophyll cells are almost three times more active in non-cyckuc electron transport system than that of bundle sheath cells.
9. For cyclic electron transport, both the cells are equally efficient.
10. Clear cut categorization of enzymes are found in C_4 cycle.
    Most of the PEP carbocylase occurs in mesophyll cells. While most of ribulose-1,5-diphosphate carboxylase and malic enzymes are found in bundle sheath cells.

The C_4 cycle is also referred as the **dicarboxylic acid cycle** or the **β -Carboxylation pathway** or **Hatch-Slack cycle** or **Co-operative Photosynthesis** (Karpilov, 1970).
In this Cycle, the characteristic point is the primary carboxylation reaction and the **phosphoenolpyruvate (PEP)** is found to be **CO_2 acceptor molecule.**

Site of Occurrence of C_4 Pathway

The C_4 pathway is known to operate in two types of cells :
i. Chloroplasts of mesophyll cells.
ii. Chloroplasts of bundle sheath cells.

The Hatch-Slack (C_4) pathway is schematically represented in Fig.19.

i. Reactions in the chloroplasts of mesophyll cells

1. Here, CO_2 is reduced by the carboxylation of PEP to form oxaloacetate (OAA). This reaction requires amolecule of water and releases a molecule of phosphoric acid as by-product. The enzyme, phosphoenolypyruvate carboxylase (PEPCase) mediates this carboxylation reaction.

2. OAA is readily converted into malate or aspartate depending upon species. Malate is derived from OAA by reduction with NADPH2 in the presence of the enzyme, malic dehydrogenase.

Malate is then transported to the chloroplasts of bundle sheath cells.

ii. Reactions in the chloroplasts of bundle sheath cells

1. Here, Malate is decarboxylated by NADP specific malate enzyme to produce pyruvate and CO_2. This CO_2 is used in the C_3 cycle for carboxylation of ribulose-1,5-diphosphate in the presence of enzyme, RuBP carboxylase (RuBP Case) and produces phosphoglycerate, the first stable product of Calvin Cycle(C_3) of photosynthesis,
2. It follows Calvin Cycle in further steps to produce starch and to regenerate ribulose 1,5-diphosphate.
3. Simultaneously, Pyruvate is transported back to the chloroplast of mesophyll cells, where it is reconverted into the phosphoenolypyruvate by utilizing ATP generated during light phase in the presence of enzyme pyruvate phosphate dikinase. The ATP is converted into AMP. Since the conversion results in the form of AMP, the requirement to regenerate ATP from AMP is **2 ATP**. This is how 12 additional ATP molecules are needed in the C_4 pathway.

Phyiological significance of C_4 plants

1. Presence of C_4 pathway offers adaptation mechanism to xerophytic plants.
2. Plant can photosynthesize even with very low concentration of CO_2 (up to 10ppm) in the atmosphere.
3. Therefore, partial closure of stomata (due to xerophytic conditions) would not affect photosynthesis much. Thus, the plants can adapt to grow at low water content, high temperature and bright light intensities. Hence, this cycle is best suited to the crops grown in dry climates in both tropics and subtropics.
4. In C_4 pathway, photosrespiration is absent; hence, photosynthetic rate remains higher.
5. C_4 plants are about twice as efficient as C_3 plants in converting solar energy into carbohydrates or dry matter.

Categories of C_4 plants

Chollet and Ogren (1975) recognized three types of C_4 plants. They are :

a. In the first category, CO_2 is initially fixed by phosphoenolpyruvate and oxaloacetate is formed. ` The malate produced from it will be transported to bundle sheath cells. Example : Sugarcane and maize.
b. Second group includes plants such as *Panicum maximum and Chloris gayana,* in which case it is aspartate, rather than malate, transported to bundle sheath cells. There it is transmitted to oxaloacetate, which is converted into pyruvate and CO_2 by PEP carboxykinase.
c. In third case, the aspartate produced in mesophyll cells is transported to bundle sheath cells where it is transmitted and reduced to malate. The malate is then decarboxylated to form pyruvate and CO_2 . The example includes *Atriplex spongiosa.*

Differences between C_3 and C_4 plants :

S.No	**C_3 Plants (Calvin Cycle)**	**C_4 Plants (Hatch-Slack Cycle)**
01.	Calvin cycle is found in all	C_4 cycle is found only in certain tropical plants.
O2.	Efficiency of CO_2 absorption at low conc. is far less. So, they are less efficient.	Efficiency of CO_2 absorpition from low conc. is quite high. So, they are more efficient plants.
03.	The CO_2 acceptor is Ribulose-1,5 diphosphate.	The CO_2 acceptor is phosphoenolpyruvate.
04.	The first stable product is Phospho-glyceric acid.	Oxaloacetate is the first stable product.
05.	All cells participating in photosynthesis have one type of chloroplast *(monomorphic type).*	The chloroplast of parenchymatous bundle sheath is different from that mesophyll cells *(dimorphic type*); The chloroplast in bundle sheath cell are centripetally arranged and lack grana. Leaves show *Kranz* anatomy.
06.	In each chloroplast, two pigment systems ()PS I abd II) are present.	In the chloroplasts of bundle sheath cells, the PS II is absent. Therefore, these are dependent on mesophyll chloroplasts for the supply of assimilatory powers ($NADPH_2$).
07.	The Calvin cycle enzymes are present in mesophyll chloroplasts. Calvin cycle occurs.	Calvin cycle enzymes are absent in the mesophyll chloroplasts. The cycle occurs only in the chloroplasts of bundle sheath cells.
08.	CO_2 compensation point is 50-150ppm CO_2 .	CO_2 compensation point is 0-10 ppm CO_2.
09.	Photorespiration is present and easily detectable.	Photorespiration is present only to a slight degree or absent.
10.	The CO_2 conc. inside leaf remains high (about 200 ppm).	The CO_2 conc. inside the leaf remains low (100ppm).
11.	The $^{13}C/^{12}C$ ratio in C-Containing compounds remains relatively low (both ^{13}C and ^{12}C are present in air.	The ratio is relatively high., C_4 plants are more enriched with ^{13}C than C_3 plants.
12.	Net rate of photosynthesis in full sunlight (10,000-12,000fc.) is 15-25mgof CO_2 dm^2 of leaf area per hl.	It is 40-80 mg of CO_2 dm^2 of leaf area per h. Thus, photosynthetic rate is quite high. The plants are efficient.
13.	The saturation intensity reaches in the range of 1000 to 4000fc.	It is difficult to reach saturation even in full sun light.
14.	Bundle sheath cells are unspecialised.	The bundle sheath cells are highly developed with unusual construction of organelles.
15.	Only C_3 cycle is found.	Both C_4 and C_3 cycles are found.
16.	The optimum temperature for the process is 10-25^{0}C.	In these plants, it is 30-45^{0}C. So, these are warm climate plants. At this temperature, the rate of photosynthesis is double than that in C_3 plants.
17.	O_2 present in air (=21%) markedly inhibits the photosynthetic process as compared to an external atmosphere containing no $O_{2.}$	The process of photosynthesis i s not inhibited in air as compared to an external atmosphere with no O_2.

18.	For synthesis of one glucose molecule, **18 ATP** are required.	In this process, **30 ATPs** are required for the synthesis of one glucose molecule.

3.Crassulacean Acid Metabolism (CAM cycle)

Under natural conditions, the acidity of green shoots of some non-halophytic succulents and semi-succulent plants increases at night and decreases during day time. This diurnal change in the acidity was first discovered in *Bryophyllum* belonging to the family Crassulaceae. Therefore, it is called as **Crassulacean Acid Metabolism (CAM)**. This metabolism occurs only in green organs and it is quite common in the plants belonging to the families like Crassulaceae, Cactaceae, Orchidaceae, Bromeliaceae, Liliaceae, Asclepiadaceae, Vitaceae and Euphorbiaceae. All such plants are called as Crassulacean Acid Metabolism plants(CAM plants). Most CAM plants possess the succulent habit. Typical example of commercial crop possessing CAM pathway is **pineapple**.

Mechanism of CAM cycle

Sequence of reaction taking place in CAM pathway is shown in Fig.20.

1. During night when stomata are open, CO2 is fixed through the action of PEP carboxylase to Malic acid. This is accomplised in two steps :
   a. PEP fixes CO_2 and is converted into oxaloacetic acid (OAA)
   b. OAA is subsequently converted into malic acid by malic dehydrogenase enzyme.

2. During light (day time), the malic acid releases one CO_2 molecule with the formation of PEP. This PEP is ultimately converted into sugars or starch.
3. The leaves of CAM plants also contain enzymes of Calvin cycle. Therefore, the released CO_2 combines with PGA and completes C_3 cycle in the light.

   Thus, two cycles (CAM and Calvin cycle) occur in the mesophyll cells and there is no differentiation between the type of cells as found in C_4 plants.

Chemosynthesis

There are certain aerobic bacteria, which do not have chlorophyll but can synthesise organic food from CO2 and H20. This process of manufacture of food materials by bacteria making use of the chemical energy is called as Chemosynthesis. Some of the common examples of **Chemosynthetic** bacteria are given below :

1. Nitrifying Bacteria

Nitrosomonas and Nitrosococcus oxidise ammonia to nitrite and chemical energy is released.

$$2NH_3 + 3O_2 \rightarrow 2HNO_2 + 2H_2O + \text{Chemical energy} + 2S$$

2. Sulphur Bacteria

Beggiatoa and *Thiothrix oxidize* hydrogen suphide to sulphur and release sufficient amount of chemical energy for subsequent food synthesis.

$$4FeSO_4 + O_2 + 6H_2O \rightarrow 4Fe(OH)_3 + 4C_2O + \text{Chemical energy.}$$

Factors Influencing Rate of Photosynthesis

The factors influencing rate of photosynthesis can be classified into two categories, the internal and external (environmental)

A. Internal Factors

1. Chlorophyll

The amount of chlorophyll present has a direct relationship with the rate of photosynthesis, since, it is the pigment, which is photoreceptive and is directly involved in trapping the light energy.

2. Photosynthetic Enzyme Systems

The amount and nature ofenzymes play a direct role on the rate of photosynthesis. Greater enzyme activity at higher light intensity increases the capacity of the leaf to absorb more light and thus increases the photosynthetic rate.

3. Leaf Resistance

Photosynthesis shows close dependence upon leaf resistance. For C4 plants, leaf resistance (primarily controlled by stomatal aperture) appears to regulate photosynthesis, but in C_3 plants, the internal resistances including carboxylation efficiency offer greater limitation to CO_2 fixation than stomatal resistance. Environmental factors such as light intensity, photoperiod, CO_2 concentration,humidity and soil moisture also affect photosynthesis via stomatal resistance.

4. Demand for Photosyntate

Because of the greater demand, the rapidly growing plants show increased rate of photosynthesis in comparison to the mature plants. However, if the demand for photosynthesis is lowered by removal of meristem, then the photosynthetic rate declines.

5. Leaf Age

The Photosynthetic rate is higher in the newly expanding leaves and reaches a maximum as the leaves achieve full size. The rate declines as the leaf ages due to reduced chloroplast functions and other anabolic reactions.

6. Role of Hormones

Photosynthesis may be regulated by some of the plant hormones. Gibberellic acid (GA) and Cytokinins (CK) increase both carboxylating activity and photosynthetic rate.

7. Genetic Control

Genetic contronl also plays a very important role on both CO_2 fixing systems and the CO_2 transport system of the leaf. The fixation of CO_2 is the function of set of enzyme present in the chloroplast, which are in turn under the control of genes of the chloroplasts.

B. External Factors

1. Carbon dioxide

CO_2 is one of the raw materials for photosynthesis; therefore, its concentration affects the rate of photosynthesis markedly. Rate of photosynthesis increases with the increase in the atmospheric CO2 concentration up to a certain extent. Because of its very low concentration in atmosphere (current level of 350-360ppm), it acts as a limiting factor in natural photosynthesis. Rate of photosynthesis increases with increase in the atm. CO_2 level of upto 1000ppm beyond which, there is a general decline in photosynthesis. At this enhanced level of CO_2, the increase in the photosynthetic rate may be 10 to 30 times more than the normal CO_2 level.

2. Light

Light affects the rate of photosynthesis in several ways. In general, photosynthesis can occur under artificial lights of sufficient intensity. Role of light on photosynthesis can be discussed under the following sub-heads:

a. Intensity of light :

With the increase in light intensity, the rate of photosynthesis increases, i.e., the rate of photosynthesis is directly proportional to light intensity. However, at stronger light intensity, increase in rate of photosynthesis is not proportional to light intensity. Except on cloudy days, light is never a limiting factor in nature.

At certain light intensity, the amount of CO_2 used in photosynthesis and the amount of CO_2 produced in respiration are volumetrically equal. This point of light intensity is known as Light Compensation Point. Light compensation point is frequently in the order of 100 to 200 f.c. for sunloving leaves; while, the value is 100f.c. for shade-loving leaves. Thus, in shade-loving plants the compensation point lasts for a much shorter period than in sun-loving plants.

b. Wavelength of light :

For photosynthesis, the visible range of spectrum of light (PAR:400 to 700 nm) is essential. Maximum photosynthesis is known to occur in the red part of the spectrum with the next peak in blue part and minimum in the green region (RED >BLUE > GREEN) The region between 575 and 750nm (yellow to red) is quite congenial for photosynthesis. Ultra violet light has a lethal effect on plants if exposure is for a prolonged period.

C. Duration of light :

Photosynthesis may be sustained for relatively long periods of time without any noticeable damaging effect on plants.

d. Photo-oxidation :

When the light intensity for photosynthesizing tissue is increased beyond a certain limit, the cells become vulnerable to chlorophyll photo-oxidation; due to this, many more chlorophyll molecules become excited than can possibly be utilized. This causes damaging effect to the chloroplast membrane system. In presence of O_2, the damaging effect of photo-oxidation is severe. It results in bleaching of chlorophyll and inactivation of some important enzyme involved in photosynthesis.

3. Temperature

Effect to temperature on photosynthesis is little than on other process. Very high and very low temperatures affect the photosynthetic rate adversely. The rate of photosynthesis increases with rise in temperature from 5 to 35^0C; beyond which, there is a rapid fall in photosynthesis. In the optimum range of temperature, the Temperature Quotient (Q_{10}) is found to be 2.0 for the rate of photosynthesis (Q_{10}=2.0).

4. Water

Water is one of the raw materials in photosynthesis. It has an indirect effect on the rate of photosynthesis. Water availability affects the water relation of plant, thus affecting the rate of photosynthesis. In scarcity of water, cells become flaccid. Depending upon the availability of water, the rate of photosynthesis may be decreased from 10 to 90%.

5. Oxygen

Oxygen is the by-product of photosynthesis. Accumulation of greater amount of oxygen molecules causes substantial inhibition of photosynthesis. Oxygen is also known to have a direct and competitive inhibition for RuBP carboxylase. As a result, glycolate synthesis is enhanced which leads to photorespiration.

6. Warburg's Effect

An increase in the concentraion of O_2 in many plants results in a decrease in the rate of photosynthesis. A German Scientist, Warburg first discovered this phenomenon of the inhibition of photosynthesis by the greater accumulation of O_2 in 1920 in green alga, Chlorella. It is now known to operate in soybean etc. (C_3 plant). But, plants like maize, sugarcane, sorghum etc. (C_4 plants) do not show the effect.

7. Mineral nutrients

Some nutrients like copper etc., which are components of photosynthetic enzymes or magnesium as component of chlorophylls also affect the rate of photosynthesis indirectly by affecting the synthesis of photosynthetic enzymes and chlorophyll, respectively. Potassium also affects the rate through the stomatal movement. Leaf N content plays a major role in inceasing the photosynthetic rate of crops.

Inhibitors of Photosynthetic Process

1. Several urea derivatives such as monuron (or CMU) and diuron (or DCMU) block electron transport between Q and PQ.
2. Simazine, atrazine, bromacil and isocil block the same step.
3. Diquat and paraquat are common photosynthetic inhibitors. These compounds (commonly referred as **viologen dyes**) accept electron from PS I before ferredoxin and produce toxic forms of O_2 (superoxide and hydroxy).

CHAPTER 8

PHOTORESPIRATION

Photorespiration is a special type of repiration shown by many green plants when they are exposed to light. The normal dark respiration (usual mitochondrial respiration) is independent of light and its rate is the same in both light and dark. The photorespiration process is carried on only in the presence of light. The term photorespiration is referred to **" the release of CO2 in respiration in presence of light during photosynthesis".**

Importance of Photorespiration

a. Photorespiration is closely related to CO_2 compensation point.
b. It usually occurs only in those plants, which have comparatively high CO_2 compensation point. (eg. Tomato, wheat, oats etc.)
c. It is insignificant or rather absent in C4 plants, which have very low CO2 compensation point] (eg. maize, sugarcane, sorghum, pearlmillet, amarantus etc.).

Sites of photorespiration

Photorespiration occurs only in chlorophyllous tissues of plants. The process of photorespiration occurs in three different organelles viz.,

1. Chloroplasts
2. Peroxisomes and
3. Mitochondria.

Substrates of Photorespiration

Glycolate (glycolic acid) is the chief substrate of photorespiration. Other important metabolites are the two amino acids such as glycine and serine.

Processes or Steps of Photorespiration

Like usual mitochondrial respiration, the photorespiration is also an oxidative process where oxidation of glycolate occurs with subsequent release of CO_2 **(post-illumination burst of CO_2)**. The process of photorespiration takes place **in three cell organelles viz., chloroplasts, peroxisomes and mitochondira.**

Various steps of the glycolate metabolism (synthesis of glycolate and its oxidation with subsequent release of CO2 (photorespiration) are given in Fig.21.

Fig.21 Digramatic representation of glycolate metabolism during photorespiration

A. Reaction in Chloroplast

1.1. Glycolate is synthesized as a side product from some intermediates of photosynthesis in **chloroplasts.** It is probably derived from 1-C and 2-C of the ketose sugar phosphates of the Calvin cycle.

1.2. It is believed that O_2 competes with CO2 for the enzyme RuBP carboxylase. When this enzyme reacts with O_2 instead of CO_2, it is called as **RuBP oxygenase**.

1.3 In the second case, one molecule of phosphoglyceric acid (PGA) and one molecule of phosphoglycolic acid are formed from RuBP.

1.4. PGA then enters into the Calvin cycle.

1.5 Phosphoglycolic acid is dephosphorylated to form **glycolate**, in the presence of the enzyme, phosphatase.

B. Reactions in Peroxisome

2.1 From chloroplasts, the glycolate migrates into peroxisome where it is oxidised (photorespired) to glyoxalate in the presence of glycolic acid oxidase. In this reaction, hydrogen peroxide (H_2O_2)is formed with the utilization of one molecule of O_2. The H_2O_2 is then removed by the enzyme catalase as follows:

$$H_2O_2 \rightarrow H_2O + 1/2O_2$$

2.2 Glyoxalate is converted into an amino acid, **glycine** by transmination reaction in the presence of L-Glutamate glyoxalate transaminase.

C. Reactions in Mitochondrion

3.1 Glycine formed in peroxisomes migrates into mitochondria

3.2 Now, two molecules of glycine react to form one molecule of another aminoacid, **Serine**, liberating **CO_2** <u>(post-illumination burst of Co2 and photorespiration)</u> and NH3. This reaction is catalyzed by serine hydroxymethyl transferase.

Serine thus formed is apparently recycled back into the pool of photosynthetic intermediates of Calvin cycle in chloroplasts. This is mediated by the formation of **hydroxypyruvate** and **glyceric acid**. On phosphorylation with ATP, glyceric acid is converted into **PGA**, which is the intermediate of Calvin cycle.

Therefore, starting from intermediates of Calvin cycle and with the synthesis of glycolate, serine is formed which is again converted into intermediates of Calvin cycle thus completing the **glycolate cycle**. The photorspiratory cycle is also called as **C_2 cycle**.

Why named as C2 Cycle?

Because glycolate and some other metabolites of this glycolate cycle, viz., glyoxalate and glycine are 2-C compounds, the glycolate metabolism or glycolate cycle is also called as **C_2 Cycle**.

Difference between two types of plants :

Characteristic	**With photorespiration (C_3)**	**Without Photorespiration (C_4)**
1. Enhancement of CO2 assimilation in light and low O_2	yes	No
2. CO_2 compensation point in light	15 to 150ppm	0ppm
3. CO_2 assimilation rate in intense light	15-35mg CO_2 /dm^2/h	50-70 mg CO_2 /dm^2/h
4. CO_2 evolution in light	High rate	Apparently NIL

5. Internal structure of leaf about vascular bundles.	Diffuse mesophyll	Mesophyll compact
6. Carbon pathway	Calvin cycle	Both Calvin cycle and Hatch & Slack pathway.
7. Carbonic anhydrase activity	High	Low
8. Translocation rate from leaf	Low	High
9. Temperature optimum for photosynthesis and growth.	Low to high	High

Significance of Photorespiration in Crop-Productivity

1. Often, presence of photorespiration is considered as a **wasteful** and **energy consuming** process in crop plants which ultimately leads to **reduction in final yield of crops.**
2. It is estimated that during C_3 photosynthesis, upto 50% of the CO_2 fixed may have to pass through photorespiratory process (glycolate pathway) to form carbohydrates such as sucrose thereby resulting in considerable decrease of photosynthetic productivity.
3. Unlike usual mitochondrial respiration, assimilatory powers (ATP or NADPH2) are not generated in photorespiration.
4. However, photorespiration is considered as metabolic adjunt to the Calvin cycle (i.e., it has been added to the **Calvin cycle** but essentially it is not a part of C_3 cycle).
5. Because photorespiration process decreases photosynthetic efficiency of crop plants, scienctists are working to increase the efficiency of C_3 plants by decreasing photorespiration.

5.1 Ways to reduce the effect of photorespiration would be :
- a. to increase the CO_2 concentration, and
- b. to develop varieties or strains of C_3 plants, which have low photorespiration rate.

Regulation of Photorespiration

Negative effects of photorespiration on crop plants can be regulated and consequently, the photosynthetic productivity can be increased manifold. Possible measures would be :

1. By manipulating different atmospheric conditions i.e., by increasing atm. CO_2 etc.
2. Use of inhibitors of glycolic acid oxidase, such as α-hydroxysulphonates.
3. Through genetic manupulation also, the process of photorespiration can be regulated.

Positive Effects of Photorespiration

Recently, photorespiration process has been considered as a **protective** and **supportive mechanism,** which **reduce O_2 injury to chloroplasts**.

Free radicals of O_2 gas are very reactive which react with membrane components and destroy them. In the absence of photorespiration, concentraion of such O_2 free radicals may reach very high and can attain a destructive level in the chloroplasts. Therefore, under such circumstances, efforts to reduce photorespiration may prove dangerous.

Factors Affecting Photorespiration

Following factors are known to influence the rate of photorespiration :

1. CO2 compensation point (higher CO2 compensation point increases photorespiration)
2. Plant species (C3 or C4 plant)
3. Higher temperature.
4. Inhibitors of glycolic acid oxidase such as α-hydroxysulphonates inhibit the process of photorespiration.

CHAPTER 9
RESPIRATION

Definition

Important plant life process such as proteins, fats and carbohydrates, require certain expenditure of energy. This energy is mainly derived from the breakdown of complex macromolecules. During photosynthesis, light energy is converted into chemical energy and stored in the bonds of complex organic molecules. The major portion of stored energy in plants is found in the form of carbohydrates like glucose and starch.

The energy stored in carbohydrate molecules during photosynthesis is released during cellular oxidation of carbohydrates into CO_2 and H_2O. This is called as **Respiration**. During this process, O2 is consumed and CO_2 released out.

In respiration, oxidation of various organic food substances like carbohydrates, fats, proteins etc., may take place. Among these, **glucose** is the **commonest**. Its oxidation proceeds a shown below in the simplestas equation :

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Energy (686 K.Cal.)}$$

Breakdown of glucose involves many steps releasing energy in the form of ATP molecules and forming a number of carbon compounds (intermediates) in a very well organized sequence.

Respiration is a complex process, which includes :

1. Absorpiton of O2.
2. Conversion of carbohydrate (comples) to CO2 and H2O and simpler substances (i.e. oxidation of food).
3. Release of energy -a part of which is utilized in various vital processes and the rest may be lost in the form of heat.
4. Formation of intermediate products playing different roles in metabolism.
5. Liberation of CO_2 and H_2O
6. Loss in weight in plants as a result of oxidation.

Therefore, **respiration is** a **reverse process of photosynthesis.**

Types of Respiration

On the basis of availability of O_2 , respiration has been divided into two categories.

a. Aerobic respiration

It is of common occurrence found in all plants and takes place in the presence of O_2 and the stored food (respiratory substrate) gets completely oxidised into CO_2 and water as,

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 686 \text{ K.Cal.}$$

Differences between these two respiration are given in P.155.

b. Anaerobic respiration

It takes place in the absence of O2 or when O2 conc. is less than 1%. The stored food is completely oxidised and instead of CO2 and water, certain other compounds are also formed. This is of rare occurC_6 rence but common among micro-organisms like yeasts and can be represented by,

$$C_6H_{12}O_6 + \quad \rightarrow 2C_2H_5OH + 2CO_2 + 56 \text{ KCal.}$$

Differences between these two respiration are given in P.155.

Mechanism of Respiration

Respiratory Substrate

The substrates, which are broken down in respiration for the release of energy, may be carbohydrates, fats or proteins. Proteins are used up in respiration only when carbohydrates and fats are not available.

As regards carbohydrates, not only simple hexose sugars like glucose and fructose but complex disaccharides particularly sucrose and polysaccharides such as lignin, inulin and hemicellulose are also used as respiratory substrates. Fats are used as respiratory substrates after their hydrolysis to fatty acids and glycerol by lipase and their subsequent conversion to hexose sugars. Proteins serve as respiratory substrates after their breakdown into aminoacids by proteolytic enzymes.

During respiration, the complex substrates are broken down into simpler ones and finally CO2 is liberated and water is formed. During oxidation of respiratory substrate, some energy is released. Part of this energy is trapped in the form of energy-rich compounds such as ATP while remaining part is lost in the form of heat. The energy trapped in ATP molecules can be used in various ways.

All complex carbohydrates are firstly converted into hexoses (glucose or fructose) before actually entering into respiratory process. The oxidation of glucose to CO_2 and water consists of three distinguishable phases:

A. Glycolysis
B. Kreb's cycle
C. Electon Transport System (ETS) or Terminal Oxidation

Details of reactions involved in these three processes are furnished below:

A. Glycolysis

(EMP=Embden Meyerhof Paranas Pathway; Common Respiratory Pathway; Cytoplasmic Respiration)

The course of step-wise degradation from glucose to pyruvic acid is termed as **glycolysis.** After the name of its tracers, the glycolytic pathway is also known as Embden Meyerhof Paranas Pathway (EMP pathway). The fate of pyruvic acid, however, depends on the presence or absence of O_2 . In the presence of O_2 the final degradation products are CO_2 and water in Kreb's cycle; while in the absence of O_2 ethyl alcohol and CO_2 in fermentation.

Glycolysis, fermentation, anaerobic respiration and lactic acid formation processes occur freely in the cytoplasm while Kreb's cycle occurs in the matrix of mitochondria in the eukaryotic cells and on the surface of mesosomes in prokaryotic cells. Enzymes of glycolysis are *found in cytoplasm* in the soluble form, called *cytosol*. These remain active through out lifetime and are required again and again. Such enzymes are called *constitutive enzymes.*

Therefore, it states that one molecule of glucose which is 6-carbon compound is broken down into two molecules of pyruvic acid which is 3-carbon compound through a large number of reactions. It occurs in the following *three important phases*.

Phase I

In the first phase of glycolysis, the glucose molecule is phosphorylated with the introudction of two phosphate groups. For this, two ATP molecules are needed.

Phase II

It involves the breaking up of 6-carbon compound, Fructose 1,6-diphosphate into two molecules of 3-carbon compounds, 3- phosphoglyceraldehyde (3-PGAld) and Dihydroxyacetone phosphate. These two 3-carbon compounds are inter-convertible.

Phase III

During this phase, degradation of 3-PGAld into pyruvic acid takes place with the production of four molecules of ATP. As in the phosphorylation of glucose during the first phase two molecules of ATP have already been used up, there is a net gain of only two molecules of ATP during glycolytic reactions.

Transphosphorylation (Phosphorylation in Glycolysis)

The kind of reaction in which a phosphate groups is transferred from another already phosphorylated compound i.e., ADP to form ATP is called as **transphosphorylation.**

With the proudction of pyruvic acid, the glycolysis comes to an end. During these reactions, two ATP moleculesd are used up; and four ATP molecules are produced. Thus, there is a net gain of two ATP molecules. However, during glycolysis, two $NADH_2$ molecules are also produced, from each, three ATP molecules are produced. As a result, 6ATP molecules are formed from Electron Transport System (ETS) chain. Thus, there is a total gain of 8 ATP molecules in glycolysis.

B. KREB'S CYCLE or TCACYCLE

This cycle is named after H.A.Kreb. The Kreb's cycle is also known as *Tricarboxylic acid,* (TCA) *cycle, Oraganic acid cycle, Mitochondrial respiration, Oxidation of pyruvate and Citric Acid cylce.* It takes place in *mitochondria* where all the necessary enzymes required for it are found on *cristae*.

In this cycle, at first the carbon atom 3or 4, then 2 or 5 and lastly carbon number 1 or 6 of the glucose molecule are released in the form of CO_2 molecules.

Various reactions of the Kreb's (TCA) cycle is schematically given in Fig.23.

Fig.23. The tricarboxylic acid (TCA) Cycle.

Significance of Kreb's cycle.

Kreb's cycle occupies a central and very important place in various metabolism of plants.

i. It provides energy in the form of ATP molecules for various metabolic activities.
ii. It is directly related to nitrogen metabolism
iii. It is also intimately related to the fat metabolism
iv. It is closely associated with glyoxalate cycle
v. It is related to other metabolic processes through its intermediates in one way or other.

C. ELECTRON TRANSPORT SYSTEM or TERMINAL OXIDATION OF REDUCED CO-ENZYME or OXIDATIVE PHOSPHORYLATION or RESPIRATORY CHAIN

The last step in the aerobic respiration is the oxidation of reduced co-enzymes produced during glycolysis and Kreb's cycle by molecular O_2 through FAD, cyt.b, cyt.c, cyt.a and cyt.a_3 (cytochrome oxidase).

Two H-atoms or electrons from the reduced coenzyme ($NADH_2$ or $NADPH_2$) travel through FAD and the cytochromes each with a more positive oxidation-reduction potential and ultimately combine with1/2 O_2 molecule to produce one molecule of H_2O. This is called as **Termination Oxidation.** Details of electron transport system is shown in Fig.24.

Fig.24. Electron transport system and oxidative phosphorylation

Oxidative Phosphorylation

During the electron transport, FAD and the iron atom of different cytochromes get successively reduced (FE^{2+}) and oxidised (FE^{3+}) and enough energy is released at some places which is utilised in the phosphorylation of ADP molecules to generate energy rich ATP molecules.

Because this oxidation accompanies phosphorylation (production of high energy phosphate bonds of ATP and Pi), it is called as **Oxidative Phosphorylation**. It takes place on stalked particles situated on cristae in mitochondria. It is inhibited by 2,4-Dinitriphenol.

During the system, one ATP molecule (contains 7.6K.Cal. energy) is synthesized at each place when electrons are transferred from :

i)reduced $NADH_2$ $NADPH_2$ to FAD, ii) reduced Cytochrome b to Cytochromec, and (iii) reduced Cytochrome a to Cytochrome a_3.

Thus, oxidation of one molecule of reduce $NADH_2$ $NADPH_2$ will result in the formation of 3 ATP molecules while oxidation of $FADH_2$ will lead to the synthesis of 2ATP molecules.

Complete oxidation of a glucose molecule (hexose sugar) results in the net gain of 38 ATP molecules as shown in the imp.15.

One glucose molecule contains about 686K.Cal. energy. The 38 ATP molecules will have 288.8K.Cal. energy. Therefore, about 40% (288.8/686) energy of the glucose molecule is utilised is utilised during aerobic breakdown and the rest is lost as heat.

Pentose Phosphate Pathway

Although glycolysis is the principal route of the conversion of carbohydrates into pyruvic acid in many biological systems, it is not the only metabolic route for breakdown of carbohydrates.

Presence of inhibitors like iodoacetate, fluorides, arsenates etc. specifically inhibit some steps of the glycolysis. Nevertheless, glucose utilization is not inhibited completely. This has led to the discovery of some other alternative routes of carbohydrate breakdown existing in plants. One such very common alternative route in plants in **Pentose Phosphate Pathway.**

It involves the oxidation of Glucose -6-phosphate to 6-Phosphogluconic acid which in turn is converted into pentose phosphates. In this pathway, glucose-6-phosphate is directly oxidised without entering glycolysis, hence it is also called as **Direct Oxidation Pathway or Hexose Monophosphate Shunt.**

In summary, 6 molecules of Glucose-6-phosphate which enter into this pathway, after oxidation produce 6 molecules of CO2 and 12 molecules of reduced coenzymes NADPH2 and while 5 molecules of Glucose-6-phosphate are regenerated as given below:

$$6(\text{Glucose-6-P}) + 12\ \text{NADP} \quad 5(\text{Glucose-6-P}) + 12\ \text{NADPH}_2 + 6\text{CO}_2$$

Significance of Pentose Phosphate Pathway

i. It provides alternative route for carbohydrate breakdown and provision of energy.
ii. It provides Ribose sugars for the synthesis of nucleic acids.
iii. It plays an important role in fixation of CO_2, in photosynthesis through Ribulose-5-P

Difference between oxidative phosphorylation and Photophosphorylation:

S.No.	Oxidative Phosphorylation	Photophosphorylation
1.	It occurs during respiration	It occurs during photosynthesis
2.	In general, it is found in mitochrondria	It takes place within the chloroplast.
3.	The process occurs on the inner membrane of cristae.	It occurs in the thylakoid membrane.
4.	Molecular O_2 is needed during terminal oxidation.	Molecular O_2 is not required.
5.	Energy released during electron transfer due to oxidation-reduction reaction is used during light.	Source of energy for conversion of ATP from ADP and Pi is external ATP formation.
6.	Process takes place in electron transport system involving cytochromes.	Pigment I and II are involved during the process.
7.	ATP molecules are released in the cytoplasm available for different metabolic reactions.	Produced ATP molecules are used up for CO_2 assimilation in the dark reaction of photosynthesis.

CHAPTER 7

PHOTOSYNTHESIS

Definition

The photosynthesis can be defined as the formation of carbon containing compounds from carbon dioxide and water with the help of green pigments and light while oxygen is liberated as the by-proudct. The simples equation for photosynthesis can be :

$$6CO_2 + 6H_2O \xrightarrow[\text{Green Pigments}]{\text{Light}} C_2H1_2O_2 + 6O_2$$

Ruben and Kamen (1941) demonstrated that the **source of liberated O_2 is the water,** through their experiment with oxygen isotope, ^{18} O as giben belwo:

$$2H_2{}^{18}O + CO_2 \xrightarrow[\text{Light}]{\text{Green cells}} \underset{\text{Carbohydrates}}{(CH_2O)} + H_2O + {}^{18}O_2$$

Requirements of Photosynthesis

Thus, the **inputs or components** of photosynethesis will be :

1. Carbon di oxide
2. Water
3. Light
4. Green pigments in chloroplasts of cells

Output of Photosynthesis

1. Carbohydrates
2. Oxygen molecules

Process of Photosynthesis

Photosynthesis is an oxidation-reduction process in which water is oxidized and CO_2 is reduced to carbohydrate and water; and O_2 molecules are by-products. The reduction of CO_2 to carbohydrate level needs **assimilatory powers such as ATP and NADPH** $_2$. Reduction of CO_2 occurs in dark **(light-independent)** but the prouction of assimilatory powers takes place in the presence of light and the process is light-dependent.

Thus, **Photosynthesis** consists of **two phases :**

1. Light dependent phast (light reaction or Hill reaction) and
2. Light-independent phase (dark reaction or Blackman's reaction).

Photosynthetic Apparatus

Simplest type of photosynthetic apparatus is observed in prokaryotic cells such as bacteria and blue-green algae in which they are represented by isolated and freely lying photosynthetic lamellae. In other algae, there is no distinction of grana and stroma regions. In these organisms, the photosynthetic lamellae are found closely arranged running parallel to each other; and carotenoid pigments dominate in the photosynthetic apparatus. These are called as Chromatophores. The pigments are evenly distributed in chromatophores and are primitive in nature.

In higher plants, well-developed photosynthetic apparatus is found which is commonly called as **Chloroplasts.** A choroplast is an advanced, well-organized and complicated photosynthetic apparatus.

Structure of Chloroplast

Each chloroplast is surrounded by a *double membrane* system. The external surface of the outer membrane is smooth whereas innner membrane is thrown into lamellated structure showing two distinct regions, called **Stroma and Grana Lamellae.**

Stroma

Stroma is the **main site** for the **dark reaction** of photosynthesis. It forms the matrix (ground substance) of the chloroplast. In the matrix, the lamellae are loosely arranged. The lamellae found in the stroma are called as **Stroma Lamellae.** Besides these structures, ribosomes and osmophilic granules are also found in the stroma.

Besides photosynthesis, lipid, protein and nucleic acid metabolisms are also found to take place in the stroma region of chloroplast.

Grana

Granum (= singular) is the most but complicated portion of chloroplast. Light reaction of photosynthesis takes place only in the grana region of chloroplasts. About 40-60 grana are present in each chloroplast.

In granum region, the lamellae are compactly arranged just like a stack of coins arranged one above the other back to back.

Grana lamellae form sac-like structures called **Thylakoids.** In higher plants, these thylakoids are closely packed together. Park and Beggins (1964,1967) observed some projections known as Thylakoid Bodies. On these thylakoid bodies, some specialised smaller and rounded particles are found which are named as **Quantasomes.** These quantasomes are known to contain chlorophyll molecules (**200-300** chlorophylls per quantasome) arranged in monolayer (single layer). Later on, these Quantasomes are referred as Photosynthetic Units.

Photosynthetic Pigments

There are **three types** of photosynthetic pigments, viz.,

1. Chlorophylls
2. Carotenoids
3. Phycobillins

Properties of photosynthetic pigments

- Chlorophylls and carotenoids are insoluble in water and can be extracted only with organic solvents like acetone, petroleum ether etc.
- phycobillins are soluble in water.
- Carotenoids include carotenes and xanthophylls (or carotenols)
- Different pigments absorb light of different wavelengths and show characteristic absorption peaks both *in vivo* and *in vitro* (refer Table below)
- They show fluorescence.
- Chlorophylls and carotenoids are insoluble in water and can be extracted only with organic solvents.
- Phycobillins are soluble in water.

Distribution of Photosynthetic Pigments in Plant Kingdom

S.No.	Pigment	Distribution in Plant Kingdom
01.	Chlorophylls : Chlorophyll a	All photosynthesizing plants except bacteria.
	Chlorophyll b	Higher plants and green algae
	Chlorophyll c	Diatoms and brown algae.
	Chlorophyll d	In some red algae
	Chlorophyll e	*In Tribonema* and Zoospores of *Vaucheria*
	*Bacterio*Chlorophyll a	Purple and green bacteria
	*Bacterio*Chlorophyll b	In a strain of purple bacterium *Rhodopseudomonas, chlorobium* Chlorophyll (Bacterioviridin). Green baceria.
02.	Carotenoids : Carotenes	Mostly in algae and higher plants.
	Xanthophylls (Carotenols)	Mostly in algae and higher plants.
03.	Phycobillins : Phycoerythrins	In blue-green and red algae.
	Phycocyanins `	-do-
	Allophycocyanin	-do-

Structure of photosynthetic Pigments

Chlorophylls :

- They are **magnesium porphyrin** compounds
- The prophyrin ring consists of four **pyrrol rings** joined together by **CH** bridges.
- A long chain of C atoms called as **Phytol chain** is attached to the porphyrin ring.
- Molecular formulae :

  Chlorophyll a : $C_{55} H_{72} O_5N_4Mg$

  Chlorophyll b : $C_{55} H_{70} O_6N_4Mg$

- Both the chlorophylls (***a*** and ***b***) consist of :

  Mg-Porphyrin head which is **hydrophilic** in nature and a

  Phytol tail possessing **lipophilic** properly.

Difference between two chlorophylls

In chlorophyll b there is -CHO (aldehyde) group, instead of a _CH_3 (acetyl) group in chlorophyll a molecule.

Synthesis of Chlorophyll

- Chlorophyll is formed from protochlorophyll in the presence of light with the loss of two hydrogen atoms as follows :

$$\text{Protochlorophyll} \xrightarrow[\text{-2H}]{\text{light}} \text{Chlorophyll}$$

Location of Chlorophyll Pigments in Chloroplast

The photosynthetic pigments are located in **grana portions of the chloroplasts** of higher plants. Chlorophyll molecules **form a monomolecular layer** between the alternative protein and lipid layers in grana lamellae (thylakoids). The **hydrophilic heads** of the chlorophyll molecules remain **embedded in the protein layer while the lipophylic phytol tails in the lipid layer.**

Absorption Spectra of chlorophylls

The chlorophyll pigments chiefly absorb in the violet-blue and red parts of the spectrum. The absorprtion band shown by the chlorophylls in violet-blue region is also called as **Soret band.**

In ether solution, chlorophyll a shows a maximum absorption peak at 663 nm in the red region and at 435nm in the violet region-while chlorophyll b has prominent absorption peak at 645nm and 453nm. The other absorption band for chlorophylla ***a*** could be 615, 578,533 and 410nm, and of chlorophyll ***b*** 595, 549 and 430nm.

Absorption Peaks of Different Chlorophylls

Type of Chlorophylls	**in vivo(nm)**	**in vivo(nm)**
Chlorophylls a	435,670,680 (several forms)	410,660
Chlorophylls b	480,650	452,642
Chlorophylls c	645	445,625
Chlorophylls d	740	450,690

in vivo : Within the living organisms or plants

in vitro : In glass vessels. This is ususally applied to biological processes when they are experimentally made to occur in isolation from the whole organisms.

* Carotenoids include carotenes and xanthophylls. The latter are also called as **carotenols.**

* Different pigments absorb light of different wavelengths and show characteristic absorption peaks.

Photosynthetic Units

The photosynthetic units are defined as the **smalles group of chlorophyll pigment molecules, which are necessary for the photochemical reaction during photosynthesis.** They absorb and migrate the quantum of light to the trapping centre where they help in the release of electron.

Emerson and Arnold (1932) found that about **2500 chlorophyll molecules** are necessary for **fixing up one molecule of CO2 or for releasing one molecule of O2; they labelled this number as Chlorophyll Unit.** Subsequently, the name has been changed to **Photosynthetic Unit.**

Since the reduction or **fixation of one molecule of CO_2 requires about 10 quanta of light,** it is assumed that **10 flashes of light are required to yield one molecule of O_2** or for reduction of one CO_2 molecule. Therefore, each unit would now contain only 1/10 of 2500, i.e., 250 chlorophyll molecules. It is the number 200-300 chlorophyll molecules per photosynthetic unit, which is widely accepted and is now considered as Physiological Unit of Function.

Later, Park and Beggins (1964) found these photosynthetic units as distinct granular structures in the grana region of chloroplast and they named these structures as **Quantasomes, which are considered**

as the morphological expression of the physiological photosynthetic units.

Quantasomes measure 180° A x 160° A and are 100 ° A thick. Park and Biggins (1964) revealed that **one quantasome contains about 230 chlorophyll molecules.** This is very close to the number of chlorophyll (200-300) contained in the **physiological photosynthetic units.**

MECHANISM OF PHOTOSYNTHESIS

The process of Photosynthesis is a complicated oxidation-reduction process ultimately resulting in the oxidation of water and reduction of CO_2 to carbohydrates.

The mechanism of photosynthesis can be studied with the following two processes :

I. *Primary Photochemical Reaction or Light reaction or Hill's reaction, and*
II. *Dark reaction or Blackman's reaction or Path of carbon in photosynthesis*

Importance of two processes photosynthesis

In the primary photochemical reaction, **assimilatory powers (NADPH2 + ATP)** are **generated** and **O_2 is released.** These assimilatory powers are utilized in the dark reaction during which process CO_2 is reduced to carbohydrates.

I. PIRMARY PHOTOCHEMICAL REACTION (OR) LIGHT REACTION (OR) HILL'S REACTION *(Activities found in **thylakoids or grana**)*

In 1937, Robert Hill demonstrated that isolated chloroplasts evolved O_2 when they were illuminated in the presence of suitable electron acceptor, such as ferricyanide. The ferricyanide is reduced to ferrocyanide by photolysis of water. This reaction is now called as **Hill Reaction.**

Photolysis of water or Hill Reaction (oxidation of water)

Splitting of water molecule into OH^- and H^+ ions in the presence of light is called as Photolysis of water. The OH^- ions unite to form water molecules again and release O^2 and electrons (e-). While, the H^+ is used in the formation of energy-rich molecules, NADPH2 during light reaction. It explains that **water is used as a source of electrons for CO_2 fixation and O_2 is evolved as a by-product.**

It is believed that photolysis of water invloves a strong oxidant, designated as **"Z".**

Primary photochemical reaction is **faster** than the dark reaction. It takes place only in the presence of light in the grana portions of the chloroplasts. It is complex process with several important events.

Primaty Photochemical Reactions can be studied under the following steps :

A. Red drop, Emerson Enhancement Effect and Two Pigment Systems :

i. Red Drop and Emerson Enhancement Effect (EEE)
ii. Two Pigment Systems.

B. Production of Assimilatory Powers

i. Electron Transport System in Photosynthesis or Reduction of NADP
ii. Photophosphorylation

They are <u>three kinds of Photophosphorylation</u>, viz.,

- i. *Cyclic Phtophosphorylation*
- ii. *Non-cyclic Photosphorylatioh, and*
- iii *Pseudocyclic Photophosphorylation*

C. Energy relationships and efficiency of photosynthesis

D. Interrelationship between light and dark reactions

A. Red drop, Emerson's Enhancement Effect (EEE) and Two Pigment Systems

Emerson and Lewis (1943) observed a sharp decrease in the quantum yield of photosynthesis at the wavelengths longer than 680nm. Beacuse this drecrease takes place in the red spectrum of the light, they called this drop as **Red Drop.**

However, Emerson and Chalmers (1951) found that this inefficient wavelength of 680 nm could be made to be fully efficient if supplemented with the light of shorter wavelengths. They also observed that the quantum yield from the combined beams of light (red and far red of shorter and longer wavelengths respectively) was found to be greater than the sum effect of both beams when used separately. This enhancement of rate of photosynthesis is called as **Emerson's Enhancement Effect.**

ii. Two Pigment Systems

With the discovery of Red Drop and Emerson Enhancement Effect, it is concluded that at least two pigment systems are involved in photosynthesis. These two pigment systems are known as **Photosytem I (PS I)** and **Photosytem II (PS II).** The wavelengths of light longer than 680 nm influence only PS I. While, wave lengths of light shorter than 680nm influence both the PS I and PS II.

Components of Photosystem I :

PS I complex consists of :

* ~ 200 chlorophylls
* ~ 50 carotenoids
* one molecule of P 700
* one cytochrome f(cyt.f)
* cyt.b 563
* **FRS** (Ferredoxin Reducing Substance)
* one or two membrane bound ferredoxin molecules etc.
* It is also rich in chl a, iron and copper
* PS I controls the process of **producing a stron reductant to reduce NADP and NADPH$_2$, besides producing ATP molecules.**

Components of Photosystem II :

* ~ 200 chlorophylls
* ~ 50 carotenoids
* a molecule of P 680
* a primary electron acceptor Q
* a Plastoquinone
* four plastoquinone equivalents
* four Mn molecules bound to one or more proteins
* two cyt.b 559
* one cyt.b6 and
* Chloride
* PS II is concerned with the **generation of strong oxidant and weak reductant** coupled with the **release of O$_2$.**

B. Production of Assimilatory Powers

Arnon (1956) used the term **assimilatory powers to refer ATP** (Adenosine TRi-Phosphate) **and NADPH2** (reduced Nicotinamide Adenine Dinucleotide Phosphate) molecules.

The process of reduction of NADP into NADPH2 may be denoted as **Electron Transport System (ETS)** inphotosynthesis or Reduction of NADP. The process of formation ATP from ADP and inorganic phosphate (Pi) in light reaction of photosynthesis is called as **Photosynthetic Phosphorylation or Photophorylation.**

i. Electron Transport System (ETS) or Reduction of NADP

The ETS involves transport of electron from water and NADP to form NADPH2 . The H+ released during the process of photolysis of water reduces NADP to $NADPH_2$ during the process of non-cyclic photo-phosphorylation (see Fig.16).

When chl a molecule receives a photon of light, it becomes excited and expels and extra energy along with an electron in both the pigment systems (PS T & II). This electron, after travelling through a number of **electron carriers,** is either cycled back or consumed in reducing NADP (Nicotinamide Adenine Dinucleotide Phosphate) to $NADPH_2$. The extra light energy carried by the electron is utilized in the formation of ATP molecules at certain places during its transport.

ii. Photophosphorylation

Arnon has contributed a lot in the understanding of the electron transport and phosphorylation in chloroplasts.

They are of two types

1. Cyclic photophosphorylation
2. Non-cyclic photophosphorylation.

1. Cyclic Photophosphorylation

This is a pathway of ATP formation during light reaction of photosynthesis. **It involves only PS I and a wavelength of light greater than 680 nm.**

* In cyclic photophosphorylation, activation of PS I by wave length greater than 680 nm (P700) causes electron to flow from P700 to ferredoxin to cyt-b6 which in turn passes back the electrons to P700 via cyt-f and plastocyanin.

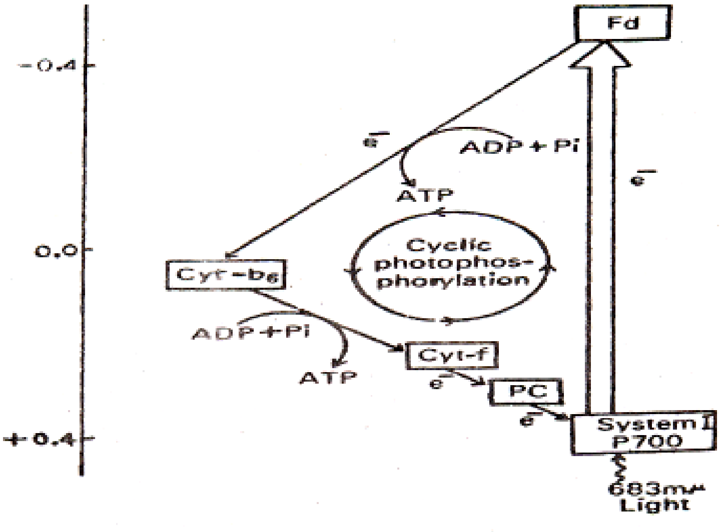


Fig.16. Schematic representation of Cyclic Photophosphorylation.

- The synthesis of ATP in this electron transport is possible at two places, i.e., between ferredoxin and cyt-b6 and cyt-f, by means of phosphorylation of one ADP molecule to form one ATP molecule in each place.
- During cyclic photophosphorylation, **2ATP molecules are produced per electron transfer.**

Since the **electron** which was **ejected from P700** in the above electron transport system is **cycled back,** the process is called as **Cyclic Electron Transport** and the accompanying phosphorylation as the **Cyclic Photophosphorylation** (Fig.16).

Significance of Cyclic Photophosphorylation

1. When cyclic photophosphorylation alone operates, the CO_2 **assimilation drops down** because of the shortage of reduced NADP ($NADPH_2$).
2. It generates **only ATP** molecules and as such **can not drive dark reactions** of photosythesis.
3. It is an **important system** in providing **ATP for synthetic processes** (other than photosynthesis)like synthesis of protein, lipids, nucleic acids and pigments within chloroplasts.

Limitations of Cyclic Photophosphorylation

1. This system operates if the activity of the PSII is blocked.
2. Under these conditions :

a. only PS I remains active
b. Photolysis of water does not take place
c. blockage of non-cylic ATP formation causes a drop in CO2 assimilation in dark reaction and, therefore,
d. there is a shortage of reduced NADP .(i.e., $NADPH_2$)

2. Non-cyclic Photophosphorylation

It occurs in green plants and **involves both PS I and PSII**

- During this process, the electron is excited by the absorption of a photon (quantum) of light by P700 form of chlorophyll ***a*** molecules in PS I.
- FRS traps this excited electron.
- The electron is now transferred to a **non-heme iron protein called ferredoxin (Fd)**.
- From Fd, the electron is transferred to NADP via the intermediate **protein electron carrier, ferredoxin-NADP-reductase** so that NADP is reduced to NADPH2 in the presence of H+ released from the reactions of photolysis of water.

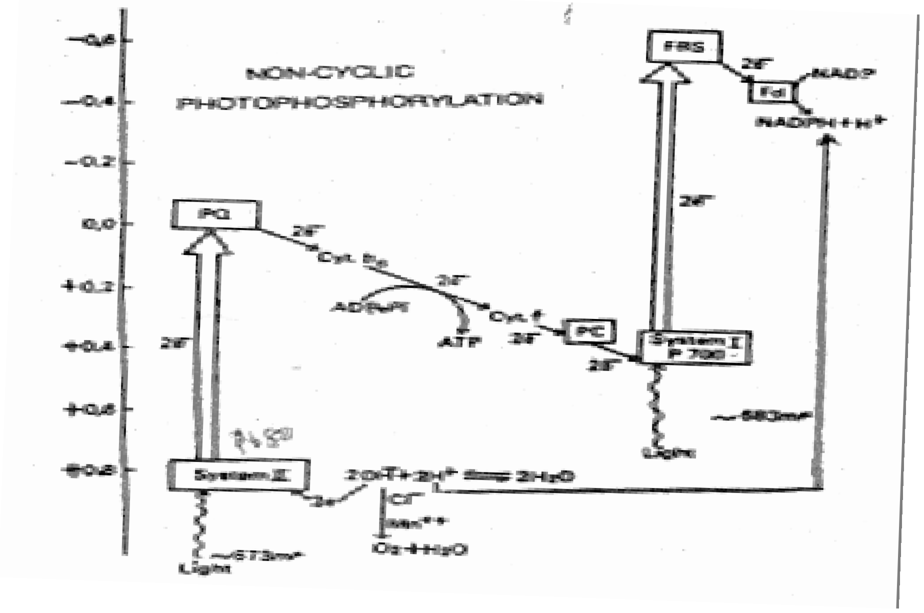


Fig.17. Schematic representation of light induced electron transport in photosynthesis showing non-cyclic photophosphorylation. Two pigment system, viz., PS PS II, Photolysis of water molecule and generation of assimilatory powers and I are indicated. (PQ: Plastoquinone; PC: Plastocyanin: FRS: Ferredoxin Reducing Substances and Fd; Ferredoxin).

* Now, when a photon (quantum) of light is absorbed by P 680 form of chlorophyll 'a" molecule in PSII, it gets excited and an electron is ejected from it so that an **electron deficiency or a 'hole" is left behind** in the P680 molecule.

* A compound of unknown identity traps this ejected electron as PQ (sometimes called as Q because it causes quenching of the characteristic **fluorescence** of chlorophyll **a in PS II**)

* From PQ the electron passes downhill along a series of compounds or **intermediate electron carriers** and is ultimately received by PS I where it **fills the "hole".**

* The series of compounds **(or electron carriers)** consists of :

i. Plastoquinone (PQ)
ii Cytochrome-b6
iii Cytochrome-f, a **copper containing Protein,** and
iv. Plastocyanin (PC)

*` During this electron transport, at one place, between cyt-b and cyt-f, phosphorylation of one molecule of ADP to form ATP molecule takes place(photophosphorylation).

* In the above scheme of Non-cyclic photophosphorylation, the electron ejected from PS II did not return toits place of origin; instead, it was taken by PSI. Similarly, the electron ejected from PS I did not cycle back and was consumed in reducing NADP. Therefore, this electron transport is called as Non-cyclic Photophos phorylation (Fig.17). However, Non-cyclic Photophosphorylation is inhibited by the chemicals like, CMU (3-(4'-dichlorophenyl)-1, 1-dimethyl urea)). This entire process is called as Hill Reaction or Light Reaction.With the production of ATP and NADPH2, the plant is now ready to reduce CO2 to form the carbohydrates. This is also called as 'Z' Scheme of Photophosphorylation.

Significance of Hill Reaction or Light Reaction

1. Light energy has been converted into chemical energy during primary photochemical reaction and is trapped in ATP and NADPH2 molecules.

2. This chemical energy is finally stored in carbohydrate molecules when ATP and NADPH$_2$ (assimilatory powers) are utilized in dark reaction of photosynthesis in reducing CO$_2$ to carbohydrates.

Differences between Cyclic and Non-cyclic photophosphorylation :

S.No.	**Cyclic photophosphorylation**	**Non-cyclic Photophosphorylation**
1.	Associated with PS I	Associated with both PS I and PSII
2.	Electron expelled from chl molecule is cycled back.	Electron expelled from chl molecule is not cycled back. Its loss is compensated by electron coming from photolysis of water.
3.	Photolysis of water and O2 evoluation do not take place	Photolysis of water and O2 evolution take place.
4.	Phosphorylation takes place at two places	Phosphorylation takes place only at one place.

5.	Two ATP molecules are produced.	one ATP and one $NADPH_2$ are produced.
6.	NADP is not reduced	NADP is reduced to $NADPH_2$ and used in assimilation of CO_2.
7.	This system is found dominantly in photosynthetic bacteria.	This is dominant in green plants
8.	The process is not inhibited by DCMU/CMU	The process is stopped by use of DCMU or CMU.

3. Pseudocyclic Photophosphorylation

Amon et al.(1954) demonstrated yet another kind of phosphorylation. They observed that:

1. In the absence of CO_2 and NADP, it can produce ATP from ADP and PI in the presence of FMN (Flavine Mono Nucleotide) or vitamin K and O_2.
2. The process is very simple.
3. It requires no chemical change except for the formation of ATP and water.
4. This is also called as **Oxygen dependent FMN catalysed photophosphorylation or pseudocyclic photophosphorylation** involving reduction of FMN with the production of O_2.

II. PATH OF CARBON IN PHOTOSYNTHESIS (OR) DARK REACTION (OR) BLACKMAN'S REACTION *(Activities found in stroma).*

The dark reaction of photosynthesis is purely enzymatic and slower than the primary photochemical reaction. It takes place in stroma portion of chloroplast and is independent of light, i.e., it can occur either in presence or absence of light. Main credit for investigating the sequences of dark reaction in photosynthesis goes to Melvin Calvin who was awarded Nobel Prize in 1961. A.A.Benson, J. Bassham and other co-workers have also contributed a lot.

By employing labelled 14C in photosynthesis, Calvin and his co-workers were able to formulate the complete metabolic path of carbon assimilation in the form of a cycle, which is called as Calvin Cycle. They observed that carbon reduction is a cyclic process and the first stable product is the 3-C compound, i.e., 3-phosphoglyceric acid. The first acceptor of labelled CO2 was found to be 5-carbon compound (ribulose 1,5-diphosphate; RuDP). The entire process of photosynthesis runs in a cyclic fashion, which includes formation of hexose or starch and regeneration of RuDP.

Different Pathway of Photosynthesis or Alternate Choices of Pathways of Dark Reactions of Photosynthesis.

They are more than one pathway of dark reactions of CO_2fixation leading to the synthesis of carbohydrate. Following are the three types of the pathways, which are well eatablished now.

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

1. C3 CYCLE (or) CALVINCYCLE

This dark reaction process of photosynthesis has been named variously such as -Calvin Cycle, Bassham and Calvin Cycle, Bassham and Calvin Cycle, Blackman reaction, Carbon assimilation, Path of carbon in photosynthesis, Reductive Pentose Phosphate Cycle.

Calvin cycle consists of two <u>important steps</u> :

i. Synthesis of carbohydrate
ii. Regeneration of RuDP

Sequences of reaction taking place in Calvin (C_3) cycle are furnished below :

i. Synthesis of Carbohydrate

1. CO_2 is first accepted by RuDP and forms an unstable 6-carbon compound from which two molecules of phosphoglyceric acid (PGA) are formed. The reaction is regulated by enzyme called carboxydismutase or RuDP carboxylase **(Rubisco)**.
2. The phosphoglyceraldehyde molecule is converted into dihydorxyacetone phosphate in presence of enzyme triose phosphate isomerase.
3. The phosphoglyceraldehyde molecule is converted into dihydorxyacetone phosphate in presence of enzyme triose phosphate isomerase.
4. Phosphoglyderaldehyde and dihydorxyacetone phosphate (one molecule each) unite to form fructose-1,6-diphosphate with the regulation of aldolase enzyme.
5. From fructose-1,6-diphosphate, different types of compounds are synthesized converted into glucose or starch.

In this Calvin Cycle, only one molecule of CO_2 is utilized at a time; therefore, the cycle has to run for 6 times totally, for carbohydrate synthesis using CO_2, and H_2O. Hence, regeneration of RuDP is essential to carry on the processes of photosynthesis.

ii. Regeneration of Ribulose diphosphate

Regeneration of ribulose diphosphate is essential to carry on the process of photosynthesis. The sequential reactions leading to the regeneration of RuDP would be as follows:

1. The so formed fructose-6-phosphate and phosphoglyceral dehyde combine and break into4-carbon compound (erythrose-4-phosphate) and 5-carbon compound (xylulose-5-phosphate) in presence of enzyme transketolase.
2. Erythrose-4-phosphate combines with a molecule of dihydorxyacetone phosphate to form sedoheptulose-17-diphosphate in presence of enzyme aldolase.
3. From sedoheptulose-1,7-diphosphate, one phosphate is removed in presence of enzyme phosphatase to form sedoheptulose-7- diphosphate.
4. Sedoheptulose-7-diphosphate and phosphoglyceraldehyde combine in presence of transketolase and produce one molecule each of xylulose-5-phosphate and ribose-5-phosphate.
5. Both these compounds convert into ribulose-5-phosphate in presence of enzyme phosphopentose isomerase. Thus, ribulose-5-phosphate is formed.
6. Ribulose-5-phosphate is converted into ribulose-1,5-diphosphate mediated by phosphopentokinase utilising ATP molecule coming from photophosphorylation. The ATP is converted into ADP.

Schematic representation of the Calvin (C_3) cycle is shown in Fig.18.

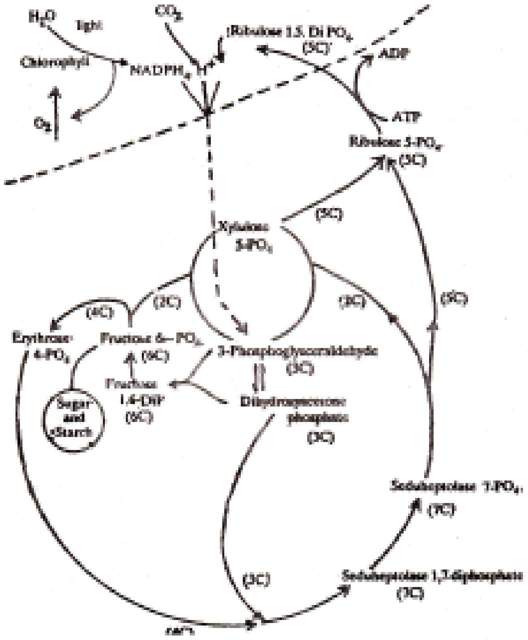


Fig.18. Simplified schematic representation of Calvin (C_3) cycle

Three Phases of Calvin Cycle

It is evident that Calvin Cycle consists of the following three phases, viz.,

i. *Carboxylative phase* (Reaction No.1 of Carbohydrate synthesis)
ii. *Reductive phase* (Reaction Nos.2-5 of Carbohydrate synthesis)
iii. *Regenerative phase* (Reaction Nos.1-6 of Regeneration of RuDP)

Enzymes involved in Calvin Cycle

In the first carboxylative phase, only one enzyme, viz., *carboxydismutase* is invloved which catalyses the carboxylation of ribulose 1,5-diphosphate to form PGA.

In the second reductive phase, three enzymes are involved for reducing PGA to triose phosphate and they are :

i. Phosphoglyceryl kinase
ii. Triose phosphate dehydrogenase, and
iii. Triose phosphte isomerase.

In the regenerative phase, seven enzymes participate and they are :

i. Aldolase
ii. Phosphatase
iii. Transketolase
iv. Transaldolase
v. Phosphoribose isomerase
vi. Phosphopentose epimerase, and
vii. Phosphorubulokinase.

Why named as C_3 cycle or C_3 Plant ?

Because **first visible product** of Calvin Cycle is **3-Phosphoglyceric acid (PGA),** Whichis **a 3-carbon compound,** Calvin cycle is popularly known as **C_3 Cycle.** The plants, which possess C_3 pathway or Calvin Cycle are called as **C_3 plants.** Most of the higher plants possess C_3 pathway for the fixatuib of CO_2 in photosynthesis, *Examples are* : rice, wheat, barley, oatt, rye, most of the pulses and oilseed crops etc.

2. C_4CYCLE (or) HATCH AND SLACK CYCLE

For many years, the Calvin cycle as described earlier was thought to be the only photosynthetic reaction operating in higher plants. But, Kortschak etal. (1965) reported that 4-carbon compound, dicarboylic acids, malate and aspartate were the major labelled proudcts when sugarcane leaves were to photosynthesize for shorter periods using $^{14}CO_2$. Later on, Hatch and Slack (1966) of Australia also confirmed this in several plants. They have proposed an alternate pathway, which is called as **C_4 Cycle** or **Hatch-Slack Pathway or Di-carboxylic Acid Cylce** (C_4), because 4-carbon containing di carboxylic acids are the earliest products after carbosylation in this pathway.

Examples of C4 Plants

Examples of C4 plants are : sugarcane, maize, sorghum, pearl millet, Amarantus, *panicum maximum, Chloris, Atriplex, Digitaria and Cyperus.*The C_4 cycke has also been reported in some members of the families, *Cyperaceae, and certain dicots belonging to Amaranthaceae, Chenopodiaceae, Compositae, Euphorbiaceae, Portulacaceae.*

Structural Peculiarities of C_4 Plants:

1. Presence of bundle sheath cells containing chloroplasts.
2. Bundle sheath cells are radially arranged around a vascular bundle.
3. Bundle sheath cells lack grana in their chloroplasts.
4. Bundle sheath cells are arranged in one or more layers consisting of large thick-walled cylindrical cells, around vascular bundle, a characteristic feature of C_4 plants.
5. Bundle sheath cells remain surrounded by one or more wreath like layers of mesophyll cells. This anatomical arrangement is called as Kranz type (Kranz=wreath, a German term). (Kranz: Wreath of mesophyll cells around bundle sheath cells)
6. Mesophyll cells have well-developed grana.(Thus, C_4 pathway constitutes an example of ***dimorphism of chloroplasts)*** But, some C_4 plants (bermuda grass)have grana in chloroplasts of bundle sheath cells).
7. The ratio of PS I : PS II activity is three times higher than the bundle sheath cells.
8. The mesophyll cells are almost three times more active in non-cyckuc electron transport system than that of bundle sheath cells.
9. For cyclic electron transport, both the cells are equally efficient.
10. Clear cut categorization of enzymes are found in C_4 cycle.
    Most of the PEP carbocylase occurs in mesophyll cells. While most of ribulose-1,5-diphos phate carboxylase and malic enzymes are found in bundle sheath cells.

The C_4 cycle is also referred as the **dicarboxylic acid cycle** or the **β -Carboxylation pathway** or **Hatch-Slack cycle** or **Co-operative Photosynthesis** (Karpilov, 1970).

In this Cycle, the characteristic point is the primary carboxylation reaction and the **phosphoenolpyruvate (PEP)** is found to be **CO_2 acceptor molecule.**

Site of Occurrence of C_4 Pathway

The C_4 pathway is known to operate in two types of cells :

i. Chloroplasts of mesophyll cells.

ii. Chloroplasts of bundle sheath cells.

The Hatch-Slack (C_4) pathway is schematically represented in Fig.19.

i. Reactions in the chloroplasts of mesophyll cells

1. Here, CO_2 is reduced by the carboxylation of PEP to form oxaloacetate (OAA). This reaction requires amolecule of water and releases a molecule of phosphoric acid as by-product. The enzyme, phosphoenolypyruvate carboxylase (PEPCase) mediates this carboxylation reaction.

2. OAA is readily converted into malate or aspartate depending upon species. Malate is derived from OAA by reduction with NADPH2 in the presence of the enzyme, malic dehydrogenase.

Malate is then transported to the chloroplasts of bundle sheath cells.

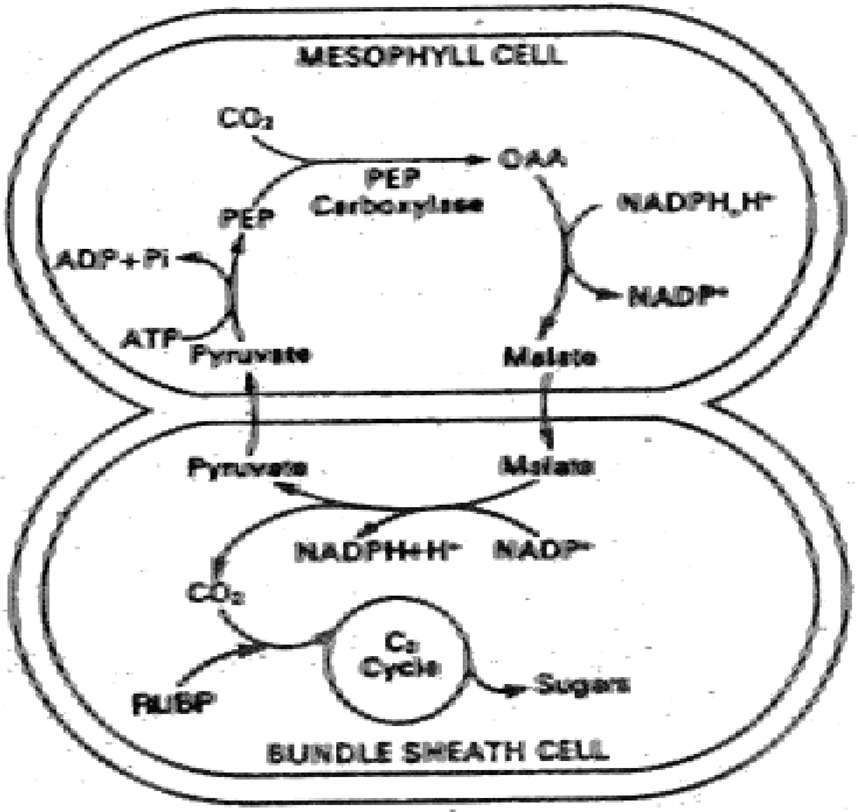


fig. 19. Hatch-Slack (C_4) pathway of carbon fixation in photosynthesis

ii. Reactions in the chloroplasts of bundle sheath cells

1. Here, Malate is decarboxylated by NADP specific malate enzyme to produce pyruvate and CO_2.This CO_2 is used in the C_3 cycle for carboxylation of ribulose-1,5-diphosphate in the presence of enzyme, RuBP carboxylase (RuBP Case) and produces phosphoglycerate, the first stable product of Calvin Cycle(C_3) of photosynthesis,
2. It follows Calvin Cycle in further steps to produce starch and to regenerate ribulose 1,5-diphosphate.
3. Simultaneously, Pyruvate is transported back to the chloroplast of mesophyll cells, where it is reconverted into the phosphoenolypyruvate by utilizing ATP generated during light phase in the presence of enzyme pyruvate phosphate dikinase. The ATP is converted into AMP. Since the conversion results in the form of AMP, the requirement to regenerate ATP from AMP is **2 ATP**. This is how 12 additional ATP molecules are needed in the C_4 pathway.

Phyiological significance of C_4 plants

1. Presence of C_4 pathway offers adaptation mechanism to xerophytic plants.
2. Plant can photosynthesize even with very low concentration of CO_2 (up to 10ppm) in the atmosphere.
3. Therefore, partial closure of stomata (due to xerophytic conditions) would not affect photosynthesis ` much. Thus, the plants can adapt to grow at low water content, high temperature and bright light intensities. Hence, this cycle is best suited to the crops grown in dry climates in both tropics and subtropics.
4. In C_4 pathway, photosrespiration is absent; hence, photosynthetic rate remains higher.
5. C_4 plants are about twice as efficient as C_3 plants in converting solar energy into carbohydrates or dry matter.

Categories of C_4 plants

Chollet and Ogren (1975) recognized three types of C_4 plants. They are :

a. In the first category, CO_2 is initially fixed by phosphoenolpyruvate and oxaloacetate is formed. The malate produced from it will be transported to bundle sheath cells.
Example : Sugarcane and maize.

b. Second group includes plants such as *Panicum maximum and Chloris gayana,* in which case it is aspartate, rather than malate, transported to bundle sheath cells. There it is transmitted to oxaloacetate, which is converted into pyruvate and CO_2 by PEP carboxykinase.

c. In third case, the aspartate produced in mesophyll cells is transported to bundle sheath cells where it is transmitted and reduced to malate. The malate is then decarboxylated to form pyruvate and CO_2 . The example includes *Atriplex spongiosa.*

Differences between C_3 and C_4 plants :

S.No	**C_3 Plants (Calvin Cycle)**	**C_4 Plants (Hatch-Slack Cycle)**
01.	Calvin cycle is found in all photosyn-thetic plants.	C_4 cycle is found only in certain tropical plants.
O2.	Efficiency of CO_2 absorption at low conc. is far less. So, they are less efficient.	Efficiency of CO_2 absorpition from low conc. is quite high. So, they are more efficient plants.
03.	The CO_2 acceptor is Ribulose-1,5 diphosphate.	The CO_2 acceptor is phosphoenolpyruvate.
04.	The first stable product is Phospho-glyceric acid.	Oxaloacetate is the first stable product.
05. `	All cells participating in photosynthesis have one type of chloroplast *(monomorphic type).*	The chloroplast of parenchymatous bundle sheath is different from that mesophyll cells The chloroplast in bundle sheath cell are centripetally *(dimorphic type*); The chloroplast in bundle sheath cell are centripetally arranged and lack grana. Leaves show *Kranz* anatomy.
06.	In each chloroplast, two pigment systems ()PS I abd II) are present.	In the chloroplasts of bundle sheath cells, the PS II is absent. Therefore, these are dependent on mesophyll chloroplasts for the supply of assimilatory powers ($NADPH_2$).

07.	The Calvin cycle enzymes are present in mesophyll chloroplasts. Calvin cycle occurs.	Calvin cycle enzymes are absent in the mesophyll chloroplasts. The cycle occurs only in the chloroplasts of bundle sheath cells.
08.	CO_2 compensation point is 50-150ppm CO_2 .	CO_2 compensation point is 0-10 ppm CO_2.
09.	Photorespiration is present and easily detectable.	Photorespiration is present only to a slight degree or absent.
10.	The CO_2 conc. inside leaf remains high (about 200 ppm).	The CO_2 conc. inside the leaf remains low (100ppm).
11.	The $^{13}C/^{12}C$ ratio in C-Containing compounds remains relatively low (both ^{13}C and ^{12}C are present in air.	The ratio is relatively high., C_4 plants are more enriched with ^{13}C than C_3 plants.
12.	Net rate of photosynthesis in full sunlight (10,000-12,000fc.) is 15-25mgof CO_2 dm^2 of leaf area per hl.	It is 40-80 mg of CO_2 dm^2 of leaf area per h. Thus, photosynthetic rate is quite high. The plants are efficient.
13.	The saturation intensity reaches in the range of 1000 to 4000fc.	It is difficult to reach saturation even in full sun light.
14.	Bundle sheath cells are unspecialised.	The bundle sheath cells are highly developed with unusual construction of organelles.
15.	Only C_3 cycle is found.	Both C_4 and C_3 cycles are found.
16.	The optimum temperature for the process is 10-25^{0}C.	In these plants, it is 30-45^{0}C. So, these are warm climate plants. At this temperature, the rate of photosynthesis is double than that in C_3 plants.
17.	O_2 present in air (=21%) markedly inhibits the photosynthetic process as compared to an external atmosphere containing no O_2.	The process of photosynthesis i s not inhibited in air as compared to an external atmosphere with no O_2.
18.	For synthesis of one glucose molecule, **18 ATP** are required.	In this process, **30 ATPs** are required for the synthesis of one glucose molecule.

3.Crassulacean Acid Metabolism (CAM cycle)

Under natural conditions, the acidity of green shoots of some non-halophytic succulents and semi-succulent plants increases at night and decreases during day time. This diurnal change in the acidity was first discovered in *Bryophyllum* belonging to the family Crassulaceae. Therefore, it is called as **Crassulacean Acid Metabolism (CAM)**. This metabolism occurs only in green organs and it is quite common in the plants belonging to the families like Crassulaceae, Cactaceae, Orchidaceae, Bromeliaceae,Liliaceae, Asclepiadaceae, Vitaceae and Euphorbiaceae. All such plants are called as Crassulacean Acid Metabolism plants(CAM plants). Most CAM plants possess the succulent habit. Typical example of commercial crop possessing CAM pathway is **pineapple**.

Mechanism of CAM cycle

Sequence of reaction taking place in CAM pathway is shown in Fig.20.

1. During night when stomata are open, CO2 is fixed through the action of PEP carboxylase to Malic acid. This is accomplised in two steps :

   a. PEP fixes CO_2 and is converted into oxaloacetic acid (OAA)

   b. OAA is subsequently converted into malic acid by malic dehydrogenase enzyme.

2. During light (day time), the malic acid releases one CO_2 molecule with the formation of PEP. This PEP is ultimately converted into sugars or starch.

3. The leaves of CAM plants also contain enzymes of Calvin cycle. Therefore, the released CO_2 combines with PGA and completes C_3 cycle in the light.

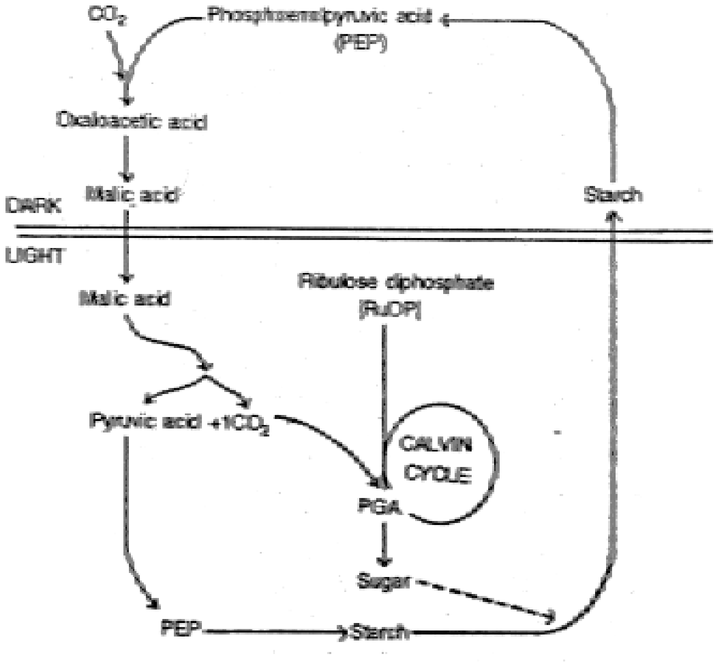


Fig. 20. CAM cycle

Thus, two cycles (CAM and Calvin cycle) occur in the mesophyll cells and there is no differentiation between the type of cells as found in C_4 plants.

Chemosynthesis

There are certain aerobic bacteria, which do not have chlorophyll but can synthesise organic food from CO2 and H20. This process of manufacture of food materials by bacteria making use of the chemical energy is called as **Chemosynthesis**. Some of the common examples of Chemosynthetic bacteria are given below :

1. Nitrifying Bacteria

Nitrosomonas and Nitrosococcus oxidise ammonia to nitrite and chemical energy is released.

$$2NH_3 + 3O_2 \rightarrow 2HNO_2 + 2H_2O + \text{Chemical energy}$$

2. Sulphur Bacteria

Beggiatoa and *Thiothrix oxidize* hydrogen suphide to sulphur and release sufficient amount of chemical energy for subsequent food synthesis.

$2H_2S + O_2 \rightarrow 2H_2O\ 4 +$ Chemical energy+ 2S

3. Iron Bacteria

Ferrobacillus and Leptothrix oxidise ferrous iron to ferric iron and release chemical energy.

$4FeSO_4 + O_2 + 6H_2O \rightarrow 4Fe(OH)_3 + 4C_2O +$ Chemical energy.

Factors Influencing Rate of Photosynthesis

The factors influencing rate of photosynthesis can be classified into two categories, the internal and external (environmental)

A. Internal Factors

1. Chlorophyll

The amount of chlorophyll present has a direct relationship with the rate of photosynthesis, since, it is the pigment, which is photoreceptive and is directly involved in trapping the light energy.

2. Photosynthetic Enzyme Systems

The amount and nature ofenzymes play a direct role on the rate of photosynthesis. Greater enzyme activity at higher light intensity increases the capacity of the leaf to absorb more light and thus increases the photosynthetic rate.

3. Leaf Resistance

Photosynthesis shows close dependence upon leaf resistance. For C4 plants, leaf resistance (primarily controlled by stomatal aperture) appears to regulate photosynthesis, but in C_3 plants, the internal resistances including carboxylation efficiency offer greater limitation to CO_2 fixation than stomatal resistance. Environmental factors such as light intensity, photoperiod, CO_2 concentration,humidity and soil moisture also affect photosynthesis via stomatal resistance.

4. Demand for Photosyntate

Because of the greater demand, the rapidly growing plants show increased rate of photosynthesis in comparison to the mature plants. However, if the demand for photosynthesis is lowered by removal of meristem, then the photosynthetic rate declines.

5. Leaf Age

The Photosynthetic rate is higher in the newly expanding leaves and reaches a maximum as the leaves achieve full size. The rate declines as the leaf ages due to reduced chloroplast functions and other anabolic reactions.

6. Role of Hormones

Photosynthesis may be regulated by some of the plant hormones. Gibberellic acid (GA) and Cytokinins (CK) increase both carboxylating activity and photosynthetic rate.

7. Genetic Control

Genetic contronl also plays a very important role on both CO_2 fixing systems and the CO_2 transport system of the leaf. The fixation of CO_2 is the function of set of enzyme present in the chloroplast, which are in turn under the control of genes of the chloroplasts.

B. External Factors

1. Carbon dioxide

CO_2 is one of the raw materials for photosynthesis; therefore, its concentration affects the rate of photosynthesis markedly. Rate of photosynthesis increases with the increase in the atmospheric CO2 concentration up to a certain extent. Because of its very low concentration in atmosphere (current level of 350-360ppm), it acts as a limiting factor in natural photosynthesis. Rate of photosynthesis increases with increase in the atm. CO_2 level of upto 1000ppm beyond which, there is a general decline in photosynthesis. At this enhanced level of CO_2, the increase in the photosynthetic rate may be 10 to 30 times more than the normal CO_2 level.

2. Light

Light affects the rate of photosynthesis in several ways. In general, photosynthesis can occur under artificial lights of sufficient intensity. Role of light on photosynthesis can be discussed under the following sub-heads:

a. Intensity of light :

With the increase in light intensity, the rate of photosynthesis increases, i.e., the rate of photosynthesis is directly proportional to light intensity. However, at stronger light intensity, increase in rate of photosynthesis is not proportional to light intensity. Except on cloudy days, light is never a limiting factor in nature.

At certain light intensity, the amount of CO_2 used in photosynthesis and the amount of CO_2 produced in respiration are volumetrically equal. This point of light intensity is known as Light Compensation Point. Light compensation point is frequently in the order of 100 to 200 f.c. for sunloving leaves; while, the value is 100f.c. for shade-loving leaves. Thus, in shade-loving plants the compensation point lasts for a much shorter period than in sun-loving plants.

b. Wavelength of light :

For photosynthesis, the visible range of spectrum of light (PAR:400 to 700 nm) is essential. Maximum photosynthesis is known to occur in the red part of the spectrum with the next peak in blue part and minimum in the green region (RED >BLUE > GREEN) The region between 575 and 750nm (yellow to red) is quite congenial for photosynthesis. Ultra violet light has a lethal effect on plants if exposure is for a prolonged period.

C. Duration of light :

Photosynthesis may be sustained for relatively long periods of time without any noticeable damaging effect on plants.

d. Photo-oxidation :

When the light intensity for photosynthesizing tissue is increased beyond a certain limit, the cells become vulnerable to chlorophyll photo-oxidation; due to this, many more chlorophyll molecules become excited than can possibly be utilized. This causes damaging effect to the chloroplast membrane system. In presence of O_2, the damaging effect of photo-oxidation is severe. It results in bleaching of chlorophyll and inactivation of some important enzyme involved in photosynthesis.

Effect to temperature on photosynthesis is little than on other process. Very high and very low temperatures affect the photosynthetic rate adversely. The rate of photosynthesis increases with rise in temperature from 5 to 35^0C; beyond which, there is a rapid fall in photosynthesis. In the optimum rangeof temperature, the Temperature Quotient (Q_{10}) is found to be 2.0 for the rate of photosynthesis (Q_{10}=2.0).

4. Water

Water is one of the raw materials in photosynthesis. It has an indirect effect on the rate of photosynthesis. Water availability affects the water relation of plant, thus affecting the rate of photosynthesis. In scarcity of water, cells become flaccid. Depending upon the availability of water, the rate of photosynthesis may be decreased from 10 to 90%.

5. Oxygen

Oxygen is the by-product of photosynthesis. Accumulation of greater amount of oxygen molecules causes substantial inhibition of photosynthesis. Oxygen is also known to have a direct and competitive inhibition for RuBP carboxylase. As a result, glycolate synthesis is enhanced which leads to photorespiration.

6. Warburg's Effect

An increase in the concentraion of O_2 in many plants results in a decrease in the rate of photosynthesis. A German Scientist, Warburg first discovered this phenomenon of the inhibition of photosynthesis by the greater accumulation of O_2 in 1920 in green alga, Chlorella. It is now known to operate in soybean etc. (C_3 plant). But, plants like maize, sugarcane, sorghum etc. (C_4 plants) do not show the effect.

7. Mineral nutrients

Some nutrients like copper etc., which are components of photosynthetic enzymes or magnesium as component of chlorophylls also affect the rate of photosynthesis indirectly by affecting the synthesis of photosynthetic enzymes and chlorophyll, respectively. Potassium also affects the rate through the stomatal movement. Leaf N content plays a major role in inceasing the photosynthetic rate of crops.

Inhibitors of Photosynthetic Process

1. Several urea derivatives such as <u>monuron (or CMU)</u> and <u>diuron (or DCMU)</u> block electron transport between Q and PQ.
2. Simazine, atrazine, bromacil and isocil block the same step.
3. Diquat and paraquat are common photosynthetic inhibitors. These compounds (commonly referred as **viologen dyes**) accept electron from PS I before ferredoxin and produce toxic forms of O_2 (superoxide and hydroxy).

CHAPTER 8

PHOTORESPIRATION

Photorespiration is a special type of repiration shown by many green plants when they are exposed to light. The normal dark respiration (usual mitochondrial respiration) is independent of light and its rate is the same in both light and dark. The photorespiration process is carried on only in the presence of light. The term photorespiration is referred to **" the release of CO2 in respiration in presence of light during photosynthesis".**

Importance of Photorespiration

a. Photorespiration is closely related to CO_2 compensation point.

b. It usually occurs only in those plants, which have comparatively high CO_2 compensation point. (eg. Tomato, wheat, oats etc.)

c. It is insignificant or rather absent in C4 plants, which have very low CO2 compensation point] (eg. maize, sugarcane, sorghum, pearlmillet, amarantus etc.).

Sites of photorespiration

Photorespiration occurs only in chlorophyllous tissues of plants. The process of photorespiration occurs in three different organelles viz.,

1. Chloroplasts
2. Peroxisomes and
3. Mitochondria.

Substrates of Photorespiration

Glycolate (glycolic acid) is the chief substrate of photorespiration. Other important metabolites are the two amino acids such as glycine and serine.

Processes or Steps of Photorespiration

Like usual mitochondrial respiration, the photorespiration is also an oxidative process where oxidation of glycolate occurs with subsequent release of CO_2 **(post-illumination burst of CO_2)**. The process of photorespiration takes place **in three cell organelles viz., chloroplasts, peroxisomes and mitochondira.**

Various steps of the glycolate metabolism (synthesis of glycolate and its oxidation with subsequent release of CO2 (photorespiration) are given in Fig.21.

Fig.21 Digramatic representation of glycolate metabolism during photorespiration

A. Reaction in Chloroplast

1.1. Glycolate is synthesized as a side product from some intermediates of photosynthesis in **chloroplasts.** It is probably derived from 1-C and 2-C of the ketose sugar phosphates of the Calvin cycle.

1.2. It is believed that O_2 competes with CO2 for the enzyme RuBP carboxylase. When this enzyme reacts with O_2 instead of CO_2, it is called as **RuBP oxygenase**.

1.3 In the second case, one molecule of phosphoglyceric acid (PGA) and one molecule of phosphoglycolic acid are formed from RuBP.

1.4. PGA then enters into the Calvin cycle.

1.5 Phosphoglycolic acid is dephosphorylated to form **glycolate**, in the presence of the enzyme, phosphatase.

B. Reactions in Peroxisome

2.1 From chloroplasts, the glycolate migrates into peroxisome where it is oxidised (photorespired) to glyoxalate in the presence of glycolic acid oxidase. In this reaction, hydrogen peroxide (H_2O_2)is formed with the utilization of one molecule of O_2. The H_2O_2 is then removed by the enzyme catalase as follows:

$$H_2O_2 \rightarrow H_2O + 1/2O_2$$

2.2 Glyoxalate is converted into an amino acid, **glycine** by transmination reaction in the presence of L-Glutamate glyoxalate transaminase.

C. Reactions in Mitochondrion

3.1 Glycine formed in peroxisomes migrates into mitochondria

3.2 Now, two molecules of glycine react to form one molecule of another aminoacid, **Serine**, liberating **CO_2** (post-illumination burst of Co2 and photorespiration) and NH3. This reaction is catalyzed by serine hydroxymethyl transferase.

Serine thus formed is apparently recycled back into the pool of photosynthetic intermediates of Calvin cycle in chloroplasts. This is mediated by the formation of **hydroxypyruvate** and **glyceric acid**. On phosphorylation with ATP, glyceric acid is converted into **PGA**, which is the intermediate of Calvin cycle.

Therefore, starting from intermediates of Calvin cycle and with the synthesis of glycolate, serine is formed which is again converted into intermediates of Calvin cycle thus completing the **glycolate cycle**. The photorspiratory cycle is also called as **C_2 cycle**.

Why named as C2 Cycle?

Because glycolate and some other metabolites of this glycolate cycle, viz., glyoxalate and glycine are 2-C compounds, the glycolate metabolism or glycolate cycle is also called as **C_2 Cycle**.

Difference between two types of plants :

Characteristic	**With photorespiration (C_3)**	**Without Photorespiration (C_4)**
1. Enhancement of CO2 assimilation in light and low O_2	yes	No
2. CO_2 compensation point in light	15 to 150ppm	0ppm
3. CO_2 assimilation rate in intense light	15-35mg CO_2 /dm^2/h	50-70 mg CO_2 /dm^2/h
4. CO_2 evolution in light	High rate	Apparently NIL
5. Internal structure of leaf about vascular bundles.	Diffuse mesophyll	Mesophyll compact
6. Carbon pathway	Calvin cycle	Both Calvin cycle and Hatch & Slack pathway.
7. Carbonic anhydrase activity	High	Low
8. Translocation rate from leaf	Low	High
9. Temperature optimum for photosynthesis and growth.	Low to high	High

Significance of Photorespiration in Crop-Productivity

1. Often, presence of photorespiration is considered as a **wasteful** and **energy consuming** process in crop plants which ultimately leads to **reduction in final yield of crops.**
2. It is estimated that during C_3 photosynthesis, upto 50% of the CO_2 fixed may have to pass through photorespiratory process (glycolate pathway) to form carbohydrates such as sucrose thereby resulting in considerable decrease of photosynthetic productivity.
3. Unlike usual mitochondrial respiration, assimilatory powers (ATP or NADPH2) are not generated in photorespiration.
4. However, photorespiration is considered as metabolic adjunt to the Calvin cycle (i.e., it has been added to the **Calvin cycle** but essentially it is not a part of C_3 cycle).
5. Because photorespiration process decreases photosynthetic efficiency of crop plants, scienctists are working to increase the efficiency of C_3 plants by decreasing photorespiration.

5.1 Ways to reduce the effect of photorespiration would be :

a. to increase the CO_2 concentration, and

b. to develop varieties or strains of C_3 plants, which have low photorespiration rate.

Regulation of Photorespiration

Negative effects of photorespiration on crop plants can be regulated and consequently, the photosynthetic productivity can be increased manifold. Possible measures would be :

1. By manipulating different atmospheric conditions i.e., by increasing atm. CO_2 etc.

2. Use of inhibitors of glycolic acid oxidase, such as α-hydroxysulphonates.
3. Through genetic manupulation also, the process of photorespiration can be regulated.

Positive Effects of Photorespiration

Recently, photorespiration process has been considered as a **protective** and **supportive mechanism,** which **reduce O_2 injury to chloroplasts**.

Free radicals of O_2 gas are very reactive which react with membrane components and destroy them. In the absence of photorespiration, concentraion of such O_2 free radicals may reach very high and can attain a destructive level in the chloroplasts. Therefore, under such circumstances, efforts to reduce photorespiration may prove dangerous.

Factors Affecting Photorespiration

Following factors are known to influence the rate of photorespiration :

1. CO2 compensation point (higher CO2 compensation point increases photorespiration)
2. Plant species (C3 or C4 plant)
3. Higher temperature.
4. Inhibitors of glycolic acid oxidase such as α-hydroxysulphonates inhibit the process of photorespiration.

CHAPTER 9

RESPIRATION

Definition

Important plant life process such as proteins, fats and carbohydrates, require certain expenditure of energy. This energy is mainly derived from the breakdown of complex macromolecules. During photosynthesis, light energy is converted into chemical energy and stored in the bonds of complex organic molecules. The major portion of stored energy in plants is found in the form of carbohydrates like glucose and starch.

The energy stored in carbohydrate molecules during photosynthesis is released during cellular oxidation of carbohydrates into CO_2 and H_2O. This is called as **Respiration**. During this process, O2 is consumed and CO_2 released out.

In respiration, oxidation of various organic food substances like carbohydrates, fats, proteins etc., may take place. Among these, **glucose** is the **commonest**. Its oxidation proceeds a shown below in the simplestas equation :

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Energy (686 K.Cal.)}$$

Breakdown of glucose involves many steps releasing energy in the form of ATP molecules and forming a number of carbon compounds (intermediates) in a very well organized sequence.

Respiration is a complex process, which includes :

1. Absorpiton of O2.
2. Conversion of carbohydrate (comples) to CO2 and H2O and simpler substances (i.e. oxidation of food).
3. Release of energy -a part of which is utilized in various vital processes and the rest may be lost in the form of heat.
4. Formation of intermediate products playing different roles in metabolism.
5. Liberation of CO_2 and H_2O
6. Loss in weight in plants as a result of oxidation.

Therefore, **respiration is** a **reverse process of photosynthesis.**

Types of Respiration

On the basis of availability of O_2, respiration has been divided into two categories.

a. Aerobic respiration

It is of common occurrence found in all plants and takes place in the presence of O_2 and the stored food (respiratory substrate) gets completely oxidised into CO_2 and water as,

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 686\ \text{K.Cal.}$$

Differences between these two respiration are given in P.155.

b. Anaerobic respiration

It takes place in the absence of O2 or when O2 conc. is less than 1%. The stored food is completely oxidised and instead of CO2 and water, certain other compounds are also formed. This is of rare occurC_6rence but common among micro-organisms like yeasts and can be represented by,

$$C_6H_{12}O_6 + \rightarrow 2C_2H_5OH + 2CO_2 + 56\ \text{KCal.}$$

Differences between these two respiration are given in P.155.

Mechanism of Respiration

Respiratory Substrate

The substrates, which are broken down in respiration for the release of energy, may be carbohydrates, fats or proteins. Proteins are used up in respiration only when carbohydrates and fats are not available.

As regards carbohydrates, not only simple hexose sugars like glucose and fructose but complex disaccharides particularly sucrose and polysaccharides such as lignin, inulin and hemicellulose are also used as respiratory substrates. Fats are used as respiratory substrates after their hydrolysis to fatty acids and glycerol by lipase and their subsequent conversion to hexose sugars. Proteins serve as respiratory substrates after their breakdown into aminoacids by proteolytic enzymes.

During respiration, the complex substrates are broken down into simpler ones and finally CO2 is liberated and water is formed. During oxidation of respiratory substrate, some energy is released. Part of this energy is trapped in the form of energy-rich compounds such as ATP while remaining part is lost in the form of heat. The energy trapped in ATP molecules can be used in various ways.

All complex carbohydrates are firstly converted into hexoses (glucose or fructose) before actually entering into respiratory process. The oxidation of glucose to CO_2 and water consists of three distinguishable phases:

A. Glycolysis

B. Kreb's cycle

C. Electon Transport System (ETS) or Terminal Oxidation

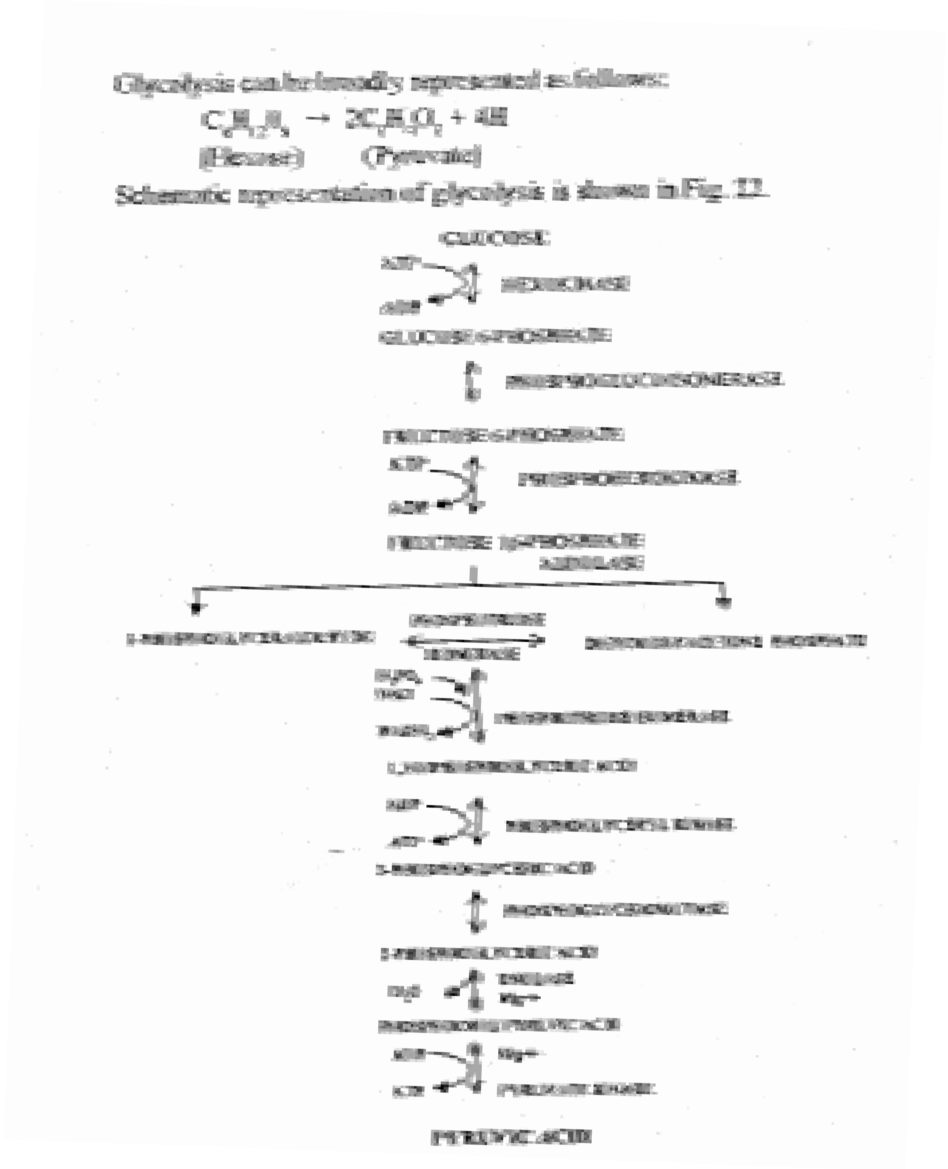
Details of reactions involved in these three processes are furnished below:

A. Glycolysis

(EMP=Embden Meyerhof Paranas Pathway; Common Respiratory Pathway; Cytoplasmic Respiration).

The course of step-wise degradation from glucose to pyruvic acid is termed as **glycolysis.** After the name of its tracers, the glycolytic pathway is also known as Embden Meyerhof Paranas Pathway (EMP pathway). The fate of pyruvic acid, however, depends on the presence or absence of O_2 . In the presence of O_2 the final degradation products are CO_2 and water in Kreb's cycle; while in the absence of O_2 ethyl alcohol and CO_2 in fermentation.

Glycolysis, fermentation, anaerobic respiration and lactic acid formation processes occur freely in the cytoplasm while Kreb's cycle occurs in the matrix of mitochondria in the eukaryotic cells and on the surface of mesosomes in prokaryotic cells. Enzymes of glycolysis are *found in cytoplasm* in the soluble form, called *cytosol*. These remain active through out lifetime and are required again and again. Such enzymes are called *constitutive enzymes.*



Therefore, it states that one molecule of glucose which is 6-carbon compound is broken down into two molecules of pyruvic acid which is 3-carbon compound through a large number of reactions. It occurs in the following *three important phases*.

Phase I

In the first phase of glycolysis, the glucose molecule is phosphorylated with the introudction of two phosphate groups. For this, two ATP molecules are needed.

Phase II

It involves the breaking up of 6-carbon compound, Fructose 1,6-diphosphate into two molecules of 3-carbon compounds, 3- phosphoglyceraldehyde (3-PGAld) and Dihydroxyacetone phosphate. These two 3-carbon compounds are inter-convertible.

Phase III

During this phase, degradation of 3-PGAld into pyruvic acid takes place with the production of four molecules of ATP. As in the phosphorylation of glucose during the first phase two molecules of ATP have already been used up, there is a net gain of only two molecules of ATP during glycolytic reactions.

Transphosphorylation (Phosphorylation in Glycolysis)

The kind of reaction in which a phosphate groups is transferred from another already phosphorylated compound i.e., ADP to form ATP is called as **transphosphorylation.**

With the proudction of pyruvic acid, the glycolysis comes to an end. During these reactions, two ATP moleculesd are used up; and four ATP molecules are produced. Thus, there is a net gain of two ATP molecules. However, during glycolysis, two $NADH_2$ molecules are also produced, from each, three ATP molecules are produced. As a result, 6ATP molecules are formed from Electron Transport System (ETS) chain. Thus, there is a total gain of 8 ATP molecules in glycolysis.

B. KREB'S CYCLE or TCACYCLE

This cycle is named after H.A.Kreb. The Kreb's cycle is also known as *Tricarboxylic acid,* (TCA) *cycle, Oraganic acid cycle, Mitochondrial respiration, Oxidation of pyruvate and Citric Acid cylce.* It takes place in <u>*mitochondria*</u> where all the necessary enzymes required for it are found on *cristae.*

In this cycle, at first the carbon atom 3or 4, then 2 or 5 and lastly carbon number 1 or 6 of the glucose molecule are released in the form of CO_2 molecules.

Various reactions of the Kreb's (TCA) cycle is schematically given in Fig.23.

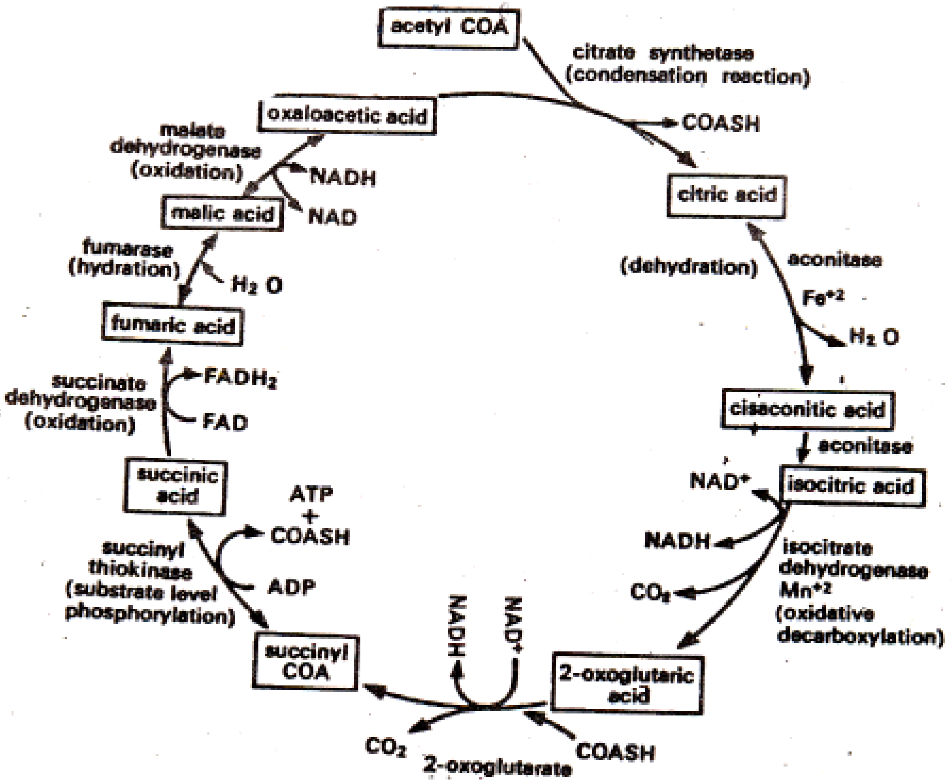


Fig.23. The tricarboxylic acid (TCA) Cycle.

Significance of Kreb's cycle.

Kreb's cycle occupies a central and very important place in various metabolism of plants.

i. It provides energy in the form of ATP molecules for various metabolic activities.
ii. It is directly related to nitrogen metabolism
iii. It is also intimately related to the fat metabolism
iv. It is closely associated with glyoxalate cycle
v. It is related to other metabolic processes through its intermediates in one way or other.

C. ELECTRON TRANSPORT SYSTEM or TERMINAL OXIDATION OF REDUCED CO-ENZYME or OXIDATIVE PHOSPHORYLATION or RESPIRATORY CHAIN

The last step in the aerobic respiration is the oxidation of reduced co-enzymes produced during glycolysis and Kreb's cycle by molecular O_2 through FAD, cyt.b, cyt.c, cyt.a and cyt.a_3 (cytochrome oxidase).

Two H-atoms or electrons from the reduced coenzyme ($NADH_2$ or $NADPH_2$) travel through FAD and the cytochromes each with a more positive oxidation-reduction potential and ultimately combine with1/2 O_2 molecule to produce one molecule of H_2O. This is called as **Termination Oxidation.** Details of electron transport system is shown in Fig.24.

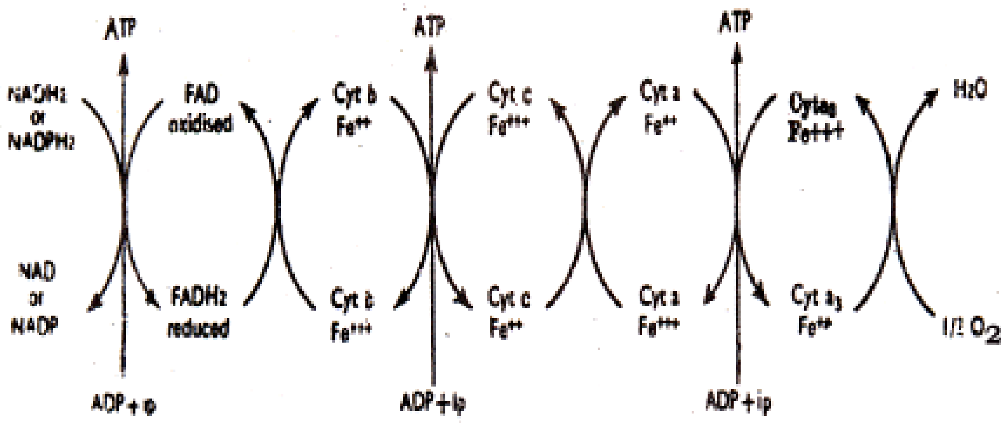


Fig.24. Electron transport system and oxidative phosphorylation

Oxidative Phosphorylation

During the electron transport, FAD and the iron atom of different cytochromes get successively reduced (FE^{2+}) and oxidised (FE^{3+}) and enough energy is released at some places which is utilised in the phosphorylation of ADP molecules to generate energy rich ATP moleculs.

Because this oxidation accompanies phosphorylation (production of high energy phosphate bonds of ATP and Pi), it is called as **Oxidative Phosphorylation**. It takes place on stalked particles situated on cristae in mitochondria. It is inhibited by 2,4-Dinitriphenol.

During the system, one ATP molecule (contains 7.6K.Cal. energy) is synthesized at each place when electrons are transferred from :

i)reduced $NADH_2$ $NADPH_2$ to FAD, ii) reduced Cytochrome b to Cytochromec, and
(iii) reduced Cytochrome a to Cytochrome a_3.

Thus, oxidation of one molecule of reduce $NADH_2$ $NADPH_2$ will result in the formation of 3 ATP molecules while oxidation of $FADH_2$ will lead to the synthesis of 2ATP molecules.

Complete oxidation of a glucose molecule (hexose sugar) results in the net gain of 38 ATP molecules as shown in the imp.15.

One glucose molecule contains about 686K.Cal. energy. The 38 ATP molecules will have 288.8K.Cal. energy. Therefore, about 40% (288.8/686) energy of the glucose molecule is utilised is utilised during aerobic breakdown and the rest is lost as heat.

Pentose Phosphate Pathway

Although glycolysis is the principal route of the conversion of carbohydrates into pyruvic acid in many biological systems, it is not the only metabolic route for breakdown of carbohydrates.

Presence of inhibitors like iodoacetate, fluorides, arsenates etc. specifically inhibit some steps of the glycolysis. Nevertheless, glucose utilization is not inhibited completely. This has led to the discovery of some other alternative routes of carbohydrate breakdown existing in plants. One such very common alternative route in plants in **Pentose Phosphate Pathway.**

It involves the oxidation of Glucose -6-phosphate to 6-Phosphogluconic acid which in turn is converted into pentose phosphates. In this pathway, glucose-6-phosphate is directly oxidised without entering glycolysis, hence it is also called as **Direct Oxidation Pathway or Hexose Monophosphate Shunt.**

In summary, 6 molecules of Glucose-6-phosphate which enter into this pathway, after oxidation produce 6 molecules of CO2 and 12 molecules of reduced coenzymes NADPH2 and while 5 molecules of Glucose-6-phosphate are regenerated as given below:

$$6(\text{Glucose-6-P}) + 12\ \text{NADP} \quad 5(\text{Glucose-6-P}) + 12\ \text{NADPH}_2 + 6\text{CO}_2$$

Significance of Pentose Phosphate Pathway

i. It provides alternative route for carbohydrate breakdown and provision of energy.
ii. It provides Ribose sugars for the synthesis of nucleic acids.
iii. It plays an important role in fixation of CO_2, in photosynthesis through Ribulose-5-P

Difference between oxidative phosphorylation and Photophosphorylation:

S.No.	**Oxidative Phosphorylation**	**Photophosphorylation**
1.	It occurs during respiration	It occurs during photosynthesis
2.	In general, it is found in mitochrondria	It takes place within the chloroplast.
3.	The process occurs on the inner membrane of cristae.	It occurs in the thylakoid membrane.
4.	Molecular O_2 is needed during terminal oxidation.	Molecular O_2 is not required.
5.	Energy released during electron transfer due to oxidation-reduction reaction is used during light.	Source of energy for conversion of ATP from ADP and Pi is external ATP formation.
6.	Process takes place in electron transport system involving cytochromes.	Pigment I and II are involved during the process.
7.	ATP molecules are released in the cytoplasm available for different metabolic reactions.	Produced ATP molecules are used up for CO_2 assimilation in the dark reaction of photosynthesis.

Anaerobic Respiration

In the absence of O2 or in anaerobic organisms, pyruvic acid is converted into either ethyl alchohol (ethanol) or lactic acid by fermentation process. This process is known as **Anaerobic Respiration.**

a. Proudction of Ethanol

In the production of ethanol, pyruvic acid is decarboxylated to acetaldehyde by pyruvate decarboxylase enzyme, followed by acetaldehyde reduction to ethanol by the enzyme alcohol dehydrogenase. During this process, 2ATP molecules are produced.

Pyruvic acid → Acetaldehyde

Acetaldehyde+ NADH → Ethanol + NAD +2ATP

Conversion glucose into ethanol is quite common in yeast cells when they are grown anaerobically. This process of fermentation is of commercial significance to produce alcoholic drinks on large scale.

b. Production of Lactic acid

In many algae and fungi, pyruvic acid is converted into lactic acid instead of ethanol under anaerobic conditions. In the reaction, pyruvic acid is converted into lactic acid by the action of lactate dehydrogenase enzyme. The reaction uses NADH generated in glycolysis. Therefore, there is no net production of NADH in lactic acid fermentation.

Pyruvic acid+ NADH2 ⇔ lactic acid + NAD

Respiratory Quotient

Respiration Quotient (RQ) may be defined as "*the ratio of the volume of CO_2 released to the volume of O_2 taken in respiration in a given period of time at constant temperature and pressure*".

$$RQ = \frac{\text{Volume of } CO_2 \text{ evolved}}{\text{Volume of } O_2 \text{ absorbed}}$$

The value of R Q depends upon the nature of respiratory substrate or on the amount of O2 present in therespiratory substrate and the extent to which substrate is broken down.

RQ of carbohydrates is **1** or unity

RQ of fats is about **0.7** or less than unity

RQ of proteins and theri derivatives is around **0.79**

RQ of succulents is **0** (Zero)

RQ of organic acids is about **1.33**

RQ of maturing fatty acids is more than unity **(>1.00)**

The following Table indicates RQ values in different plant parts and substrates:

S.No.	**Types of Plants**	**RQ**
1.	Leaves rich in carbohydrates	1.00
2.	Darkened shoots of Opuntia	0.30
3.	Germinating starchy seeds	1.00
4.	Germinating linseed (high fat)	0.64
5.	Germinating buckwheat(high protein)	0.54
6.	Germinating peas	1.54-2.40

Significance of RQ

By determining the value of RQ, the nature of respiratory substrate can be known. For example, if the value of RQ is one, it indicates that carbohydrates are being oxidised during respiration. Similarly, if the value is less than one, if will be concluded that macro molecules like fats constitute the respiratory substrate.

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Differences between Respiration and Photosynthesis :

S.No	**Respiration**	**Photosynthesis**
1.	Oxygen is absorbed in the process.	Oxygen is liberated in the process.
2.	CO_2 is liberated as a result of oxidation. of carbon containing compounds.	CO_2 is absorbed and is fixed inside to form carbon containing compounds.
3.	The proces occurs day and night.	Proces occurs only in the presence of light.
4.	Light is not essential for the process.	Proces occurs only in the presence of light.
5.	During the process, potential energy is converted into kinetic energy.	During the process, radiant energy (light energy) is converted into potential energy.
6.	Raw materials used are glucose and O_2 .	Raw materials used are CO_2 and water.
7.	The presence of chlorophyll is not necessary.	Chlorophyll is a pre-requisite for the process.
8.	Energy is released during the process, hence, it is an exothermic process.	Energy is stored during the process, hence, it is endothermic process.
9.	Due to respiration, the plant suffers with the loss of weight.	By the process, the weight is gained.
10.	It is a catabolic process and includes the destruction of stored food.	It is an anabolic process and includes the manufacture of food.
11.	The process includes dehydrolysis and and decarboxylation.	It includes the processes like hydrolysis and carboxylation.
12.	During the breakdown of one glucose molecule, **38 ATP** molecules are formed.	During the synthesis of one glucose molecule, **38 ATP** molecules are utilized.

Energy Budgeting or Balance Sheet of ATP Molecules in Glycolysis and Kreb's Cycle :

Reaction	**Gain of ATP per**	**Net gain of ATP**
A. Glycolysis		
1. Fructose 6P to Fructose		
2. Fructose 6P to Fructose	-	
3. 1,3 Diphospho glyceraldehyde to	-	
1,3 Diphosphoglyceric acid	2x3=6	
4. 1,3-Diphosphoglyceric acid to		
3-phosphoglyceric acid		Net gain
5. 2- Phosphoenol pyruvic acid to pyruvic acid	2x1=2	(10-2)=8ATP molecules.
	2x1=2	
	10	
B. Kreb's cycle		
6. Pyruvic acid to Acetyl CoA	2x3=6	
7. Isocitric acid to oxalosoccinic acid	2x3=6	
8. α–ketoglataric acid to succinyl acid	2x3=6	Gain of 30 ATPmolecule.
9. Succin CoA to Succinic acid	2x1=2	
10. Succinic acid to Fumaric acid	2x2=4	
11. Malic acid to oxaloacetric acid	2x3=6	
Grand total	**30**	**38 ATP molecules.**

Factors Affecting Respiration]

The rate of respiration is affected by two types of factors. These are discussed below:

A. Internal Factors

1. Protoplasmic factors

The amount of protoplasm in the cells and its state of activity influence rate of respiration. Rate of respiration is higher in young meristermatic cells, which divide actively and require more energy. In older tissues, the rate of respiration is lower because of lesser amount of not very active protoplasm.

2. Concentration of respiratory substrate

The rate of respiration depends much on the presence of the respiratory materials. Increased conc.of food materials brings about an increase in the rate of respiration, if other factors are not limiting.

B. EXternal Factors

1. Temperature

Like any other processes, the rate of respiration is also much affected by the temperature within certain limit Optimum temperature for respiration is about 30^0C, minimum 0^0C, and maximum about 45^0C.

At low temperatures, the respiratory enzymes become inactive, consequently the rate of respiration falls. An increase in temperature from 0 to 30^0C or more brings about an increased rate of respiration, the Q^{10} for respiration being 2.0 to2.5. But, at very high temperatures, respiration slows down and may even bestopped due to the denaturation of the respiratory enzymes.

2. Oxygen

When sufficient amount O_2 is available, the rate of aerobic respiration will be optimum while anaerobic respiration will be completely stopped. This is called as Extinction Point. In complete absence of O_2 , anaerobic repiration takes place while the aerobic respiration stops. If some amount of O_2 is available, the aerobic respiration will start and anaerobic respiration will slow down.

3. Carbon dioxide

Higher conc. of CO_2 in the atmosphere has retarding effect on the respiration especially in the poorly aerated soil.

4. Inorganic salts

The rate of respiration increases when a plant or tissue is transferred to salt solution.
This is called as “ Salt Respiration”.

5. Water

Rate of respiration decreases with decreased amount of water, so much so, that in dry seeds the respiration is at its minimum, mainly due to the inactive respiratory enzymes. Therefore, proper hydration of respiring cells is essential for respiration.

6. Light

Its effect is indirect on the rate of respiration through the synthesis of organic food matter (substrate) in photosynthesis.

7. Injury

Wounding of plant organs stimulates respiration in that organ. Because these wounded cells become meristermatic to form new cells to heal up the wound, and hence they require more energy which is supplied by increased respiration. After the wound is healed up, the rate of respiration becomes normal.

8. Pasteur's Effect

Presence of O_2 may sometimes lower down the rate of breakdown of sugar. This phenomenon is called Pasteur's Effect named after great scientist which is defined as " the inhibition of sugar breakdown due to the presence of O_2 under aerobic conditions" and the reaction is called as Pasteur's reaction. Pasteur's effect is said to occur due to the following reasons:

a. Pasteur's effect inhibits glycolytic enzymes and stops glycolysis
b. Formation of excess of CO_2 from degradation of compounds other than respiratory substrate.
c. Increased glycolysis with decreased O_2 tension.
d. Occurrence of partial oxidative glycolic products and oxidative anabolism.

light Compensation Point

In green plants, respiration occurs continuously for 24 hours, while photosynthesis occurs only during the day when light is available. The rate of photosynthesis is higher at about noon when light intensity is higher and is slower during morning or evening when the light intensity is low. However, there will be a time either in the morning or evening, when the rate of photosynthesis is equal to rate of respiration. This is called as **Light Compensation Point.**

Differences between Datk respiration and Photorespiration :

S.No.	Dark respiration	Phtorespiration
1.	Respiratory substrate may be carbohydrate, fat or protein.	The substrate is glycolate
2.	Substrate may be recently formed or stored one.	Substrate is always recently formed.
3.	Process occurs in cytoplasm and mitochondria.	It occurs between chloroplast, cytosol, peroxisomes and mitochondria.
4.	H_2O_2 is not formed during the process.	It is formed.
5.	In the process, several ATP molecules are produced.	ATP molecules are not formed.
6.	NAD is reduced to $NADH_2$	Here, $NADH_2$ is oxidised to NAD
7.	Transamination reaction does not occur.	Such reactions are involved in the process.
8.	It is dependent on O2 conc. only to a limited extent.	It shows close positive relation with $O_{2.}$
9.	Process is not so sensitive to rise in temperature.	Its rate is hightly accelerated in between 25 and35^{0}C.
10.	Process is found in all living cells	Its rate is highly accelerated in between 25 and35^{0}C.
11.	It is foud in dark as well as light.	It is found only in the presence of light.

Differences between aerobic and anaerobic respiration :

S.No.	**Aerobic respiration**	**Anaerobic respiration**
1.	It is common to all plants.	It is of rare occurrence.
2.	It goes on throughout the life.	It occurs for a temporary phase of life.
3.	Energy is liberated in larger quantity. In total, 38 ATP molecules are formed.	Energy is liberated in lesser quantity. Only 2 ATP molecules are formed.
4.	The process is not toxic to plants.	It is toxic to plants.
5.	O_2 is utilized during the process.	It occurs in the absence of $O_{2.}$
6.	Carbohydrates are oxidised completely and are broken down into CO_2 and water.	The carbohydrates are oxidised incompletely and ethyl alcohol and CO_2 are formed.
7.	The end products are CO_2 and water	The end products are ethyl alcohol and CO_2 .
8.	Process takes place partly (glycolysis) in cytosol and partly (Kreb's cycle) inside mitochondira.	The process occurs only in the cytosol.

Role of Respiration

Respiration is an important process because :

* It releases energy, which is consumed in various metabolic processes essential for plant life and activates cell division.

* It brings about the formation of other necessary compounds participating as important cell constituents.

* It converts insoluble food into soluble form.

* It liberates CO2 and plays active role in maintaining carbon balance in nature.

* It converts stored energy (potential energy) into usable energy (kinetic energy).