

Fundamentals of Crop Physiology

(PPY-121)

Prepared By

Dr. Mubeen



Faculty of Agriculture
Mohammad Ali Jauhar University, Rampur,

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1. Introduction to crop physiology and its importance in agriculture

Crop physiology is concerned with the processes and functions of the crops at cellular, sub-cellular and whole plant levels in response to environmental variables and growth. In short, physiology is the study of functional aspects of crop plants.

Crop physiology is important in agriculture because:

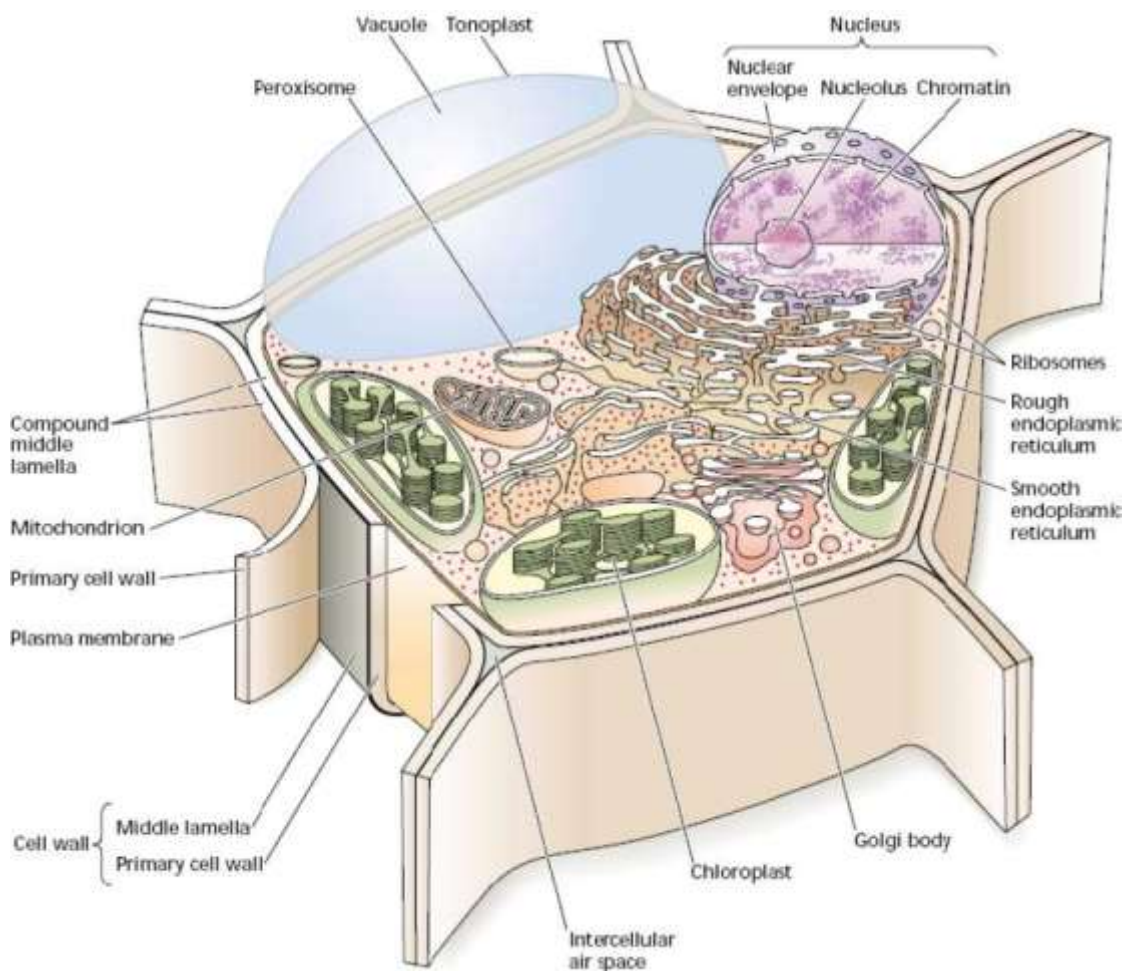
1. It studies the entire plant and its communities.
2. They deal with a plant in terms of knowledge from the different field such as soil science, plant physiology, botany etc.
3. One of the most important advantage in crop physiology was the elucidation of the subtle processes that regulate energy metabolism in green plants. Photosynthesis and respiration were found to be two related aspects of the same function—the metabolism of nutrients and energy.
4. It aims to "increase the yield" of the plant economically.

Crop yield is controlled by the interaction between the genetic potentialities of crop plants and the environment in which they grow. Variations in the genotype and in the environment, including weather and cultural practices, act through physiological processes to control growth. Thus the physiological processes of plants are the machinery through which both the genetic potentialities and the environment operate to produce the quantity and quality of growth or phenotype which we term yield.

1. Plant Cell: Cell structure and physiological functions of cell wall, cell inclusions

Cell: Plants are multicellular organisms composed of millions of cells with specialized functions. At maturity, such specialized cells may differ greatly from one another in their structures. However, all plant cells have the same basic eukaryotic organization: They contain a nucleus, a cytoplasm, and sub cellular organelles, and they are enclosed in a membrane that defines their boundaries.

In plants, cell migrations are prevented because each walled cell and its neighbor are cemented together by a middle lamella. As a consequence, plant development unlike animal development, depends solely on patterns of cell division and cell enlargement. Plant cells have two types of walls: primary and secondary. Primary cell walls are typically thin and are characteristic of young, growing cells. Secondary cell walls are thicker and stronger than primary walls and are deposited when most cell enlargement has ended. Secondary cell walls owe their strength and toughness to lignin, a brittle, glue-like material. The evolution of lignified secondary cell walls provided plants with the structural reinforcement necessary to grow vertically above the soil and to colonize the land.



Plant anatomy

There are two categories of seed plants, gymnosperms and angiosperms. Gymnosperms are the less advanced type. Angiosperms, the more advanced type of seed plant which dominate the landscape. About 250,000 species are known, but many more remain to be characterized. The major innovation of the angiosperms is the flower; hence they are referred to as flowering plants. Three major tissue systems are found in flowering plants; in all plant organs contain dermal tissue, ground tissue, and vascular tissue. The vegetative body is composed of three organs: leaf, stem, and root. The primary function of a leaf is photosynthesis, that of the stem is support, and that of the root is anchorage and absorption of water and minerals. Leaves are attached to the stem at nodes, and the region of the stem between two nodes is termed the internode. The stem together with its leaves is commonly referred to as the shoot.

Cell structure and functions

Cell has non living outer layer called Cell wall found only in plant cells. Below cell wall is cell membrane encloses protoplasm. Protoplasm has semi fluid matrix called Cytoplasm and large membrane bound structure called Nucleus.

Cytoplasm has many membrane bound organelles like Endoplasmic reticulum , Golgi Bodies Mitochondria ,Plastids and vacuoles. They also have non membrane bound structures called Ribosomes and Centrosomes. Cytoplasm without Cell organelles are called Cytosol.

The plant cell and the animal cell can be differentiated by the presence of organelles in them. Although both are classified as Eukaryotes, the presence of the cell wall, vacuoles, and chloroplasts are the most remarkable and distinguishing components of the plant cells which are absent in the animal cells. Even the size of the animal cell is smaller than the plant cell.

The concept of cell originated from the historical work done by the Schleiden and Schwann in 1838. Cells exist in an amazing variety of sizes and shapes. Likewise the

living beings, the individual cells that form the body can grow, reproduce, process information, as well respond to stimuli. Despite the differences among different kinds of the cells whether it is plant cell or animal cell, single celled or multi cell, they all share certain common features and carry out different complicated process in the almost same way. The multicellular organisms contain billions or trillions of cells organized complexly, while unicellular consist of the single cell only. But even that single cell organisms will define itself by exhibiting all the remarkable properties that a cell needs to become a fundamental and structural unit of life. In this content, we will take-up the salient features of the plant cells and animal cells and how they differ from each other.

Difference between Plant cell and Animal cell

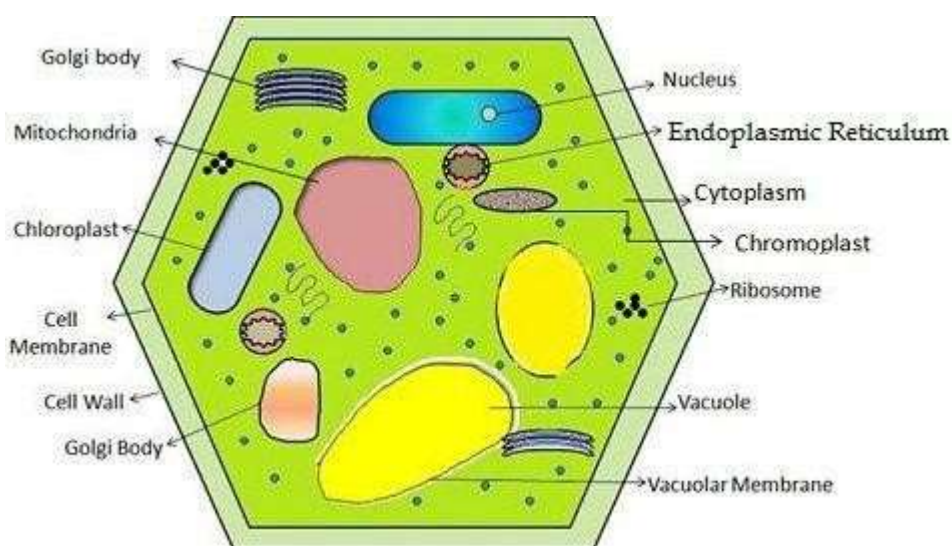
BASIS FOR COMPARISON	PLANT CELL	ANIMAL CELL
Meaning	The fundamental and functional unit of Kingdom Plantae of the Eukaryotic cells, having true nucleus along with the many organelles, specially the cell wall, chloroplast and the vacuoles.	Animal cells are also the basic unit of life of Kingdom Animalia of the Eukaryotic cells, having all the necessary organelles with specified functions.
Cell Size	Usually larger, which is fixed.	Smaller in size and irregular.
Cell Shape	Rectangular.	Round.
Enclosed by	A plant cell is enclosed by rigid cell wall along with the plasma membrane.	The animal cell is enclosed by a flexible, thin plasma membrane only.
Nucleus	Present and lies on one side of	Present and lies in the

	the cell.	centre of the cell wall.
Centrosomes/Centrioles	Absent	Present
Plastids	Present with chloroplast in them.	Plastids are absent.
Cilia	Absent.	Usually present.
Glyoxysomes	May be present.	Absent.
Plasmodesmata	Present.	Absent.
Desmosomes/Tight junction	Absent.	Present.
Mitochondria	Present in fewer number.	Present in large number.
Vacuoles	Only one huge vacuole.	Animal cells contain many in numbers.
Lysosomes	Rarely noticed in plant cells.	Present.
Chloroplast	Plant cell contains chloroplast, which they use in storing energy.	Animal cells lack chloroplast and use mitochondria for energy storing purpose.
Reserve food	Present as starch.	Present as glycogen.
Synthesis of nutrients	They can synthesize all amino acids, vitamins and coenzymes.	They are not able to synthesize any amino acids, vitamins and coenzymes required by them.

Cytokinesis	Occurs by cell plate only.	Occurs by furrowing or constrictions.
Hypotonic/Hypertonic Solutions	Plant cell does not burst if placed in hypotonic solution.	Animal cells burst in hypertonic solution as they do not have the cell wall.

Plant Cell

Mainly Kingdom Plantae consists of multi-cellular eukaryotes living things, which are autotrophic by nature. As we discussed above that the organelles in plant cell like – chloroplast, cell wall, and vacuoles distinguishes them from the animal cells. Till yet around 400,000 number of plants species have been identified, and there is the lot remain undiscovered.



Normally the range of plant cells varies from 10-100 μm in size. Plant cell carries out the function of photosynthesis, due to which the green plants are called as autotrophs. This is done by the presence of chlorophyll in the chloroplast of the plant cells. The cell wall is made up of cellulose, which provides support and rigidity to the cells.

Types of Plant Cells:

1. Parenchyma - These are the structurally simplest cells, and have thin walls. They are used for storage of organic products.
2. Collenchyma - These have thin walls, with thickening at some parts of the cell. These cells provide structural support to the cell.
3. Sclerenchyma - The cell wall of this cell are embedded with lignin.
4. Water Conducting Cells – The vascular tissue in plants known as Xylem, helps in transmitting water from roots to other parts of the plants.
5. Sieve Tube Members – The another plant tissue known as Phloem, helps in transporting food and nutrients. This (food) is prepared in the green leaves by the process of photosynthesis.

3. Cell organelles and their physiological functions.

Functions of some important organelles:

Plasma Membrane – As discussed above that it controls the movement of the molecules in and out of the cell and function in cell-cell signaling and cell adhesion. It is the outermost layer of the cell and the protect the internal organelles also.

Mitochondria – It is called as ‘the powerhouse of the cell’ as ATP (adenosine triphosphate) is generated by oxidation of glucose and fatty acids.

Lysosomes - It has the acidic lumen which degrades material engulfed by the cell, and worn out cellular membranes and organelles. They are regarded as the digestive tract of the cell.

Nuclear envelope – This is the double layer membrane, protecting the contents of the nucleus.

Nucleus – It contains the hereditary material and is filled with chromatin made up of DNA and proteins.

Endoplasmic reticulum (ER) – It is of two types Smooth endoplasmic reticulum and Rough endoplasmic reticulum. In Smooth endoplasmic reticulum, lipids are synthesized, and detoxification occurs of the hydrophobic compounds. In Rough endoplasmic reticulum protein synthesis, processing takes place.

Golgi Complex – This organelle processes and sorts lysosomal proteins, secreted proteins and membrane proteins synthesized on the rough endoplasmic reticulum.

Secretory vesicles - It stores secreted proteins and fuse with the plasma membrane to release their content.

Peroxisomes – Also known as microbodies and are the single membrane cellular bodies. They are oval or spherical and contain the enzyme catalase. Peroxisomes detoxify the molecules and break down the fatty acids to produce acetyl groups for biosynthesis.

Cytoskeletal fibers – It forms the network and bundles that support cellular membrane, and help organize organelles and supports the cell movement. The cellular matrix is collectively referred to as cytosol. The cytosol is a compartment containing several metabolites, enzymes, and salts in an aqueous gel like the medium.

Microvilli – It increases the surface area for absorption of the nutrients from surrounding medium.

cell inclusions: They are the products of cell metabolism, appearing and disappearing at various stages of cell's life-cycle. In majority of cases they are waste products of simple chemical nature compared to protoplasmic components which are more complex.

These ergastic substances may be present in the cell walls or vacuoles or in the organelles of protoplasm. They may be present in soluble or insoluble state and may be organic or inorganic in nature.

These substances belong to three categories:

1. Reserve food,
2. Inorganic Materials (Mineral matter)
3. Secretory products
4. Excretory products.

1. Reserve food:

They occur in the form of starch, glycogen, fat droplets and aleurone grains.

- (i) Starch grains (Fig. 3.48):

Starch is the most important storage food. It is insoluble in water. Starch grains are found in all parts of the plant although in storage organs, e.g., seeds, fruits, rhizome etc., these are found in larger amount.

It is of two types:

- (a) Temporary starch and
- (b) Permanent starch.

The temporary starch, which is also known as assimilatory starch, is formed in the process of photosynthesis during day and converted to sugar during night. The permanent starch, which is also known as reserve starch, is found mostly in rhizome, seeds and fruits. Sugar above a certain level is converted to permanent starch.

- (i) Glycogen Granules:

They are minute granules of storage carbohydrates which occur in animal cells. The granules are flattened, circular or oval bodies which may form rosette-shaped aggregates.

(ii) Fat droplets:

They occur in both plant and animal cells. In plants fat droplets or globules occur abundantly inside the seeds either in endosperm (e.g., castor, coconut) or cotyledons [e.g., groundnut, mustard].

(iii) Aleurone Grains : They are insoluble storage proteins occur inside special leucoplasts called aleuroplasts. They occur in the outer endosperm cells of cereals, such as wheat, rice, maize grains.

. Inorganic Materials (Mineral Matter).

The accumulation of inorganic materials within the plant and their cells mostly takes place in the form of calcium salts or anhydrous silicate salts. One very important type of deposit is that of calcium oxalate which is common in plants of many families. Their crystalline particles are of various shapes such as prismatic, needle-shaped, rhomboidal (diamond-shaped) etc. Very often the crystals occur as compound aggregates called druses, sphaerites etc. Elongated crystals are called styloids and raphides. Raphides occur in the form of bundles. Some crystals occur in special type of cells such as in case of idioblast cells.

Very often, crystals of calcium carbonate are also found in some plants. One very well known example is the cystolith found in some plants [e.g., Ficus leaves), which are found on an outgrowth of cell wall towards the interior of cell and this outgrowth bears CaCO_3 depositions. Leaves of Ficus species have cystoliths in their epidermal cells.

3. Secretory Products:

Many substances secreted by special glands and organs, are found in plants, such as:

(i) Colouring matter:

Plants possess green colouring matter because of the presence of chlorophyll a and chlorophyll b. They also contain orange and yellow pigments, carotene and xanthophyll.

The flowers and fruits become differently coloured because of the presence of carotene and xanthophyll. Blue, purple and pink colours are due to anthocyanin pigments which are found in vacuolar sap of fruits and petals of flowers and young leaves of some plants.

(ii) Enzymes:

Enzymatic proteins occur in colloidal state in the protoplasm. These enzymes convert complex organic food into simple compounds. For instance, enzyme diastase converts starch into glucose.

(iii) Nectar:

Nectar, secreted by nectaries in plants, attracts insects for pollination because it is sweet and contains sucrose, glucose and fructose.

4. Excretory Products:

Several chemical substances which are of no use to plants are produced during metabolic reactions. These waste products are called excretory products, but the plants do not have any special mechanism to remove these substances. However, some of these are thrown away by way of dropping of old leaves, bark and flowers. These excretory products are found as cell inclusions.

Some of the excretory products are:

(i) Alkaloids: These are nitrogenous compounds, made up of carbon, hydrogen, oxygen and nitrogen. They are found in storage organs of plants such as seeds, bark and leaves. They are insoluble in water but soluble in alcohol. They have sour taste and

some are poisonous. However, a large number of alkaloids, such as quinine, reserpine, nicotine, caffeine, thein, strychnine, morphine, atropine, are used as medicines.

(ii) Glucosides:

These are degradation products of carbohydrates. Some, such as digitoxin used in heart diseases, are used as medicine.

(iii) Tannins:

They are sour in taste and related to glucosides. They occur in vacuolar sap, cell wall, bark and leaves of some plants. They are found mostly in unripe fruits. They are used on a large scale for hardening of leather, a process called tanning of leather.

(iv) Latex:

It is a milky substance secreted by latex glands. Rubber secreted by the rubber tree *Hevea brasiliensis* is an important example.

(v) Essential oils:

These are volatile oils produced by special glands and cells. Aromatic flowers, leaves and bark are due to essential oils.

(vi) Resins:

Produced by the oxidation of essential oils. These are found in some special glands or canals either alone or in combination with essential oils. These are insoluble in water but soluble in ether and alcohol. These are used in the manufacture of paints and varnishes.

(vii) Gums:

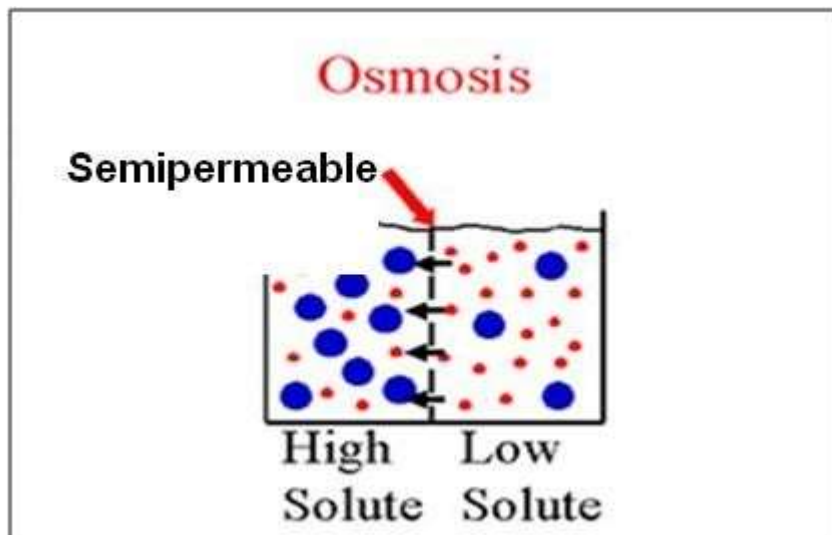
Produced by the disintegration of cellulose cell wall. They are soluble in water. Used for sticking purposes, and also as medicine.

(viii) Organic Acids:

These are found in leaves and fruits. Tartaric acid is found in fruits of Tamarindus, oxalic acid in Oxalis and citric acid in Citrus fruits.

4. Diffusion of water: Diffusion, osmosis and imbibition, plasmolysis measurements of water status in plants, water potential and its components.

osmosis and imbibition: These are unique phenomena and play an important role in plant development. Osmosis may be considered as special type of diffusion characterized by the movement of water through a differentially permeable membrane. Imbibition is a special type of diffusion in which an adsorbent is involved.



Osmotic potential and Pressure

Osmotic pressure is actually a potential and is not usually reached or measured in plant cells. It is a measure of the absence of energy to do work or capacity to flow in an ideal osmotic situation. The osmotic pressure can be demonstrated and measured

by a simple apparatus termed as **osmometer** in which two compartments are separated by differentially permeable membrane.

$$\Pi = \frac{N}{V} \times RT \text{ or } \Pi = CRT$$

Where: Π = osmotic potential, N = numbers of moles, V = volume in liters, R = gas constant, T = absolute temperature,

$$C = \frac{N}{V} = \text{Concentration}$$

The negative sign is inserted to denote osmotic potential that it characterizes a solution in several ways. It indicates the maximum pressure (osmotic) that might develop if the solution were allowed to come to equilibrium with pure water in an ideal osmotic system, and it is proportionately related to the amount of solutes in a solution and to the decrease in chemical potential (total free energy) due to solute- solvent interactions.

Turgor pressure

Turgor pressure (or hydrostatic pressure) which can also be defined as pressure exerted by protoplast over cell wall which pushes the plasma membrane against the rigid cell wall and provides a force for cell expansion.

The cell wall, being rigid, exerts an equal and opposite pressure called **wall pressure** which can also be defined as pressure exerted by the cell wall over protoplast. The first, easily observed sign of a water deficit in a plant is a decrease in the turgor of its leaf cells giving the leaves wilted appearance.

Diffusion pressure deficit – Wall pressure tends to force the water out of cell and acting against the osmotic entry of water in the cell. Now absorption of water takes place which depends upon difference between osmotic pressure and wall pressure. During this time wall pressure equals turgor pressure. This difference is called **diffusion**

pressure deficit which is the difference between osmotic pressure and turgor pressure and can be expressed as follows:

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

Water potential

The chemical potential is the free energy per mole of any substance in a chemical system. Generally chemical potential of water is referred as **Water potential (Ψ_w)**. When water potential is expressed it is expressed as the difference between the chemical potential of water at any point in a system (μ_w) and that of pure water under standard conditions (μ_w°) with the expression:

$$\Psi_w = \mu_w - \mu_w^\circ = RT \ln \frac{e}{e^\circ}$$

Where, R is the gas constant (erg/mol /degree), T is the absolute temperature ($^\circ\text{K}$), e the vapour pressure of the solution in the system at temperature T and the vapour pressure of pure water at the same temperature. The expression RT in (e / e°) is zero which indicates that pure water has a potential of zero. In a biological system (e / e°) is less than zero making in (e / e°) negative. Pure water is defined as having a potential of zero, any dilution of water with a solute establishes a potential that is less than that of pure water and expressed as a negative number. Both water potentials and chemical potentials can be expressed in energy units. It is more convenient to express water potentials in pressure units (atmosphere, bars, megapascal) which can be obtained by dividing water potential by partial molar volume of water (V_w):

$$\frac{\mu_w - \mu_w^\circ}{V_w} = \frac{RT \ln \frac{e}{e^\circ}}{V_w}$$

The units of the above equation are:

$$\frac{\text{Erg/mole}}{\text{Cm}^2/\text{mole}} = \frac{\text{erg}}{\text{cm}^3} = \text{dyne/cm}^2$$

1bar = 0.987 atm. = 10^6 dynes/cm²,

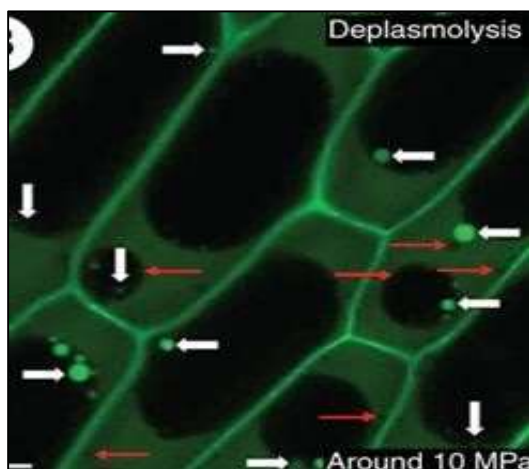
10bars = 1megapascal (mpa)

If some substance such as sugar is dissolved in pure water contained in a beaker, the resulting solution has an osmotic potential that is lower (more negative) than that of pure water. Since this is an unconfined solution (not under the pressure of a piston or cell wall, the turgor pressure is zero. An increase in solute decreases the free energy and will produce more negative osmotic hence water potential.

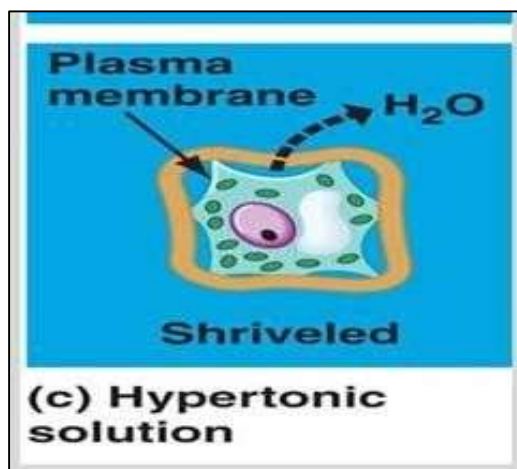
Plasmolysis:- Shrinking of the protoplasm of a cell placed in an hypertonic (low osmotic potential) solution, away from the cell wall, due to loss of water. If we place a living plant cell in a solution with an osmotic potential to that of its own cell sap (**an isotonic solution**), the appearance of the cell remains normal in every respect. However, if the water potential of the surrounding solution is less negative than that of cell sap (hypotonic) or more negative than that of cell sap (**hypertonic**), we can easily observe several changes in cell structure. If we immerse epidermal tissue from the leaves of Rhoeo or Zebrina in a hypertonic solution of sucrose, we can observe the plasmalemma pulling away from the cell wall which is referred to as **plasmolysis**. In the first case the water inside the cell has a greater free energy and thus a greater tendency to flow outward. Second, the cell and vacuolar membranes are practically impermeable to sucrose but readily permeable to water. Third, the cell wall will allow the free passage of both sucrose and water. Thus there will be a net movement of water out of the cell vacuole and into the external solution, water will move from a region of less negative (high) to a region of more negative (low) water potential. This movement of water results in a loss of turgor, a shrinking of the vacuole, and pulling away of the cell membrane from the cell wall. **Incipient plasmolysis** is the initial pulling away of the membrane from the cell wall. At this point the turgor pressure is zero. If the process continues, there will be a tendency for the cell wall to be pulled towards the cytoplasm because of cohesive and adhesive properties of water between the cell wall and plasmalemma. This cell is then said to be under tension, and the turgor pressure becomes negative. Eventually, the forces exerted by the retracting plasmalemma will become greater than

those between the water molecules of the cell wall. Complete plasmolysis follows, with the plasmalemma being pulled entirely away from the wall.

Hypotonic



Deplasmolysis



Plasmolysis

Deplasmolysis

Regaining of original state by plant cell or tissue as a result of water absorption. Plasmolysed cells can be deplasmolysed, if a cell that has been plasmolysed is placed in a hypotonic solution, it will regain its turgidity.

Osmotic potential measurements

The boiling point of an aqueous solution is higher than that of pure water, the vapour pressure of the water in a solution is lower than that of pure water, and a solution freezes at a lower temperature (**freezing point depression**) than pure water. These factors, called the colligative properties of solutions, are interrelated, and the extent to which each factor is affected is directly proportional to the number of dissolved particles (molecules or ions) present. Therefore, a measure of any one of these factors is an indirect measure of the osmotic potential because it is also one of the colligative properties of the solutions. Generally, we do not use boiling point elevation to measure the osmotic potential of the cell sap. However, we can measure the vapour pressure depression and freezing point depression of expressed plant juices with a considerable degree of accuracy. For example, the theoretical freezing point depression of a 1 molal

solution composed of nonionised solute has a freezing point depression of -1.86°C and a theoretical osmotic potential of $-22.7\text{ bars}(-22.4\text{ atm.})$. We can easily arrive at an equation relating these two factors, freezing point depression and osmotic potential and we can use this equation to determine the osmotic potential of a solution of unknown concentrations.

Therefore,

$$\psi_s = \frac{-22.7 \times \Delta \text{fp}}{-1.86}$$

In this equation, Δ stands for the observed freezing point depression of the unknown solution. If, for example, some plant juice is expressed and found to have a freezing point depression of 1.395 , the osmotic potential of this solution would be:

$$\psi_s = \frac{-22.7 \times 1.395}{-1.86} = 17.025\text{ bars}$$

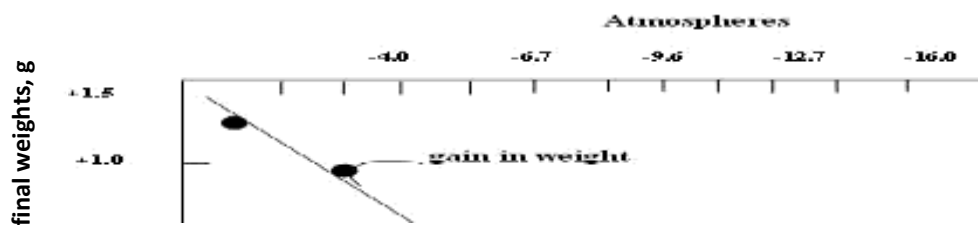
The determination of a solution's osmotic potential by determination of its freezing point is called **Cryoscopy** and the technique is referred to as **Cryoscopic** method.

Water potential measurements

Volume method

The volume method of measuring water potential is based on changes in linear dimensions (length) of a tissue when it is placed in solutions of different osmotic potentials. When solutions are placed in a beaker, there is no turgor pressure and solutions are unconfined and at this moment $\psi_w = \psi_s$. This situation is not true for plant cells. Strips of root, fruit or leaf tissue, 3 to 4 cm long and of the same width, are measured and placed in the series of different concentrations of sucrose solutions for about 1 hour. The tissues are removed and remeasured. The change in length is then plotted against the known osmotic potentials of the solution. The water potential of the solution $\psi_w = \psi_s + 0$ in which the tissue does not change in length is the same as the water potential ($\psi_w = \psi_s + ?$) of the tissue.

Gravimetric method



This method involves the placement of preweighed plant tissue into a graded series of solutions of sucrose or other **osmoticum (osmotically active solute)** at known osmotic potential.

A representative sampling of tissue is incubated for predetermined time in the solutions, removed and reweighed. The weight gain or loss is plotted against the water potential of each solution. When the points are connected, the intercept at the abscissa (through zero) represents the water potential of the tissue, with the zero weight gain or loss. The water potential of the solution corresponding to the intercept point is equal to that of the tissue.

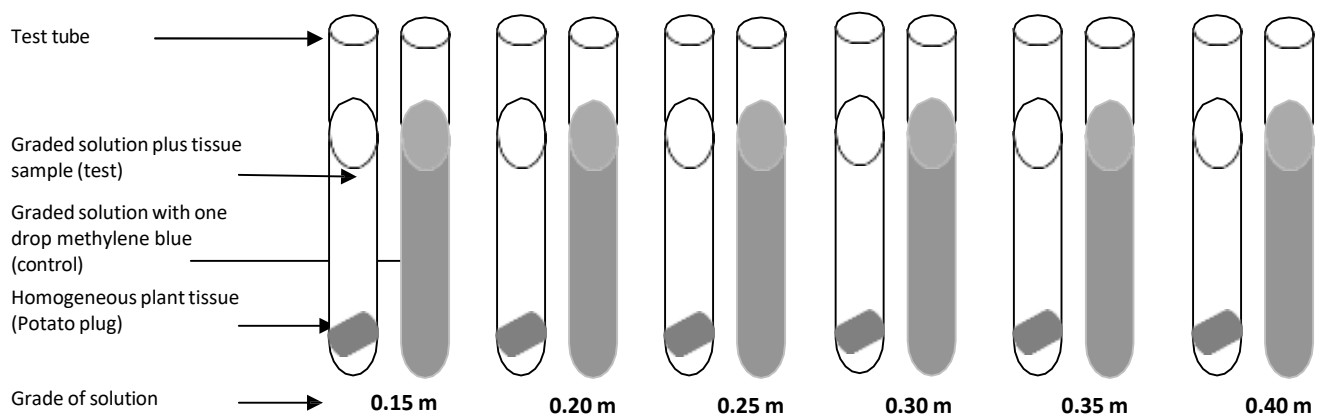
Chardakov's or Falling drop method

Two graded series of sucrose solutions (ranging from 0.15 to 0.50 molal in increments of 0.5 molality) are placed in test tubes set up in duplicate. Homogenous plant tissue is placed into each test tube of one of the series (test series). Only one drop of methylene blue is mixed into each solution of the second series (control series). Plant tissue is not added to the control series and the dye does not appreciably change the osmotic potentials.

After the tissue has incubated for 15 to 30 minutes, it is removed from each tube. The actual time of incubation can be just long enough for osmosis to proceed and change the concentration of each solution in the test series, the attainment of equilibrium is not necessary. After the tube is removed, a small drop of the respective control series solution is introduced below the surface of its corresponding test solution.

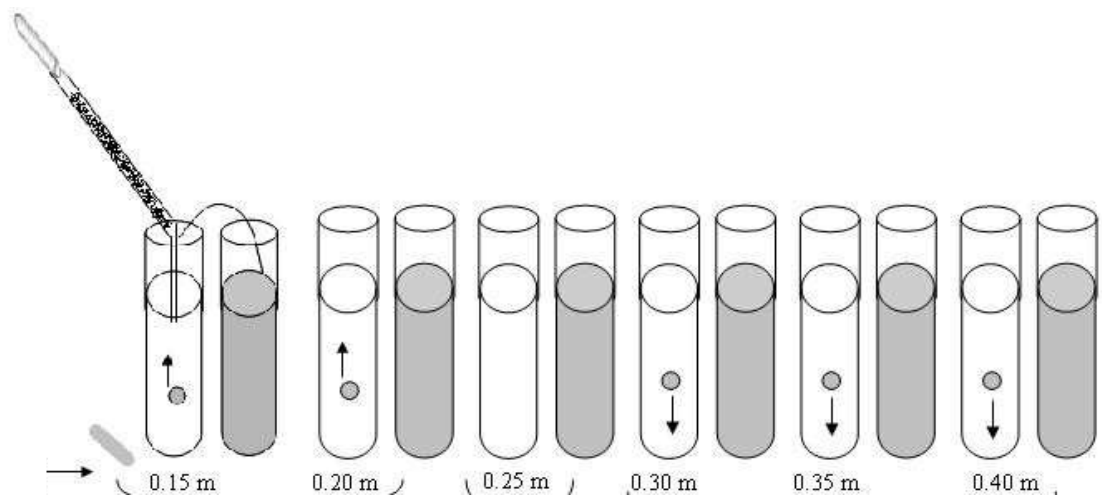
If the drop rises in the test solution, it means that the drop is lighter and that the tissue inoculation solution is more concentrated and this indicates that water from the solution entered the tissue. If drop falls, it means that the test solution is lighter which indicates that water has left the tissue and diluted the solution. In the latter instance, the water potential of the solution initially is more negative than that of the tissue. Accordingly, if the density of the drop from the methylene blue solution is the same as that of the test solution, the drop will diffuse into the solution uniformly. At this point (called the null point), the water potential of the tissue and solution is equal.

It is also possible to determine the solution changes with a refractometer (refractometer method) instead of falling drop. The refractometer is used to measure directly the concentration changes that take place in the tissue incubation solutions. No change in concentration indicates that the solutions have the same water potential as that of the tissue cells. This method does not require the methylene blue dye and experimental error due to technique is minimized.

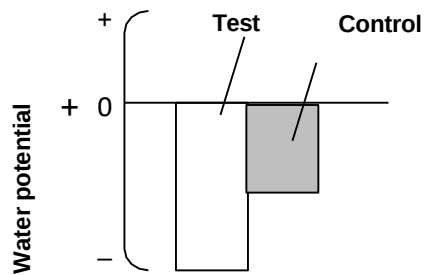


Step 2 - Incubate series for 15 to 30 minutes

Step 3 - Remove tissue and introduce drop of control solution into test solution



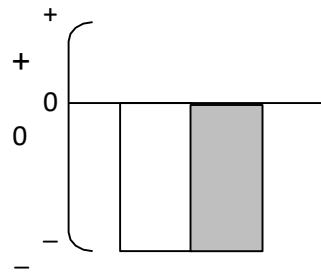
Tissue removed



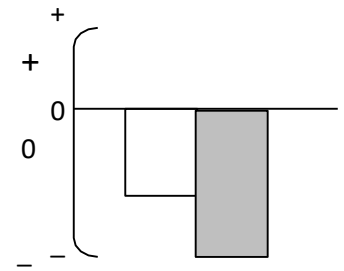
Conclusions about
initial water potentials

Test $>$ Control

vapour pressure



Test = Control



Test $<$ Control

(Thermocouple Psychrometer Method)

The vapour pressure is based on the fact that tissue will not gain or lose water to the atmosphere when the vapour pressure of air corresponds to the water potential of the tissue. The most extensively used apparatus is constructed for measurements of humidity inside a closed chamber containing two thermocouple junctions. One remains at the temperature of the air in the chamber, the other cools rapidly when a weak current is passed through the two junctions. Moisture from the air in the chamber will eventually condense on the cooling thermocouple. The drop of moisture then acts as a wet bulb. The water potential of the air in the chamber is equal to the difference between the temperature of the wet bulb and that of the dry thermocouple.

Pressure Bomb

Pressure bomb is the device that is used to determine the plant moisture stress and the water potential of a leafy shoot and is based on the assumption that the water column in a plant is almost always under tension because of the pull exerted by the osmotic influences (water potential) of the living cells of the leaves. If the tension is high, the water potential of the leaf cells is very negative. When a stem is cut, the water column is

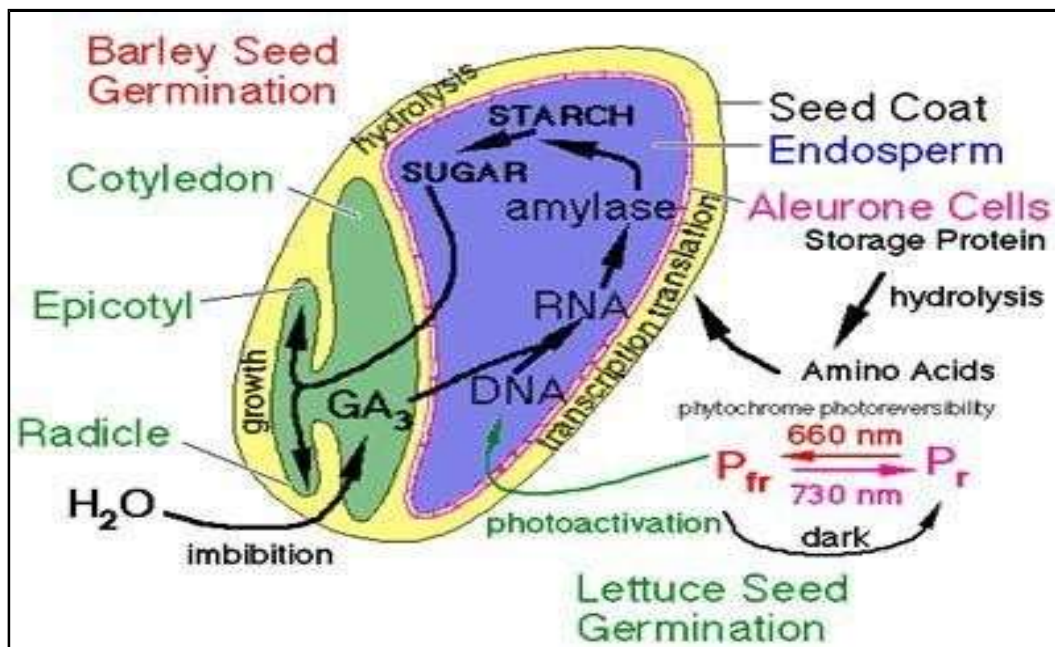


disrupted, and because the water column is under tension, it will recede back into the stem towards the leaves. The shoot is placed in the chamber, with the cut end protruding through an air tight hole. Pressure is increased within the chamber, and the water column within the twig is forced back to the cut surface. The pressure in the chamber is then carefully recorded.

The pressure required to force the water to appear at the cut surface is equal to the tension (but with the opposite sign) of the water column at the time the shoot was cut. If low pressure is sufficient to force water to appear at the cut surface of the shoot, the living cells mainly of the leaves have slightly negative water potentials, with the shoot being under relatively low moisture stress. But high pressure is required to force water to the cut surface the moisture stress(tension) is relatively high due to very negative water potentials of the leaf cells.

Imbibition

It is the adsorption of water by the hydrophilic colloids of plant material. As with osmosis, imbibition may be considered a special type of diffusion since the net movement of water is along a diffusion gradient. In this case an adsorbent is involved. If dry plant material is placed in water, a noticeable swelling takes place and sometimes amounts to a considerable increase in volume. Tremendous pressures can develop if an



adsorbent is confined and then allowed to imbibe water. For example, dry wooden stakes, driven into a small crack in a rock and then soaked, can develop enough pressure to split the rock.

Conditions necessary for imbibitions

Two conditions are necessary for imbibition to occur 1. A water potential gradient must exist between the surface of the adsorbent and the liquid imbibed. 2. A certain affinity must exist between components of the adsorbent and the imbibed substance.

Matric potential

The term Matric potential is analogous to osmotic potential in that it represents the potential maximum pressure that an adsorbent will develop if submersed in water. The actual pressure that develops when water is imbibed may be thought of as turgor pressure (pressure potential). Accordingly the following equation can be presented:

$$\Psi_w = \Psi_m + \Psi_p$$

Factors affecting rate and extent of imbibitions

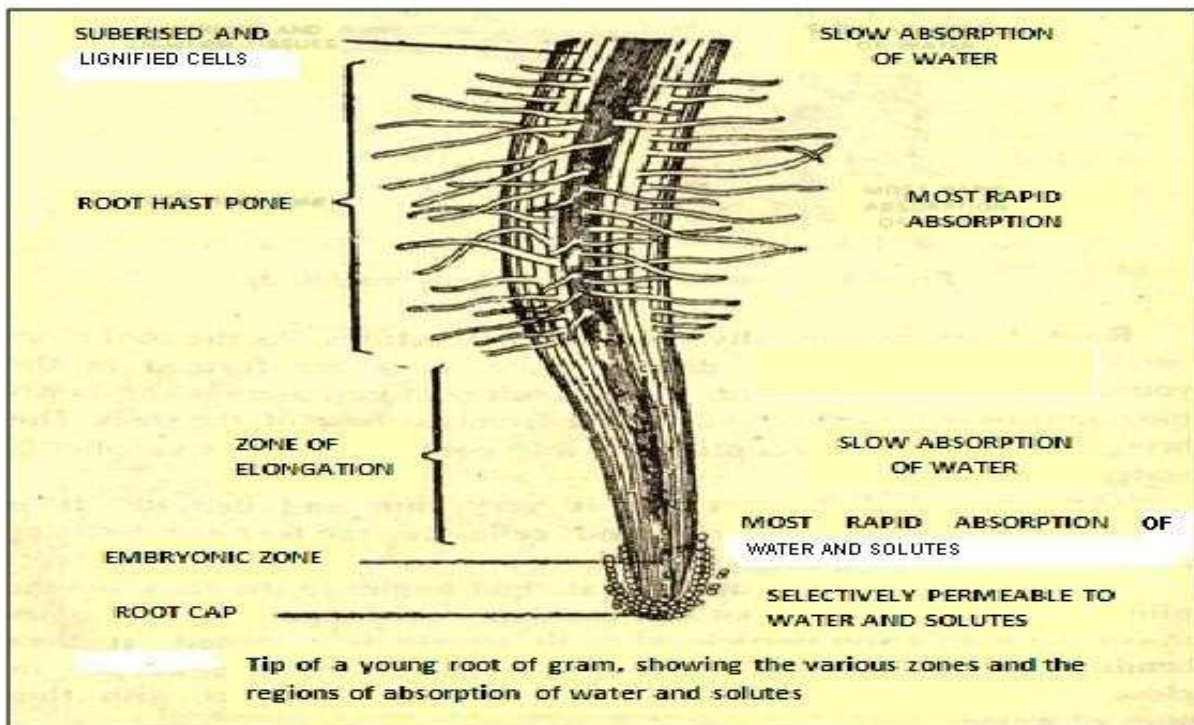
The rate and extent of imbibition is affected primarily by the temperature and by the osmotic potential of the substance to be imbibed. Temperature does not affect the amount of water taken up by the adsorbent, but it does have a definite effect on the rate of imbibition. An increase in temperature causes an increase in the rate of imbibition. Both the amount of water imbibed and the rate of imbibition are affected by the osmotic potential of the substance to be imbibed. The addition of solute to pure water causes a more negative water potential. This addition has the effect of altering the water potential gradient between the solution water and the adsorbent. The water potential gradient is less steep than it would be if the same adsorbent was submersed in pure water. Similarly, a decrease in the water potential gradient will bring about a decrease in the rate at which water is imbibed and thus the amount of water taken up.

5. Absorption of water: Water absorbing system of plant, Kinds of soil water in relation to water absorption.

Water absorbing system of plant

The main part of the plant which is concerned with water absorption is the root. The depth deep root system, whereas, some are surface feeders associated with shallow root system.

The absorption of water does not take place from entire surface of root. Only younger portions near the tip are active in absorption of water and mineral substances. More



number of tips in roots favour higher absorption. Extensively developed root system is associated with higher absorption rates in view of more number of active tips in roots. Most of the water absorption is carried out by the younger part of the roots. Just behind the growing tip of a young root is the piliferous region, made up of hundreds of projections of the epidermal tissue, the root hairs.

Terms contacted with soil water

Soil water: Soil is a great reservoir of water for plants.

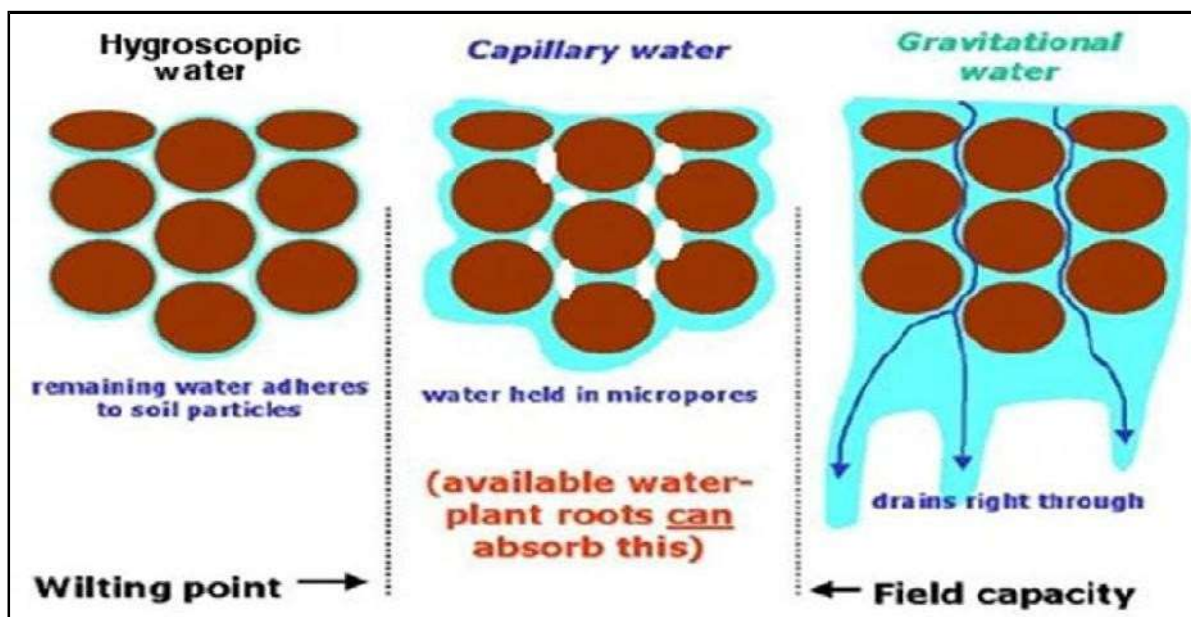
Run away water: After a heavy rainfall or excess irrigation some part of water drains away along slopes which is not available for plant growth.

Gravitational water: Some part of water percolates downward through larger pores between soil particles under influence of gravitational force till it reaches the water table, not available for plant growth.

Hygroscopic water: Water adsorbed on the surface of soil colloids in the form of tightly held thin film. Not available for plant growth.

Capillary water: Water fills the spaces between noncolloidal smaller soil particles and forms films around them, available for plant growth.

Chemically combined water: Water bounds to soil minerals by strong chemical bonds, not available for plants.



Field capacity or water holding capacity

Much of the rain water is retained by soil particles against the force of gravity and makes the soil wet. The amount of water which soil retains after the excess amount of water is removed is also called field capacity.

Water table

At some depth in soil all the pore spaces are filled with water. If a hole is bored in the soil water will appear at this point.

Water use efficiency

Ratio between the gain of (above-ground) biomass in growth or CO_2 in photosynthesis and transpirational water loss.

Wilting coefficient or wilting point

Amount of moisture left in soil after a plant has wilted. This is expressed as a percentage of dry weight of soil. It is lowest for sandy soil, high for loam soils and still higher for clayey soils. Osmotic pressure at permanent wilting becomes 15 atmosphere.

Temporary or transient wilting

Despite of sufficient moisture in soil plants may exhibit sign of wilting. This happens generally in a afternoon of a hot summer day when transpiration exceeds absorption but plants recover again at night automatically when transpiration is reduced and absorption is still continued. This type of wilting is associated with the diurnal fluctuation of water content.

6. Mechanism of water uptake and transport by apoplastic and symplastic methods, factors affecting water absorption.

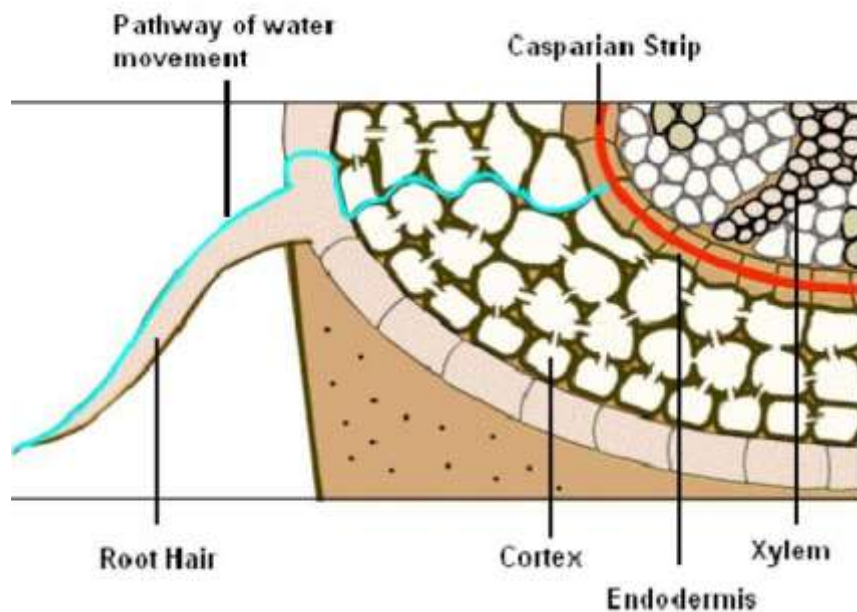
WATER MOVEMENT MECHANISM IN PLANTS

In plants, following two pathways are involved in the water movement. They are

- (1) Apoplastic pathway
- (2) Symplastic pathway
- (3) Transmembrane pathway

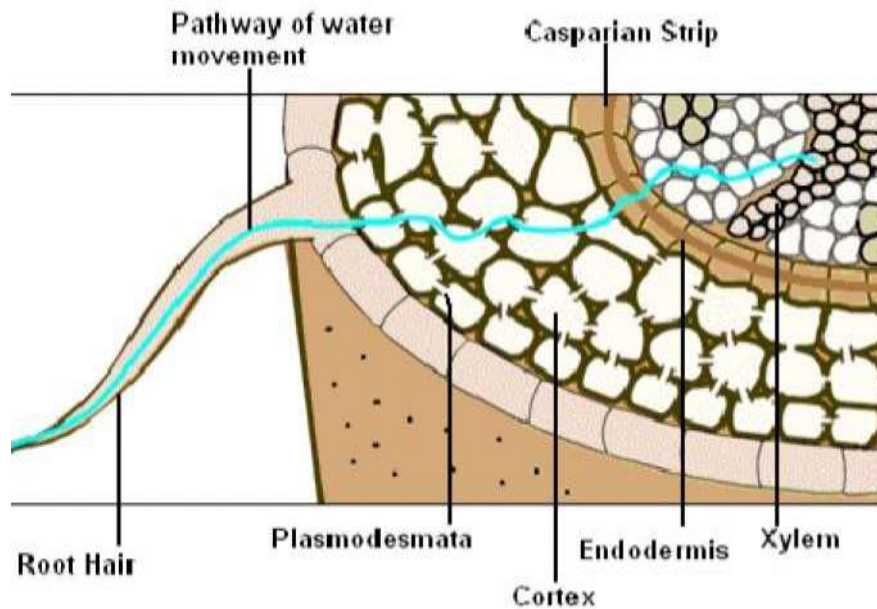
1. Apoplastic pathway

The apoplastic movement of water in plants occurs exclusively through the cell wall without crossing any membranes. The cortex receive majority of water through apoplastic way as loosely bound cortical cells do not offer any resistance. But the movement of water in root beyond cortex apoplastic pathway is blocked by casparian strip present in the endodermis.



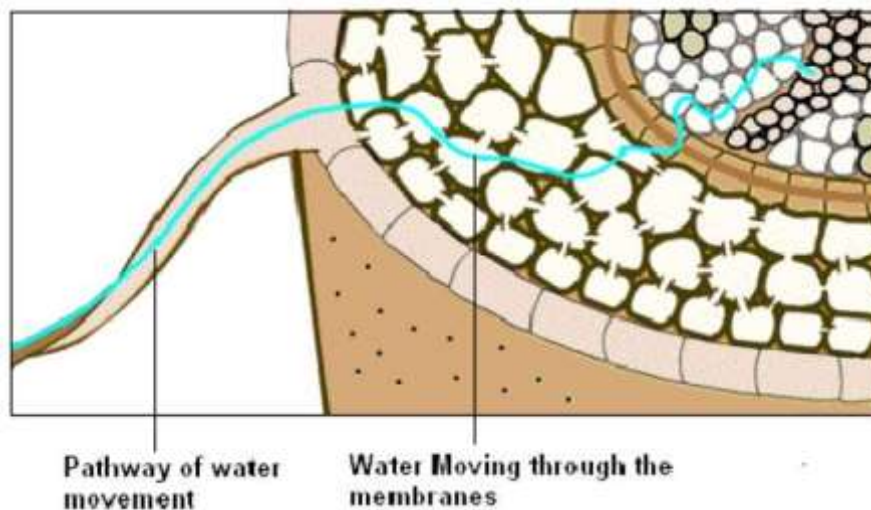
2. Symplastic pathway

The movement of water from one cell to other cell through the **plasmodesmata** is called the symplastic pathway of water movement. This pathway comprises the network of cytoplasm of all cells inter-connected by plasmodesmata.

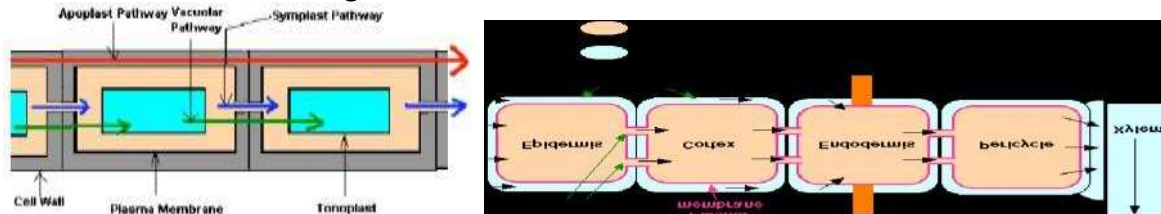


3. Transmembrane pathway

In plant roots, water movement from soil till the endodermis occurs through apoplastic pathway i.e. only through cell wall. The casparian strips in the endodermis are made-up of wax -like substance suberin which blocks water and solute movement through the cell wall of the endodermis. As a result water is forced to move through cell membranes and may cross the tonoplast of vacuole. This movement of water through cell membranes is called transmembrane pathway.

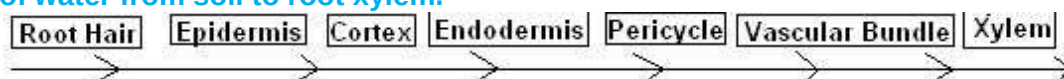


Following schematic diagram showing the apoplastic and symplastic pathway of water movement through root



Apoplastic (Red) and symplastic (Blue) and transmembrane (green) pathways of movement of substances in a plant cell

With the help of the following schematic arrow flow chart, you can understand the path of water from soil to root xylem.



MECHANISM OF WATER ABSORPTION

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.

Steps involved in the active osmotic absorption of water

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

Now the cortical cells adjacent to root hairs have high OP, SP & DPD in comparison to the root hairs. Therefore, water is drawn into the adjacent cortical cells from root hairs by osmotic diffusion. In the same way, by cell to cell osmotic diffusion gradually reaches the inner most cortical cells and the endodermis. Osmotic diffusion of water into endodermis takes place through special thin walled passage cells because the other endodermis cells have casparian strips on thin walls which are impervious to water. Water from endodermis cells is down into the cells of pericycle by osmotic diffusion which now become turgid and their suction pressure is decreased. In the last step, water is drawn into xylem from turgid pericycle cells (In roots the vascular bundles are

radical and protoxylem elements are in contact with pericycle). It is because in the absence of turgor pressure of the xylem vessels, the SP of xylem vessels become higher than SP of the cells of the pericycle when water enters into xylem from pericycle a pressure is developed in the xylem of roots which can raise the water to a certain height in the xylem. This pressure is called as root pressure

(A) Osmotic absorption

Water is absorbed from the soil into the xylem of the roots according to osmotic gradient.

(B) Non-osmotic absorption

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

2. Passive absorption of water

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

STEPS:

1. Transpiration creates tension in water in the xylem of the leaves
2. Tension is transmitted to water in xylem of root thro' xylem of stem and water rises upward to reach transpiring surface
3. Hence soil water enters cortical cells thro' root hairs to reach xylem of roots to maintain the supply of water.
4. The force for entry of water in leaves is due to rapid transpiration and root cells remain passive

2. Passive absorption of water

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

roots to maintain the supply of water. The force of this entry of water is created in leaves due to rapid transpiration and hence, the root cells remain passive during this process.

External factors affecting absorption of water

1. Available soil water

Sufficient amount of water should be present in the soil in such form which can easily be absorbed by the plants. Usually the plants absorb capillary water i.e water present in films in between soil particles other forms of water in the soil eg. Hygroscopic water, combined water, gravitational water etc. is not easily available to plants. Increased amount of water in the soil beyond a certain limit results in poor aeration of the soil which retards metabolic activities of root cells like respiration and hence, the rate of water absorption is also retarded.

2. Concentration of soil solution

Increased concentration of soil solution (due to presence of more salts in the soil) results in higher OP. If OP of soil solution will become higher than the OP of cell sap in root cells, the water absorption particularly the osmotic absorption of water will be greatly suppressed. Therefore, absorption of water is poor in alkaline soils and marshes.

3. Soil air

Absorption of water is retarded in poorly aerated soils because in such soils deficiency of O_2 and consequently the accumulation of CO_2 will retard the metabolic activities of roots like respiration. This also inhibits rapid growth and elongation of the roots so that they are deprived of fresh supply of water in the soil. Water logged soils are poorly aerated and hence, are physiologically dry. They are not good for absorption of water.

4. Soil temperature

Increase in soil temperature up to about $30^\circ C$ favours water absorption. At higher temperature water absorption is decreased. At low temperature also water absorption decreased so much so that at about $0^\circ C$, it is almost decreased. This is probably because at low temperature.

1. The viscosity of water and protoplasm is increased
2. Permeability of cell membrane is decreased

3. Metabolic activity of root cells are decreased
4. Root growth and elongation of roots are checked.

7. Transpiration Mechanism of transpiration, driving force, soil-plant-atmosphere continuum, advantages of transpiration, factors affecting transpiration, anti-transpirants.

Transpiration

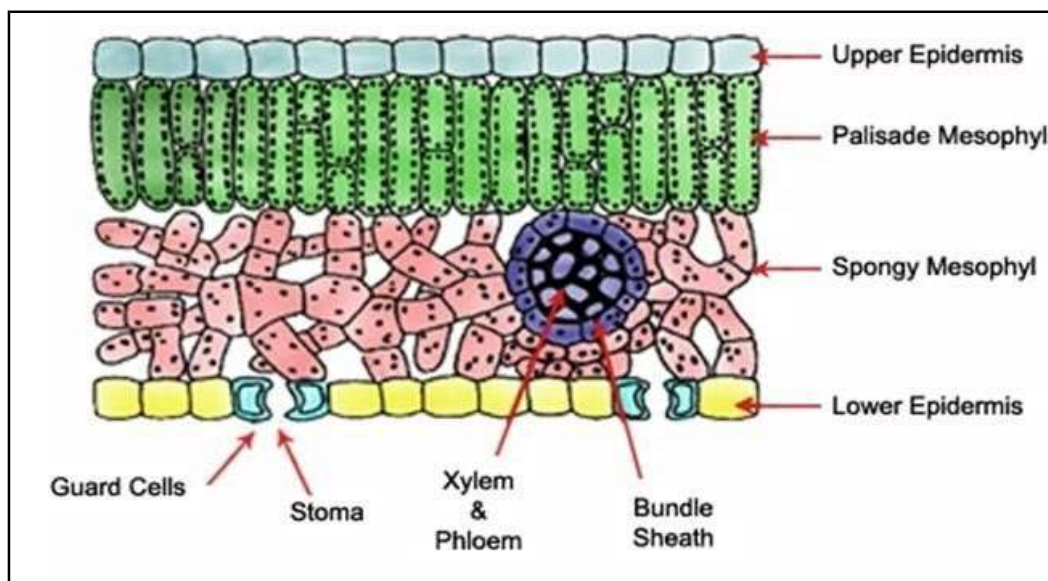
Diffusion of water vapours from aerial parts of the plants to the ambient atmosphere occurs through stomata, cuticle and lenticels is called transpiration. It is a vital phenomenon and regulated by cells, whereas, evaporation is a physical phenomenon. Generally 20-50 pounds of water is transpired for every ounce of dry matter produced.

Kinds of transpiration

Most of the transpiration takes place through leaves also called foliar transpiration. Foliar transpiration is of two kinds stomatal and cuticular. Some transpiration also takes place through lenticels called lenticular transpiration.

Mechanism

The transpiration takes place through stomata when the walls of mesophyll cells become saturated with water.



Between upper and lower epidermis of leaf mesophyll cells exist which consists of palisade cells and spongy parenchyma cells. The spongy parenchyma cells are connected with the atmosphere by means of stomata mostly found in lower epidermis. The xylem of leaf veins supply water to the mesophyll cells by osmotic diffusion. They become turgid and saturated with water. Water evaporates from then moist cells into internal atmosphere of intercellular spaces of mesophyll which becomes saturated with water. The outer atmosphere is usually unsaturated. This results in the diffusion of water from intercellular spaces of leaf into outer atmosphere through stomata. This is referred as stomatal transpiration .Due to loss of water the concentration of water vapour into intercellular spaces decreases which results in the more evaporation of water from mesophyll cells. Some transpiration also takes place by direct evaporation from the outer walls of epidermal cells through cuticle. This constitutes cuticular transpiration. The cuticle being impervious to water the amount of water loss is comparatively less between 3-10%. In stems, fruits and flower parts the transpiration is mostly cuticular. In herbaceous plans where cuticle is poorly developed cuticular transpiration almost equals stomatal transpiration. In woody stems some transpiration also takes place through lenticels (certain cracks developed on the bark) .This constitutes lenticular transpiration.

Driving force in transpiration

- Difference in water vapour concentration between leaf mesophyll cells (air space) and external air.
- Diffusional resistance of this pathway which includes resistance associated with diffusion through stomatal pore and leaf boundary layer resistance depends on layer of unstirred air next to the leaf surface through which water vapour must diffuse to reach turbulent air of atmosphere.

Soil – plant – atmosphere continuum

It includes the following:

- In the soil and xylem water moves by bulk flow in response to a pressure gradient ($\Delta \Psi_p$).
- In a vapour phase water moves primarily by diffusion, at least until it reaches the outside leaf, where convection (a form of bulk flow) becomes dominant.
- When water is transported across membranes, the driving force is water potential difference across the membrane. Such osmotic flow occurs when cells absorb water and when roots transport water from soil to the xylem.
- In all of these situations water moves towards region of low water potential or free energy.

To increase CO₂ uptake and reduce transpiration plants must have:

- An extensive root system to extract the water from the soil.
- A low resistance pathway through xylem vessel and tracheids to bring water to the leaves.
- A hydrophobic cuticle covering surfaces of the plant to reduce evaporation.
- Microscopic stomata on the leaf surface to allow gas exchange.
- Guard cells to regulate the diameter (and diffusional resistance) of stomata aperture.

Advantages of transpiration

- It creates a suction force to absorb water and minerals from the soil.
- The main force in ascent of sap is brought about by transpirational pull.
- It affects DPD thus helps diffusion through cells.
- Plants generally absorb excess quantities of water. If this water is not transpired it will disturb the osmotic balance between cells and also bring about decay of tissues. Transpiration stream helps in solute transport from one part of plant to another.

- Transpiration stream helps in the translocation of solutes from one part of the plant to another.
- Metabolic activities in plants increases the temperature and transpiration brings down the temperature. Thus the temperature within the plant is maintained.
- Opening and closing of stomata during transpiration indirectly influences the photosynthesis and respiration.
- It enhances the movement of molecules within the plants.

Disadvantages

In some soils where water availability is in scarcity the excess transpiration may even kill the plant. It has been found out that plant cells can maintain their turgidity even in absence of transpiration.

Anti-transpirants

Substances which reduce transpiration rate by causing stomatal closure partially. Examples - Colourless plastics, silicone oil, low viscosity waxes, abscisic acid, CO₂ when concentration increases from 0.03% to 0.05%.

Magnitude of transpiration

The amount of water a plant actually uses is small as compared to the large quantities it transpires. Transpiration rates of some herbaceous plants are so great that, under favourable conditions, the entire column of water in a plant may be replaced in the course of the single day (Stiles 1924). For example, it has been estimated that a single corn plant may transpire up to 54 gallons of water in a growing season. At this rate, a single acre of corn could transpire the equivalent of 15 inches of water during one growing season. The amount of water loss varies from species to species.

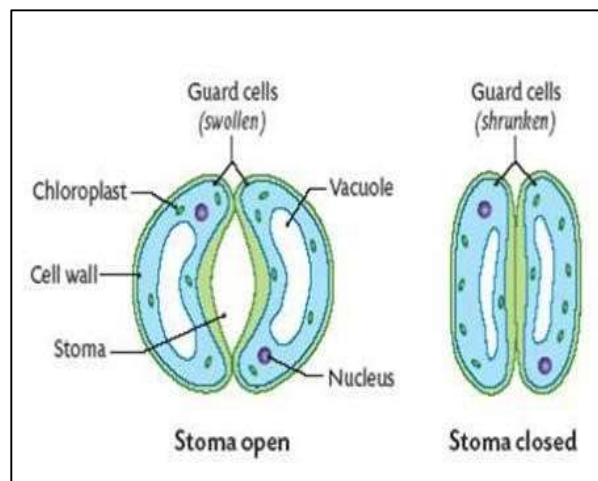
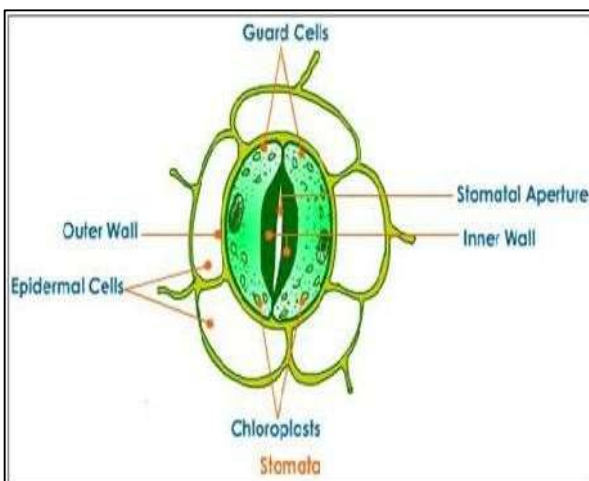
8. Stomatal physiology: Structure of stomata and mechanism of opening and closing, classification of stomata, theories of mechanism of opening and closing of stomata, bleeding, guttation

STOMATAL REGULATION

Structure of stomata and mechanism of opening and closing

Stomata are important structures which facilitates transpiration and gaseous exchange during photosynthesis and respiration. They are minute structures generally elliptical (oval in shape) and found mostly in lower surface of leaf. A stoma is covered with two epidermal cells called guard cells. They are smaller in size than other epidermal cells. In dicots they are kidney shaped. In some species examples grasses adjacent to guard cells some specialized structures exist called accessory or subsidiary cells. A stoma contains nucleus, vacuoles and chloroplasts.

Stomata open with increase in turgidity of guard cells and with decrease in turgidity stomata become narrow and closes. The walls surrounding the pore are thick and inelastic and resists stretching. The outer walls are thin, elastic and are stretched when subjected to pressure. When water enters guard cells they become turgid . The outer thin walls are stretched, while inner thick walls fail to stretch and are pulled away and become concave. This increases the gap between the guard cells resulting in opening of stomata. With the loss of turgor, the inner walls of guard cells become straight and approach each other, the pore becomes narrow and finally closes. Light, external CO₂ concentration and water content of leaf cells affect stomatal opening. Generally stomata open in light and close in dark.



Stomatal frequency

This is calculated as number of stomata per unit area of leaf. Stomatal index is however more accurate as it gives the relative ratio of epidermal cells to stomata.

This can be calculated as follows:

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

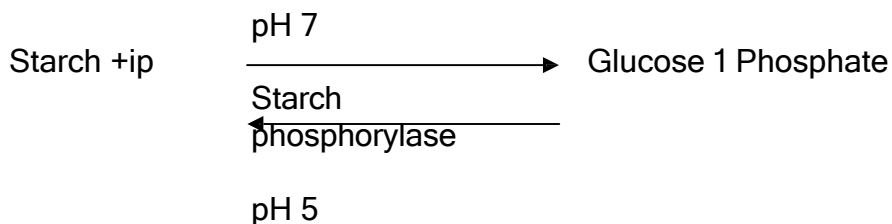
Where I = Stomatal index, E = Number of epidermal cells, S = Number of stomata / unit area.

The size of stomatal aperture varies from $7 \times 3 \mu$ in *Phaseolus vulgaris* to $38 \times 8 \mu$ in *Avena sativa*. The number of stomata / sqm. of leaf surface varies between 50- 300 .

Theories of mechanism of opening and closing of stomata

Starch – sugar hypothesis

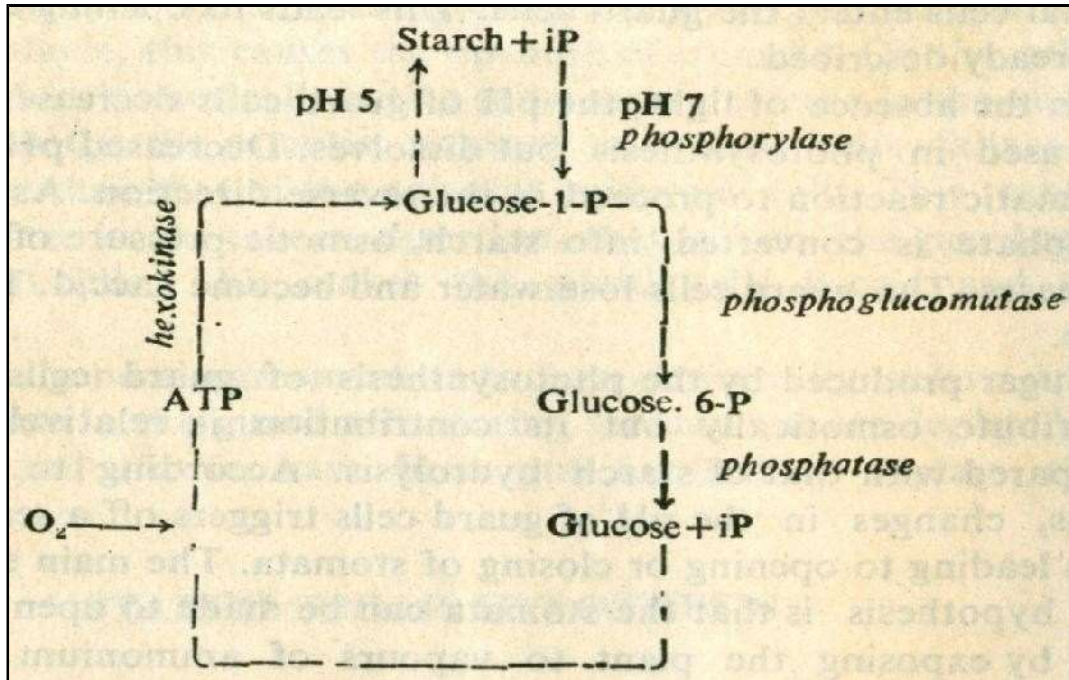
Given by J.D. Sayre (1926); Scarth (1932) and Small and Clarke (1942). In dark CO_2 which is not utilized in photosynthesis found dissolved in guard cells forming the carbonic acid (H_2CO_3) due to this pH decreases. In day photosynthesis starts due to light in guard cells carbonic acid is removed, as a result pH of guard cells increases. Starch formed during night converted to glucose 1 phosphate. Starch is insoluble and osmotically inactive but glucose phosphate is soluble and increases osmotic pressure of guard cells. As a result of this water will enter from surrounding cells to guard cells increasing the turgor pressure of guard cells resulting in opening of stomata.



Steward's (1964) scheme

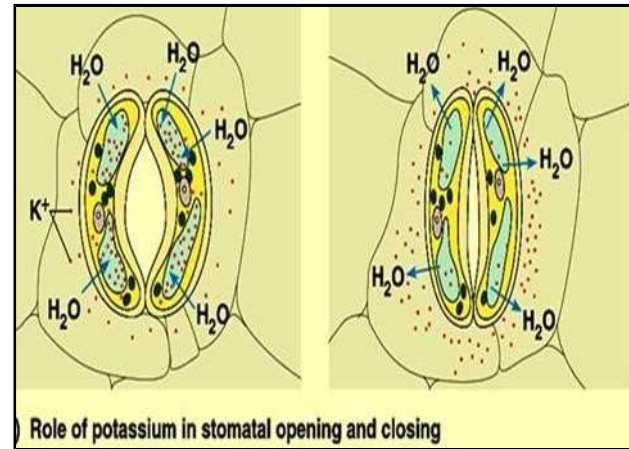
Steward had proposed some modified scheme over Starch - sugar mechanism.

Glucose 1 phosphate splits into glucose and ip both are osmotically active and together give to the guard cells a higher osmotic pressure. During closing of stomata in dark glucose converted back to glucose 1 phosphate. This transformation requires O_2 and metabolic energy in the form of ATP.

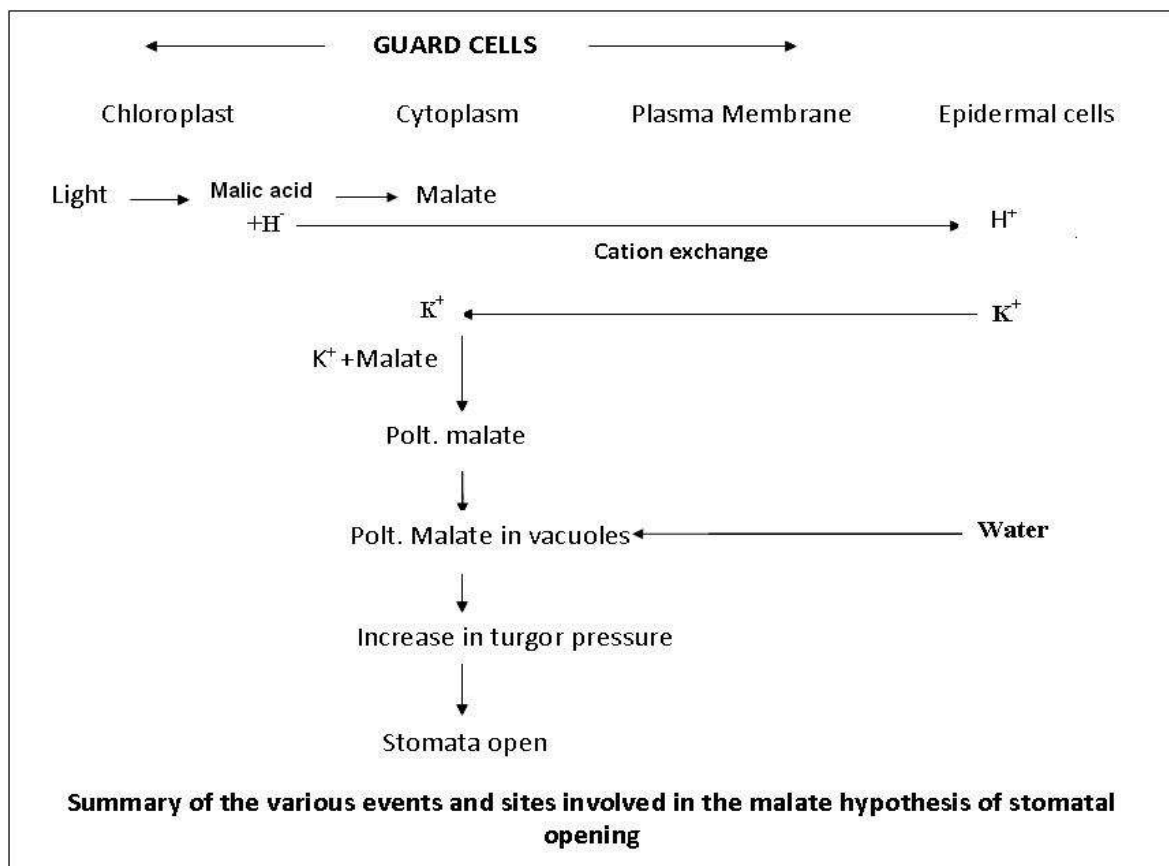


Malate hypothesis or potassium ion exchange theory

This is also called Potassium ion exchange theory. According to this theory (Levitt 1974) starch produces malic acid during respiratory pathway in presence of light in chloroplast. Malic acid is then excreted into cytoplasm of guard cells. It is a weak acid therefore dissociates into Malate and H^+ ions. The H^+ ions are removed from guard cells into surrounding



cells and K^+ ions take place of them in guard cells. This increases the osmotic pressure of guard cells. In dark H^+ ions would enter into guard cells replacing the K^+ ions. H^+ ions combines with malate to form malic acid which is used in respiration.



Stomatal movement in succulent plants

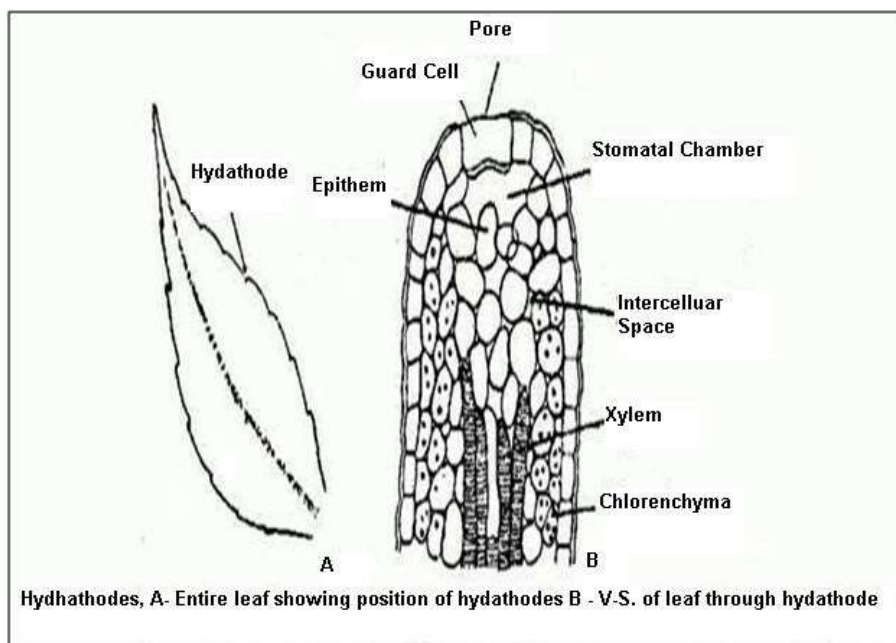
Succulent plants like Bryophyllum, Cactus form malic acid in night which increases the osmotic pressure of guard cells which results in opening of stomata. During the day the malic acid disappears. Due to formation of malic acid in dark pH should become low and stomata should remain closed but malic acid being strong solute increases the osmotic pressure of guard cells resulting in opening of stomata. In such plants starch sugar mechanism may not operate.

Bleeding

Exudation of liquid water, sap and dissolved substances from injured part of plant.

Guttation

Oozing of water drops from leaf tip where principal vein ends. Conditions that promote active absorption of water and hinder transpiration favour guttation. Phenomenon is seen in oat, potato, tomato, garden nasturtium and grasses. The water of



guttation contains carbohydrates, nitrogenous compounds, organic acids and mineral salts. Guttation takes place from more or less spherical structures called hydathode or water stomata. They are commonly found in plants inhabiting humid tropics. It consists of large sized pore which always remains open. Beneath the pore is an air cavity. Below it a loose tissue called epithem made up of small cells without chlorophyll. Underneath this are tracheids. The liquid is forced out of tracheids into intercellular spaces of epithem by root pressure.

9. Mineral nutrition of plants: Criteria of essentiality of elements, essential elements, Physiological role of elements in plants in plants.

The term, *mineral nutrient* is generally used to refer to an inorganic ion obtained from the soil and required for plant growth. The chemical form in which elements are applied to plants is called as *nutrient*. Nutrition may be defined as the supply and absorption of chemical compounds needed for plant growth and metabolism. The nutrients indispensable for the growth and development of higher plants are obtained from three sources viz., atmosphere, water and soil. The atmosphere provides carbon and oxygen as carbon dioxide. Carbon is reduced during photosynthesis and oxygen is utilized during aerobic respiration. Soil provides the mineral ions.

Criteria of essentiality of elements

Many scientists have given the criteria on that basis the elements are designated as essential elements as described below:

Arnon and Stout (1939)

- The element must be essential for growth and reproduction of plant. Its absence hinders such activities.
- The requirement for the element must be specific and can not be replaced by any other element.
- The element must be acting directly inside the plant not simply causing other element to be readily available or reversing the toxic effect of other elements.

Epstein (1972)

- An element is essential, if plant can not complete its life cycle due to its absence.
- An element is essential, if it is a part of molecule which itself is essential in plants like nitrogen in protein and magnesium in chlorophyll.

Essential elements

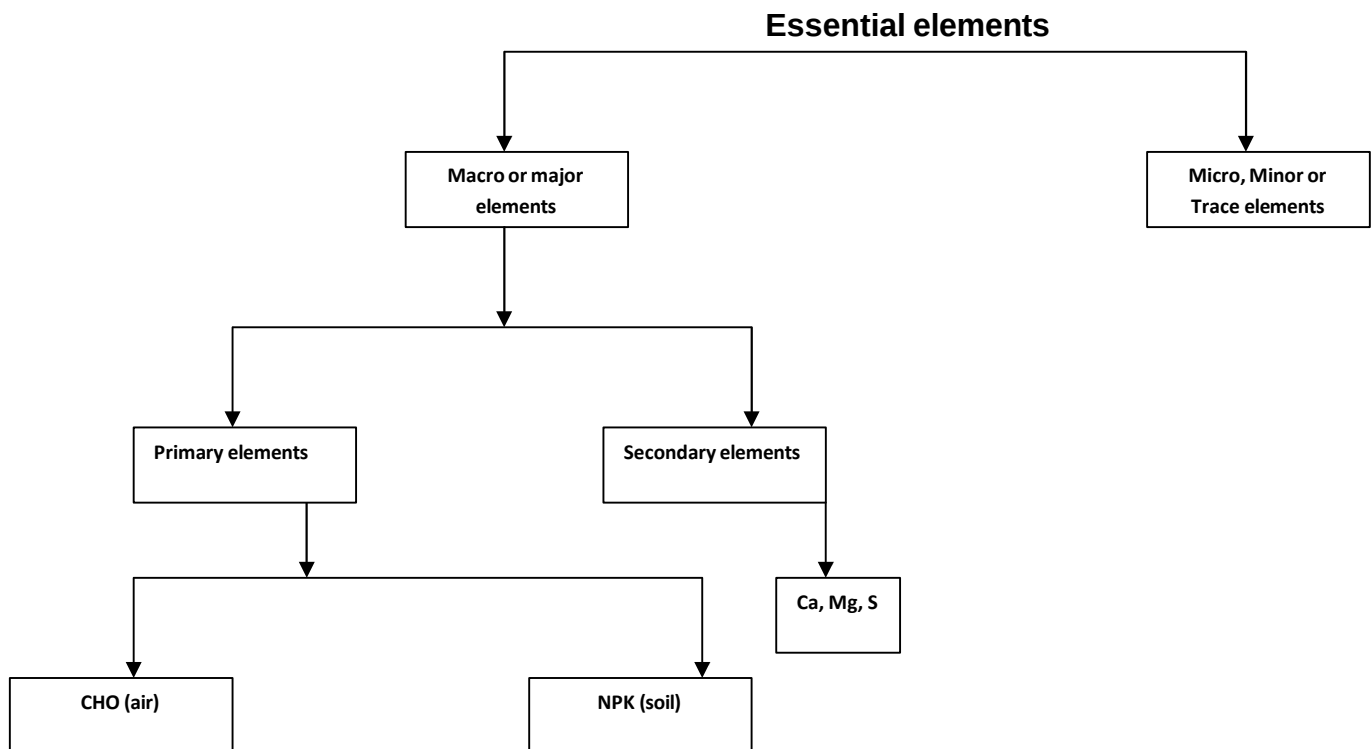
Macro or major elements: Elements required by plants in huge quantities i.e. 1000 mg/kg of dry matter. Examples – CHO NPK Ca Mg S.

Micro, Minor or Trace elements: Elements which are required by plants in less quantities i.e. 100 mg/kg of dry matter, Example Iron, Molybdenum, Copper, Chlorine, Boron, Manganese, Zinc and Nickel.

Beneficial and other elements

Strontium, Selenium, Germanium, Fluorine, Gallium, Cobalt etc.

Classification of elements



Classification of elements according to their role and physiological functions (Mengel and Kirkby, 1987)

Plant nutrients have been divided into four basic groups.

1. The first group of essential elements form the organic (carbon) compounds of the plant. Plants assimilate these nutrients via biochemical reactions involving oxidation and reduction.
2. The second group is important in energy storage reactions or in maintaining structural integrity. Elements in this group are often present in plant tissues as

phosphate, borate and silicate esters in which the elemental group is bound to the hydroxyl group of an organic molecule (i. e. sugar- phosphate).

3. The third group is present in plant tissues as either free ions or ions bound to substances such as pectic acid present in the cell wall of particular importance or their roles as enzyme cofactors and in the regulation of osmotic potentials.
4. The fourth group has important roles in reactions involving electron transfer.

Classification of plant mineral nutrients according to biochemical functions

Group 1: This group includes nutrients that are part of organic compounds.

Nitrogen: Constituent of amino acids, amides, proteins, nucleic acids, nucleotides, coenzymes, hexoamines etc.

Sulphur: Component of cysteine, methionine and proteins. Constituents of lipoic acid, coenzyme A, thiamine pyrophosphate, glutathione, biotin, adenosine-5 phosphosulphate and 3 phospho adenosine.

Group 2: Nutrients that are important in energy storage or structural integrity.

Phosphorus: Component of sugar phosphates, nucleic acids, nucleotides, coenzymes, phospholipids, phytic acid etc. Has a role in reactions that involve ATP.

Silicon: Deposited as amorphous silica in cell walls. Contributes to cell wall mechanical properties including rigidity and elasticity.

Boron: Forms complexes with mannitol, mannan, polymannuronic acid and other constituent of cell walls. Involved in cell elongation and nucleic acid metabolism.

Group 3: Nutrients that remain in ionic form.

Potassium: Required as a cofactor for more than 40 enzymes. Principal cation in establishing cell turgor and maintaining cell electro neutrality.

Calcium: Constituent of middle lamella of cell walls. Required as a cofactor by some enzymes involved in the hydrolysis of ATP and phospholipids. Acts as a second messenger in metabolic regulation.

Magnesium: Required by many enzymes involved in phosphate transfer. Constituent of the chlorophyll molecule.

Chlorine: Required for the photosynthetic reactions involved in O₂ evolution.

Manganese: Required for activity of some dehydrogenase, decarboxylase, kinases, oxidases and peroxidases. Involved with other cation- activated enzymes and photosynthetic O₂ evolution.

Sodium: Involved in the regeneration of phospho enol pyruvate in C₄ and CAM plants . Substitute for potassium in some functions.

Group 4: Nutrients that are involved in redox reactions.

Iron: Constituent of cytochromes and nonheme iron proteins involved in photosynthesis, nitrogen fixation and respiration.

Zinc: Constituent of alcohol dehydrogenase, carbonic anhydrase etc.

Copper: Component of ascarboic acid oxidase, tyrosine, monoamine oxidase, uricase, cytochrome oxidase, phenolase, laccase and plastocyanin.

Nickel: Constituent of urease. In nitrogen fixing bacteria constituent of hydrogenases.

Molybdenum: Constituent of nitrogenase, nitrate reductase and xanthine dehydrogenase.

Essential elements for most higher plants and internal concentrations considered adequate

Elements	Chemical symbol	Form available to plants	Atomic Wt.	Concentration in Dry Tissue		Relative No. of Atoms Compared to Molybdenum
				Mg/Kg	(%)	
Molybdenum	Mo	MoO_4^{2-}	95.95	0.1	0.00001	1
Nickel	Ni	Ni^{2+}	58.71	?	?	?
Copper	Cu	$\text{Cu}^+, \text{Cu}^{2+}$	63.54	6	0.0006	100
Zinc	Zn	Zn^{2+}	65.38	20	0.0020	300
Manganese	Mn	Mn^{2+}	54.94	50	0.0050	1,000
Boron	B	H_3BO_3	10.82	20	0.002	2,000
Iron	Fe	$\text{Fe}^{3+}, \text{Fe}^{2+}$	55.85	100	0.010	2,000
Chlorine	Cl	Cl^-	35.46	100	0.010	3,000
Sulfur	S	SO_4^{2-}	32.07	1,000	0.1	30,000
Phosphorus	P	$\text{H}_2\text{PO}_4^-, \text{HPO}_4^{2-}$	30.98	2,000	0.2	60,000
Magnesium	Mg	Mg^{2+}	24.32	2,000	0.2	80,000
Calcium	Ca	Ca^{2+}	40.08	5,000	0.5	125,000
Potassium	K	K^+	39.10	10,000	1.0	250,000
Nitrogen	N	$\text{NO}_3^-, \text{NH}_4^+$	14.01	15,000	1.5	1,000,000
Oxygen	O	$\text{O}_2, \text{H}_2\text{O}$	16.00	450,000	45	30,000,000
Carbon	C	CO_2	12.01	450,000	45	35,000,000

Hydrogen	H	H ₂ O	1.01	60,000	6	60,000,000
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10. Method of detection of elements, deficiency symptoms of elements in plants, hydroponics, aeroponics, nutrient solutions, foliar spray and basal application of nutrients.

Method of detection

Ash Analysis

To detect some mineral elements of a plant, the plant materials are subjected to high temperatures (about 600°C) and then analyze its ash content. In the ash only the mineral elements are present, all of the organic compounds have been decomposed and passed off in the form of gases. The primary elements (carbon, hydrogen and oxygen) are given off as CO₂, water vapour and oxygen. Accurate determination of elemental nitrogen through this method is not possible as some part of it is given off in the form of ammonium or nitrogen gas. The elements in the ash are not present in their pure state but rather in the form of oxides. The qualitative and quantitative analysis of the ash for the different elements present is dependent on various chemical treatments. The chance of cumulative erroneous results gathered from these treatments is too great to allow heavy reliance on quantitative data for the majority of minerals obtained from the ash

Table - Ash analysis of pride of Saline corn plants grown at Manhattan, Kansas

Source- From Plant Physiology by E. C. Millar

Element	Weight (g)	Total Dry Weight (%)
Nitrogen	12.2	1.459
Phosphorus	1.7	0.203
Potassium	7.7	0.921
Calcium	1.9	0.227
Magnesium	1.5	0.179
Sulphur	1.4	0.167
Iron	0.7	0.083
Silicon	9.8	1.172
Aluminum	0.9	0.107
Chlorine	1.2	0.143
Manganese	0.3	0.035
Undetermined elements	7.8	0.933

Analysis of plant tissue. Finally, we must emphasize that, although ash analysis provides information concerning the relative amounts of minerals present in or taken up (e.g., aluminum and silicon) by the plant. These are not reliable methods for determining the extent of the utilization of these minerals by the plant.

Physiological role of essential elements and their morphological and physiological deficiency symptoms

Nitrogen

It is absorbed in the form of NO_3^- , NO_2^- and NH_4^+ .

Functions

It is very important due to its presence in protein molecule. Nitrogen is found in important molecules as purines, pyrimidines, porphyrins and cytochromes. Purines, pyrimidines are found in nucleic acids, RNA and DNA which are important for protein synthesis. The porphyrin structure is found in metabolically important compounds as the chlorophyll pigments and the cytochromes essential in photosynthesis and respiration. Coenzymes are essential to the function of many enzymes. Cytochromes act as electron carrier in photosynthesis and respiration processes.

Deficiency symptoms

Most important symptom is yellowing of leaves (chlorosis) due to loss of chlorophyll. The symptoms appear first in older leaves then in new or growing leaves due to its high mobility. The younger leaves retain their nitrogen and obtain N from older leaves as well through translocation. Under severe conditions of nitrogen deficiency the lowermost leaves on plants like in tobacco or beans dries. It also indirectly involved in anthocyanin pigment synthesis besides chlorophyll. In tomato due to nitrogen deficiency purple colour in leaf petioles and veins was noticed. If a plant is supplied higher concentration of N cell size and number increased due to increase in protein synthesis with an overall increase in leaf production (Morton and Watson 1948; Neish 1957). Lutman (1934) noted a decrease in leaf epidermal size due to N deficiency in millet and buckwheat.

Phosphorus

It is absorbed as H_2PO_4^- and HPO_4^{2-} form. Low pH favours H_2PO_4^- and vice versa.

Functions

It is constituent of nucleic acid, phospholipids, the coenzyme NAD & NADP, constituent of ATP and other high energy compounds. Meristematic cells show high P concentration where P is involved in nucleoprotein synthesis. It is also involved in activation of amino acids through ATP for the synthesis of protein moiety. Phospholipids along with protein are constituent of cell membranes. The coenzymes NAD & NADP are important in oxidation reduction reactions in which hydrogen transfer takes place which controls photosynthesis, respiration, carbohydrate metabolism and fatty acid synthesis. Due to deficiency of P maturity is often delayed.

Deficiency symptoms

Phosphorus deficiency may cause premature leaf fall and purple and red anthocyanin pigmentation. Its deficiency causes development of necrotic areas on the leaves, petioles or fruits. The parts become stunted in appearance, leaves may have dark to blue green colouration. Older leaves show the deficiency symptoms first. Symptoms of zinc and phosphorus deficiencies may sometimes look alike for example, lack of either one of these elements may cause distortion in shape of leaves of some plants (Hewitt, 1963). Lyon and Garcia (1944) found increase in pith size with decreased vascular tissues during anatomical studies in tomato. Central pith cells had disintegrated and remaining cells were large and thin walled with abnormally large intercellular spaces. Phloem and xylem were thin walled with least development of these cells.

Potassium

It is absorbed in K^+ form.

Functions

Deficiency affects processes like respiration, photosynthesis, chlorophyll development and water content of leaves. The important function of potassium is opening and closing of stomata. It is found in abundance in meristematic region. It activates the enzymes involved in the formation of certain peptide bonds. Accumulation of carbohydrates was often observed during the early stage of deficiency which is due to impaired protein synthesis (Eastin 1952). The carbon skeletons which normally go into protein synthesis are accumulated as carbohydrates. It activates several enzymes involved in carbohydrate metabolism. Apical dominance is lacking in K deficient plants.

Deficiency symptoms

Due to K deficiency chlorosis first occurs on the leaves followed by the development of necrotic areas at the tip and margin of leaf. The symptoms are first seen on the older leaves due to translocation of K into new leaves. There is a tendency for the leaf tip to curve downward in potato and French bean. Marginal regions may roll inward towards the upper surface. Stunted growth due to shortening of internode was also noticed in K deficient plants. In tomato K deficiency causes disintegration of pith cells which results in conversion of phloem parenchyma into sieve tubes and companion cells (Pfeffer 1990; Phillis and Mason 1940).

Calcium

It is absorbed as Ca^{++} .

Functions

It is constituent of cell wall in the form of calcium pectate. The middle lamella is composed of calcium and magnesium pectates. Therefore, it is essential for formation of cell membranes and lipid structures. Calcium in small amounts is necessary for mitosis. Hewitt (1963) has suggested that calcium may be involved in chromatin or mitotic spindle organization. Abnormal mitosis may develop because of an effect of calcium deficiency on chromosome structure and stability. It plays a role as an activator of enzyme phospholipase in cabbage leaves (Davidson and Long 1958). It also activates enzymes arginine kinase, adenosine triphosphates, adenyl kinase and potato

apyrase (Mazia 1954). Florell (1956, 1957) found reduction in mitochondria number in wheat roots. In cotton deficiency results in increased levels of carbohydrates in the leaves and decreased levels in the stems and roots.

Deficiency symptoms

Due to deficiency of calcium meristematic regions of stem, leaf and root tips are greatly affected and die. Roots may become short, stubby and brown in calcium deficient plants (Kalra 1956). Chlorosis occurs along the margins of younger leaves, areas become necrotic. Malformation or distortion of the younger leaves was also noticed in calcium deficient plants. Hooking of leaf tip is also seen. Deficiency symptoms appear first in younger leaves and growing points due to immobility of calcium. Cell walls may become brittle or rigid (Davis 1949; Kalara 1956). Lutman (1934) observed vacuolation of cells in root apex of Calcium deficient rape and buckwheat plants.

Magnesium

It is absorbed as Mg^{++} .

Functions

It is constituent of chlorophyll molecule without which photosynthesis would not occur. Magnesium acts as activator of enzymes involved in carbohydrate metabolism. It activates the enzymes involved in synthesis of nucleic acids (DNA, RNA) from nucleotide phosphates.

Some enzymes involved in carbohydrate metabolism which require Mg^{2+} as an activator

Enzyme	Reactants		End products
Glucokinase	- Glucose + ATP	————→	Glucose 6 phosphate
Fructokinase	- Fructose + ATP	————→	Fructose 1 - P
Galactokinase	- Galactose + ATP	————→	Galactose 1 – P
Hexokinase	- Glyceraldehyde + ATP	————→	Phosphoglyceraldehyde
Gluconolactonase	- 6 Phosphogluconolaction	————→	6 Phosphogluconate

6 Phosphogluconic dehydrogenase - 6 Phosphogluconate $\longrightarrow$ Ribulose - 5 - P

Enolase - 2 Phosphoglycerate + ATP $\longrightarrow$ Phosphoenol pyruvate

Pyruvic kinase - Phosphoenol pyruvate + ATP $\longrightarrow$ Pyruvate

Carboxylase - Pyruvate $\longrightarrow$ Acetaldehyde

Phosphoglyceric kinase - 1,3 Diphosphoglycerate + ADP $\longrightarrow$ 3 Phosphoglycerate

Deficiency symptoms

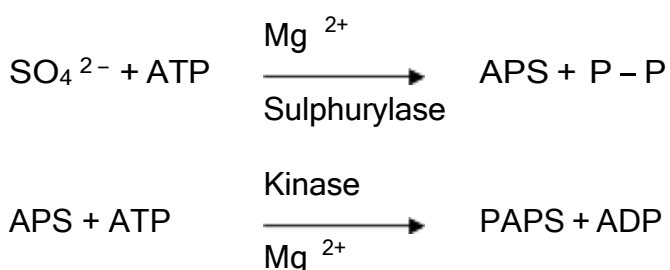
It is constituent of chlorophyll, hence deficiency causes interveinal chlorosis in leaves. Initially yellowing is seen in the basal leaves, as the deficiency becomes more acute the yellowing is seen in new leaves also. Chlorosis is sometimes followed by the appearance of anthocyanin pigments in leaves. At more acute deficiency necrotic spots may be seen over leaves. Lyon and Garcia (1944) observed in tomato plants that excess supply of Mg caused a depression of internal phloem development and an increase in size of parenchymatous cells adjacent to endodermis. A deficient supply caused extensive chlorenchyma development with decrease in cell size though greater in number and densely packed with chloroplasts. Smaller pith cells were also observed under deficient conditions.

Sulphur

It is absorbed as (SO_4^{2-}).

Functions

Its main function is its participation in protein structure in the form of sulphur bearing amino acids Viz; cystine, cysteine and methionine. It is taken up by the plants as sulphate (SO_4^{2-}) which is later on reduced via an activation step involving the compound 3 phosphoadenosine - 5 - phosphosulphate (PAPS) and ATP. PAPS is synthesized in two steps 1. An activation of sulphate by ATP 2. The enzyme sulphurylase to form Adenosine 5 phosphosulphate (APS) followed by conversion of APS to PAPS by a specific kinase (Robbins and Lipmann 1956). S favours root formation. It is also necessary for chlorophyll formation.



The activated sulphate is eventually reduced and incorporated into cystine, cysteine and methionine and finally into protein structure. Sulphur is involved in the metabolic activities of vitamins Biotin, Thiamine and Coenzyme A. Sulphur forms cross links in the protein molecule and in conjunction with peptide and hydrogen bonding, acts to stabilize protein structure. It is a component of S adenosyl – methionine which is important in lignin and sterol biosynthesis. It is also important in Fe – S proteins in photosynthesis, N metabolism and ferredoxin synthesis.

Deficiency symptoms

Deficiency symptoms of S are similar to N deficiency in certain respects like in N deficient plants, there is a chlorosis in leaves followed by production of anthocyanin pigments in some species (Easton 1951). Unlike N deficient plants sulphur deficient plants show chlorosis on the younger leaves first. However, under severe conditions all leaves may be affected (Gilbert 1951). Hall and co-workers (1972) found sulphur deficiency results in decrease in stroma lamellae and increase in grana stacking in corn plants. Easton (1935, 1941, 1942 and 1951) found accumulation of starch, sucrose and soluble nitrogen under deficient conditions in tomato, sun flower, black mustard and soybean but reducing sugars were lower than normal. He suggested that the increase in soluble nitrogen resulted from an inhibition of protein synthesis.

Manganese

It is absorbed as Mn^{++} .

Functions

It acts as an activator of enzymes involved in the respiration and nitrogen metabolism. Enzymes of Krebs cycle, malic dehydrogenase and oxalo succinic

decarboxylase requires the presence of manganese as an activator. Manganese acts as an activator for enzyme nitrate reductase and hydroxyl amine reductase (Nason 1956; Sadana and Mcelroy 1957). It is also involved in the destruction or oxidation of IAA (Goldacre 1961; Kenton 1955). Rate of photosynthesis decrease was also observed due to Mn deficiency in chlorella (Wiessner 1962). Mn is involved in electron transfer from water to chlorophyll during light reaction of photosynthesis.

Deficiency symptoms

Deficiency of Mn^{++} is characterized by the appearance of chlorotic and necrotic spots on the interveinal areas of the leaves. Symptoms first appear on young leaves in some species, whereas in some species on older leaves. Hewitt (1945) and Piper (1942) noted brown necrosis in cotyledons of pea and bean seeds in Mn deficient plants. Eltinge (1941) found in tomato leaves that due to Mn deficiency chloroplasts lose chlorophyll and starch grains, become yellow green in colour, vacuolated and granular and finally disintegrate.

Iron

It is taken up by the plants in the form of Fe^{+++} (ferric) and Fe^{++} (ferrous). The latter is metabolically more active.

Functions

Iron is directly incorporated into cytochromes as well as in compounds necessary for the electron transport in the mitochondria and into ferredoxin which is important for light reaction in photosynthesis. It is essential for chlorophyll synthesis. It is required in the synthesis of chloroplast proteins and enzymes involved in chlorophyll synthesis (Gauch and Duggar 1954). Price and Corell (1964) found increase in chlorophyll synthesis in Euglena cells with addition of iron. It is the component of various flavoproteins, metalloproteins involved in biological oxidations. It is also found in iron – porphyrin proteins, like cytochromes, peroxidases and catalases.

Deficiency symptoms

Important symptom is interveinal chlorosis in leaves. The younger leaves are most affected. More mature leaves show no chlorosis because of the immobility of iron in plants. Chlorosis sometimes followed by chlorosis of veins so that whole leaf becomes yellow. In severe cases the young leaves even become white with necrotic lesions. Lack of iron may inhibit formation of chloroplasts through inhibition of protein synthesis.

Copper

Copper is absorbed as Cu^{++} .

Functions

It acts as component of phenolases, laccase, and ascorbic acid oxidase (Nason and Kaplan 1939). Grem and co-workers (1939) and Neish (1939) observed that copper is involved in the photosynthesis. Loustalot and others (1945) found that CO_2 absorption is decreased in copper deficient tung trees. The chloroplasts possess a copper containing protein called plastocyanin that is essential as an electron carrier in photosynthesis. Plastid enzymes namely phenolases contain copper that is essential to their functioning.

Deficiency symptoms

Deficiency brings Exanthema disease that is characterized by Gummosis (Gummy exudates) accompanied by dieback and glossy brownish blotches on leaves and fruits. Its deficiency also causes reclamation that is disease of cereals and characterized by chlorotic leaf tips and failure to set seeds. Copper deficiency causes a necrosis of tip of young leaves that proceeds along the margin of leaf and gives it a withered appearance. Under more severe conditions leaves may be lost and whole plant may appear wilted.

Zinc

It is absorbed as Zn^{++} .

Functions

It is involved in biosynthesis of auxins. Skoog (1940) observed a decrease in auxin content in zinc deficient tomato plants. Scientists also concluded that zinc deficiency reduces auxin content through its involvement in the synthesis of tryptophan, a precursor of auxin (Tsui 1948). It participates in the metabolism of plants as an activator of several enzymes like carbonic anhydrase which converts carbonic acid into carbon di oxide and water. Other enzymes dependent on presence of zinc are alcohol dehydrogenase and pyridine nucleotide dehydrogenase (Hewitt et al. 1963; Nason et al. 1953). Zn may also acts as an indicator of some phosphorus transferring enzymes, such as hexose kinase or triose phosphate dehydrogenase. Zn deficiency causes accumulation of soluble nitrogen compounds such as amino acids and amides (Possingham 1956).

Deficiency symptoms

The first sign of Zn deficiency is an interveinal chlorosis of older leaves starting at tips and margins, white necrotic spotting soon follows as in cotton (Brown and Wilson 1952). Leaves smaller, internode shortened resulted in stunted growth. Distorted appearance of leaves is also one of the deficiency symptoms. These are generally smaller in size, distorted in shape and appearance and may be clustered on short branches known as rosettes. This disease is referred as little leaf disease. Seed production in beans and peas and development of fruit in citrus is also affected adversely in Zinc deficient plants.

Boron

It is absorbed as H_3BO_3 .

Functions

Gauch and Duggar (1954) observed that boron is involved in carbohydrate transport within the plant. Uptake and translocation of sugar is retarded in Boron deficient plants. It also plays an important role in DNA synthesis in meristems. Important for cellular differentiation and development, nitrogen metabolism, fertilization, active salt

absorption, hormone metabolism, water relations, fat metabolism and photosynthesis. It is involved indirectly through translocation of sugar.

Deficiency symptoms

Death of root and shoot tip due to its requirement for DNA synthesis. Leaves may have thick coppery texture and some curl and become quite brittle. Flowers do not form and root growth is stunted. Disintegration of internal tissues results in abnormalities such as heart rot of sugarbeet, internal cork formation in apples, water core development in turnips, stem crack in celery, drought spot of apple .

Molybdenum

It is absorbed as MoO_4^{2-} .

Functions

It acts as catalyst in the reduction of nitrates. It is required for functioning of enzyme nitrate reductase which reduces nitrates to nitrites and subsequently to Ammonia. Its deficiency also causes drop in the concentration of ascorbic acid in the plant (Hewitt et al. 1950). It is also involved in phosphate metabolism. Lime increases its availability.

Deficiency symptoms

Deficiency causes chlorotic interveinal mottling of leaves, followed by marginal necrosis and infolding of leaves. Under more severe conditions mottled areas may become necrotic and may cause leaf to wilt. Flower formation is inhibited, if forms then drops down before fruit setting. Its deficiency causes whip tail disease in cauliflower plants. The leaves first show interveinal mottling, margin becomes grey and flaccid and finally brown. The leaf tissue collapses leaving only mid rib and small pieces of leaf blade which appears as whip or tail.

Chlorine

It is absorbed as Cl^- .

Functions

It is necessary for photosynthesis. It acts as an activator of enzymes concerned with photolysis of water in which water splits up and O_2 is evolved. It also accelerates activation of amylase which converts starch into soluble sugars. It is essential for roots, for cell division in leaves and as an osmotically active solute (Terry 1977; Flowers 1988).

Deficiency symptoms

Cl⁻ deficiency causes reduced growth, wilting and development of chlorotic and necrotic spots. Leaves may attain a bronze colour. Roots become stunted in length but thickened or club shaped near the tip.

Nickel

It is absorbed as Ni^{++} .

Functions

It is part of enzyme urease which catalyses hydrolysis of urea to CO_2 and NH_4^+ . In plants urea has the toxic effects and hydrolysis is necessary which is done by enzyme urease which contains nickel. It is also essential for germination of seeds (Brown et al. 1987).

Deficiency symptoms

Deficiency causes necrotic spots on leaves due to increase in ureides concentration in leaves.

Metal toxicity and resistance

There is considerable genetic variation in the abilities of various species to tolerate otherwise toxic amounts of Cadmium, Silver, Mercury, Aluminum, Tin and other metals (Woolhouse 1983). In some species the elements are absorbed only to a limited extent, so this more accurately represents avoidance rather than tolerance (Taylor

1987). In other cases the elements accumulate in roots with little transport to shoots. In still others, both roots and shoots contain much higher amounts of such elements than nontolerant species or varieties could live with. This represents the true tolerance.

Recently, an important and phylogenetically widespread mechanism of tolerance was discovered (Reviewed by Gekeler et al. 1989; Steffens 1990 and Rauser 1990). Metals are detoxicated by chelation with phytochelatins, small peptides rich in the sulphur containing amino acid cysteine. These peptides generally have two to eight cysteine amino acids in the centre of the molecule and a glutamic acid and a cysteine at opposite ends. The sulphur atoms of cysteine are almost certainly essential to bind the metals, but other atoms such as nitrogen or oxygen likely also participate.

Phytochelatins are produced in numerous species, but so far they have only been found when toxic amounts of a metal are present, so they can detoxify even essential metals. Their formation therefore represents a true adaptive response to an environmental stress. They act similarly to the far larger metallothionein proteins that detoxify metals in humans and other animals, but in contrast phytochelatins do not represent direct gene products. Still genetic control of their production will no doubt prove essential in understating how various species live on mine wastes and other soils.

Among various metals Silver, Mercury and Copper are the most toxic metals. Inorganic salts of the same metal may vary in their toxicity effects on microorganisms. Thus copper as a cupric ammonium sulphate is more firmly bound by spores than in copper sulphate and silver iodide is less toxic than any other silver halides. Substances secreted by fungal spores eg. amino acids and hydroxy acids, form soluble chelate complexes with copper which then readily penetrate the spore. It has been observed that the organic mercurials are more toxic than inorganic ones in bacteria and fungi. This may be due to more effective uptake of organic mercuric compounds, although phenyl mercuric acetate is more toxic in the ionic form. The primary toxic action of metal cations is the formation of nonionized complexes with surface inorganic groups eg. phosphate, carboxyl and sulphydryl and that the different toxicities of the metals can be related with the varying strengths of surface binding. The hypothesis put forward to account for accumulation of metals in spores suggests that the entire spore protoplasm accumulates the metal so that it moves freely across the semipermeable barriers.

Hydroponics

It is the technique of growing plants with their roots immersed in nutrient solution without soil.

Advantages

- Mineral salts can be provided in the desired requirements.
- By using distilled water in the nutrient solution contamination can be avoided.

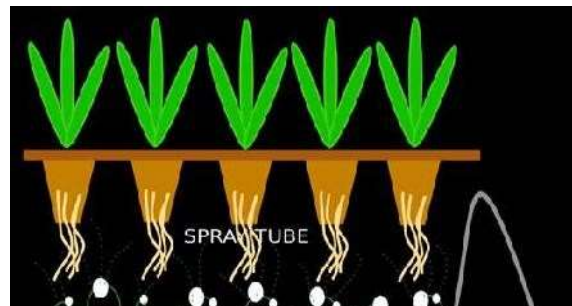
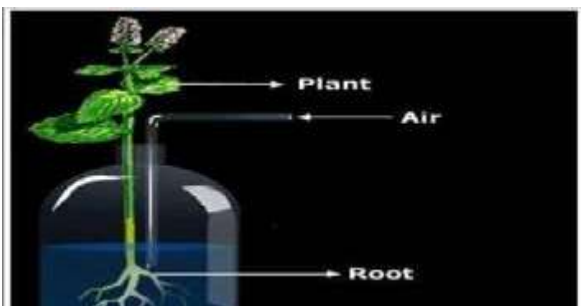
Technique of using

Several studies have shown that the best containers for solution cultures are made of borosilicate glass or natural polythene (Florell 1956). Still we can not say that these containers are contamination free due to presence of boron in borosilicate glass and molybdenum and cobalt in polythene. Water distilled in metal is also contaminated with trace amounts of copper, zinc and molybdenum.

In early studies of plant nutrition the nutrient reagent used presented a major source of contamination. These reagents had to be purified by various means before trace elements deficiencies could be demonstrated. Since most of the contamination is due to trace elements, study of major elements is not the serious problem as they are required in large quantities. One of the satisfactory methods of reversing the contamination is method of ridding water of contaminating trace elements is to pass it over cation and anion exchange resins (Florell, 1957). Next step is to prepare stock solution from inorganic salts containing necessary elements for normal plant growth.

For studying the deficiency symptoms of a particular element that element should be left out of solution. In this technique the roots of the plant are submerged in the nutrient solution and stem projects through an opening cut in the container cover. For keeping stem more tight padding material like cotton may be used. For obtaining good results aeration should be provided. Container needs to be covered to avoid the contamination due to atmospheric dust.

Aeroponics:- System in which roots are suspended over the nutrient solution, which is whipped into a mist by a motor driven rotor.



Nutrient solutions can be prepared by using the formulae given by various scientists as follows:

Various nutrient solutions

Composition of some of the nutrient solutions				
Sach's Solution (1860)		Knop's Solution (1865)		
Salts	Grams per litre	Salts	Grams per litre	per
KNO ₃	1.00	Ca(NO ₃) ₂	0.8	
Ca ₃ (PO ₄) ₂	0.50	KNO ₃	0.2	
MgSO ₄ .7H ₂ O	0.50	KH ₂ PO ₄	0.2	
CaSO ₄	0.50	MgSO ₄ .7H ₂ O	0.2	
NaCl	0.25	FeSO ₄	0.1	
FeSO ₄	Trace			
Hoagland's Solution modified by Arnon, 1940				
Salts	Grams per litre	Salts	Grams per litre	per
KNO ₃	1.02	CuSO ₄ .5H ₂ O	0.08	
Ca(NO ₃) ₂	0.49	ZnSO ₄ .7H ₂ O	0.22	
NH ₄ H ₂ PO ₄	0.23	H ₂ MoO ₄ .H ₂ O (molybdic acid)	0.09	
MgSO ₄ .7H ₂ O	0.49	FeSO ₄ .7H ₂ O 0.5%	} 0.6 ml/litre	
H ₃ BO ₃	2.86	Tartaric acid 0.4%		
MnCl ₂ .4H ₂ O	1.81	(3 x weekly)		

E.J. Hewitt and P. C. Steward (1963)

(2) Salt	Gram / liter	ppm	mM / liter
KNO ₃	0.505	K, 195; N, 70	5.0
Ca(NO ₃) ₂	0.820	Ca, 200; N, 140	5.0
NaH ₂ PO ₄ . 2H ₂ O	0.208	P, 41	1.33
MgSO ₄ . 7H ₂ O	0.369	Mg, 24	3.0
Ferric nitrate	0.0245	Fe, 5.6	0.1
MnSO ₄	0.002230	Mn, 0.550	0.01
CuSO ₄ . 5H ₂ O	0.000240	Cu, 0.064	0.001
ZnSO ₄ . 7H ₂ O	0.000296	Zn, 0.065	0.001

H ₃ BO ₃	0.001860	B, 0.370	0.033
(NH ₄) ₆ Mo ₇ O ₂₄ . H ₂ O	0.000035	Mo, 0.019	0.0002
CoSO ₄ . 7H ₂ O	0.000028	Co, 0.006	0.0001
NaCl	0.005850	Cl, 3.550	0.1

Solid medium culture

Through solid medium such as sand, crushed quartz or gravel is easier to work than liquid medium but purification problem do exists. However, we can obtain purified silica and crushed quartz that are low in trace elements.

In this technique nutrient solutions are added into the solid culture. This is done in three ways: By pouring nutrient solution over the surface (slop culture), by dripping over the surface (drip culture) and by forcing solution up from the bottom of the container by using pumping apparatus (sub-irrigation system). In all three systems the excess solution should be drained out through an opening at the bottom of the container.

Foliar or aerial spray

Technique of spraying the mineral nutrients and other substances over the leaves.

Advantages

- Insecticides, pesticides, herbicides, fungicides and growth regulating substances can be sprayed by this technique.
- In dry weather aerial spray is better than soil application.
- Leaching losses can be avoided.
- Requirement of nutrient or mineral is less.
- Quick absorption of nutrients and elements by plants.
- More economical than soil application.
- Certain elements like Mn, Zn, Cu and Fe gets precipitated in alkaline soil. By foliar application they can be easily made available.

- Availability of fertilizers to deep rooted crops is little late and this can be overcome by foliar spray.

11. Outer space and apparent free space, theories of active and passive absorption viz; Donnan's equilibrium, contact exchange, carrier concept, ion-exchange or cytochrome pump theory.

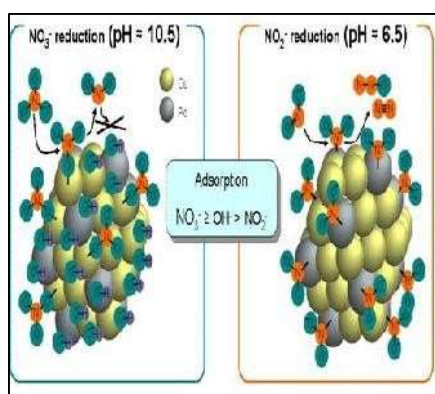
Certain terms are associated with the uptake of substances.

Sorption: When a molecule, ion or atom come in contact with some surface.

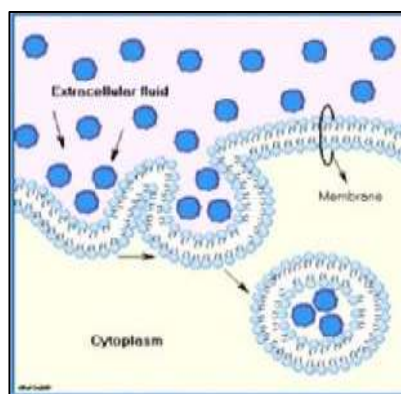
Divided into two parts

Adsorption: Binding of ions or molecules to a surface (e.g., of a soil particle or a root).

Absorption: When a molecule, ion or atom enters inside the cell.



Adsorption



Absorption

Absorption- It can be divided in two parts:

- 1. Active absorption:** Absorption process which involves metabolic energy. The movement of substances may occur against or up gradient or chemical potential. Sometimes anions and cations accumulate against the concentration gradients which does not include the Donnan's effect. Ion transport requires metabolic energy. Ion accumulation is retarded due to decrease in metabolic energy which may be due to low temperature, low O_2 tension, metabolic inhibitors etc.
- 1. Passive absorption:** The spontaneous downhill movement of molecules or ions without involvement of metabolic energy.

2. Passive absorption can be divided in two parts

1. **Diffusion:** Random movement of ions, molecules or atoms from area of higher concentration to lower concentration or from area of high kinetic energy to low kinetic energy influenced by kinetic energies of diffusing molecules.
2. **Mass flow:** Movement of ions, molecules or atoms in mass due to transpirational stream or pull.

Some scientists believe that ions can move inside roots along with the mass flow of water due to transpirational pull. According to this theory an increase in transpiration increases the absorption of ions (Russel and Barber 1960). Lopushinsky (1964) noted in the experiments with tomato using radioactive isotopes ^{32}P and ^{45}Ca that increase in absorption increases the salt absorption. Accumulation of ions against a concentration gradient is possible under mass flow mechanism due to an ion exchange mechanism or Donnan effect and equilibrium. The mass flow of ions through root tissue may also be possible with the aid of transpirational pull.

Outer space and apparent free space

The outer space is defined as part of plant cell or tissue which allows free diffusion to take place, whereas apparent free space is the apparent volume of plant tissue for accommodating the diffusion of ions freely.

Salt absorption takes place through the intimate contact of the root system with the soil colloids or soil solution. Investigations have shown that ions are also absorbed through passive process also called nonmetabolic absorption. It has been observed that when a plant cell or tissue is transferred from a medium of low salt concentration to a medium of high salt concentration, there is an initial rapid uptake of ions, followed by a slow steady uptake that is under metabolic control. The initial rapid uptake is not affected by temperature or metabolic inhibitors indicates noninvolvement of metabolic energy. If the above tissue is returned to the low salt volume, some of the ions taken up will diffuse out into the external medium. In other words, a part of cell or tissue immersed in the salt solution is opened to free diffusion of ions. Since free diffusion implies that ions can move freely in or out of the tissue, the part of the tissue opened to

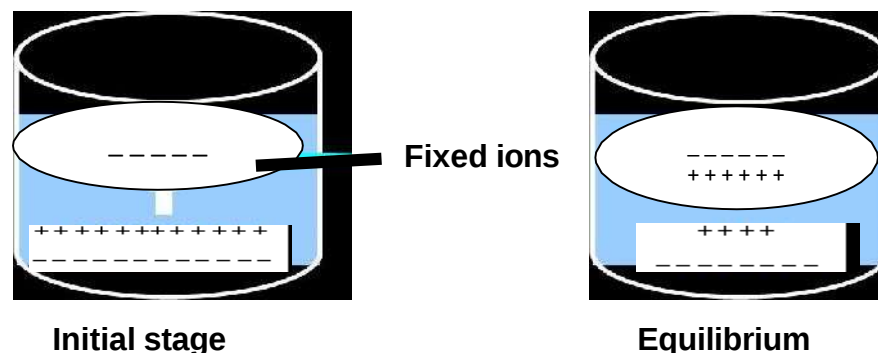
free diffusion will reach an equilibrium with the external medium and the ion concentration of this part will be the same as that found in the external medium. In response to the concept of outer space researchers turned to the task of calculating the volume of plant cell or tissue involved. They immersed a tissue in a solution of known concentration, allowed it to come to equilibrium and then determined the amount of salt taken up. Hope and Stevens (1952) found that bean root tips, when immersed in KCl solution, reached equilibrium in 20 minutes. The reversible diffusion of KCl took place in the absence of metabolic energy and the volume of tissue involved was considered to include a part of the cytoplasm. Hope (1953) demonstrated that the measured volume of the tissue allowing free diffusion increased when the concentration of KCl in the external solution is increased and since active transport was inhibited, it is assumed that a passive accumulation of ions against concentration gradient have occurred. The term apparent free space was introduced to describe the apparent volume accommodating the free diffusion of ions.



Theories (Passive absorption)

Donnan's equilibrium:

The absorption takes place in response to fixed ions.



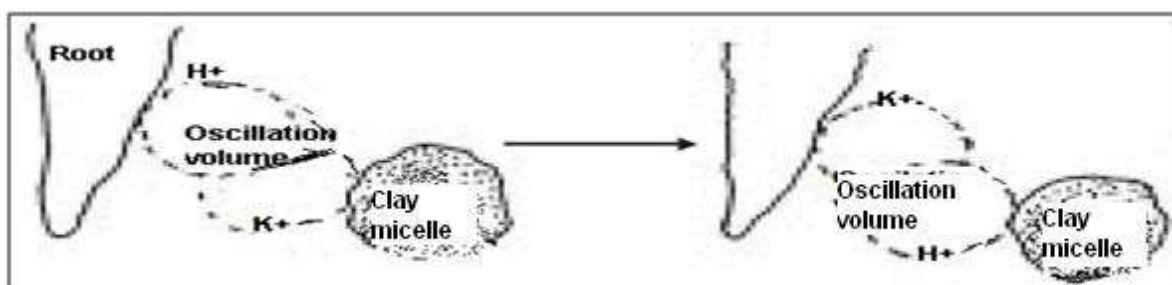
Suppose one cell is placed in the nutrient solution. Inside the cell membrane there is concentration of anions to which the membrane is impermeable. Suppose this membrane is permeable to anions and cations of the outer solution equal number of anions and cations will move across the membrane till equilibrium is reached. However, additional cations are needed to neutralize the fixed ions. Therefore, concentration of cations will be more inside the cell whereas, concentration of anions will be more in external solution. Therefore, ions can move inside the cell without involvement of energy against the concentration gradient in response to electrochemical potential gradient. When product of anions and cations in the internal solution is equal to that of anions and cations in the external solution the Donnan equilibrium is attained.

$$\text{At equilibrium} - C_i + A_i^- = C_o + A_o^-$$

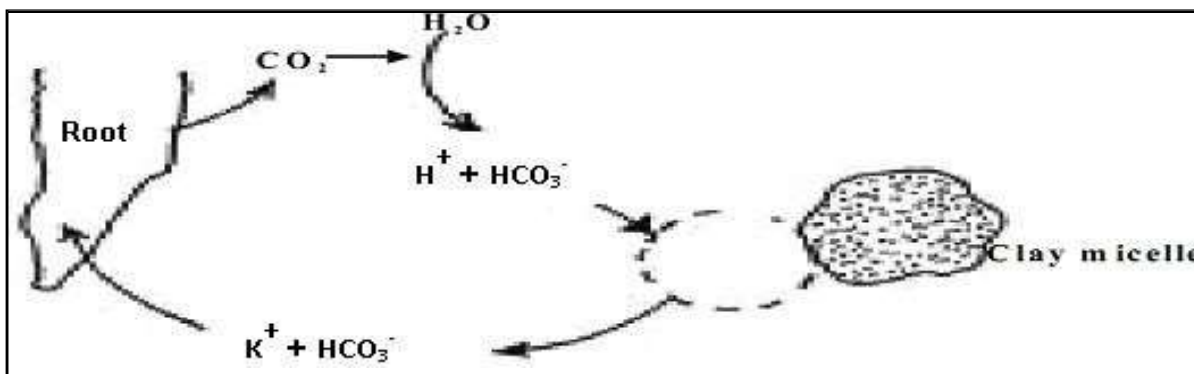
C_i = Cations Inside, A_i = Anions Inside

C_o = Cations outside A_o = Anions outside

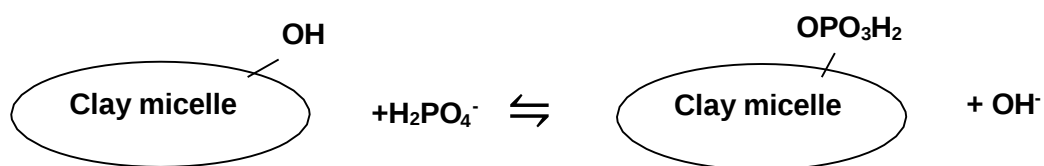
Contact exchange theory: Exchange of ions of the same charge held on the surface of soil colloids or root surface.



According to this theory (Jenny and Overstreet 1939) the ions adsorbed by the root surface or clay particles are not held very tightly but oscillates within certain volume of space, if two adsorbents are so close that oscillation volume of one ion overlaps oscillation volume of other ion, exchange takes place. Ions like K^+ are adsorbed on the surface of clay particles in the soil. These can be replaced if ions of same charge are made available. The H^+ ions held over the root surface are easily exchanged by the other ions of the same charge.



Anion exchange: Anion exchange may takes place between the minerals present in the micelles of soil and the phosphate ion. The anion $H_2PO_4^-$ replaces a hydroxyl anion from the surface of the clay micelle under mild acid conditions.



The addition of hydroxyl ions to the soil releasing the phosphate anion and raising the pH, thus also releasing phosphate from aluminum and iron complexes. However, over limiting which may cause a pH rise to over 7 could again tie up phosphate in the form of insoluble calcium phosphate.

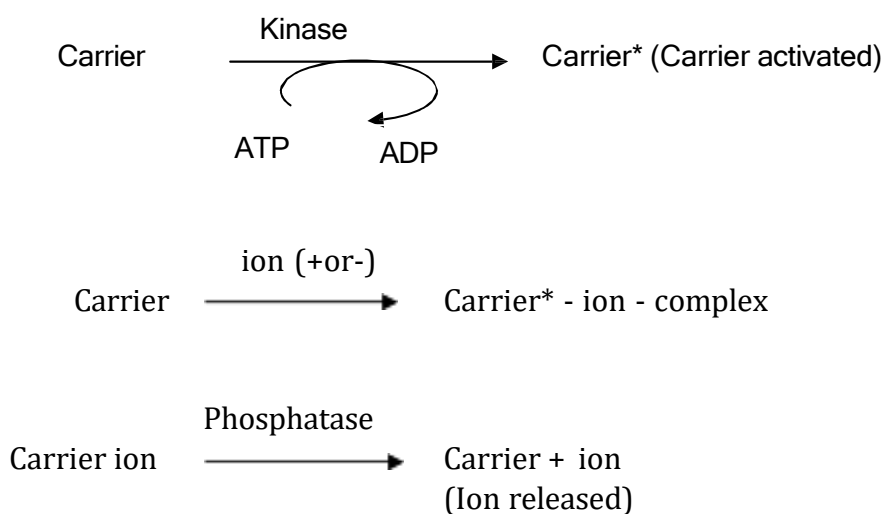
Carbonic exchange theory

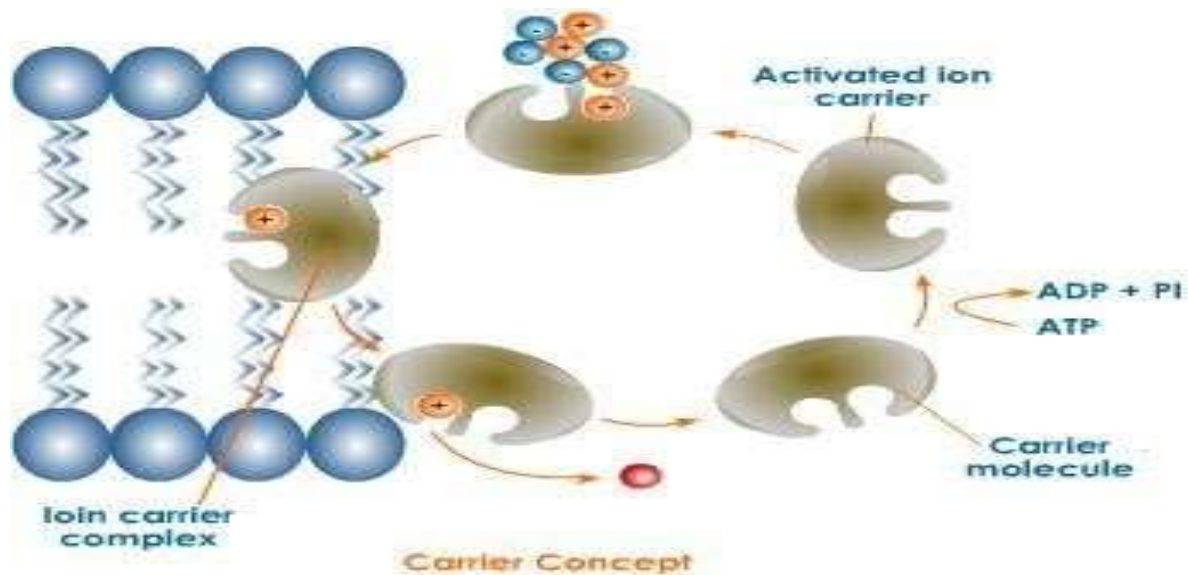
It is assumed that ions first dissolve in the soil solution, CO_2 released during the respiration dissolves in soil water forming carbonic acid (H_2CO_3) which is a weak acid, it is converted into H^+ and HCO_3^- ions. H^+ ions reach the clay particles and release other cations like K^+ from clay by exchange process. The released cations go to the soil solution. From the soil solution cations reach the root surface. The ion exchange does not require metabolic energy. Therefore, it is a physical process.

Theories of active absorption

Carrier hypothesis

It is believed that within the membrane there are some ion carriers. Outside the membrane an ion combines with the carrier forming an ion-carrier complex. Now the complex moves across the membrane and reaches at the inner surface. Finally the complex is broken down on the inner face of the membrane through the action of phosphatase enzyme, the ion is released into the cytoplasm. The whole process requires the ATPs which are obtained through the respiration. The ATPs become available to the carrier by action of kinase enzyme, the process is called phosphorylation. In the process the ADPs are formed and the carrier becomes activated, reaches to the outer surface of the membrane and again gets ready to accept the other ion.





Isotopic exchange

The carrier concept can be supported by using radioactive isotopes. Leggett and Epstein (1956) studied absorption of sulphate labeled with ^{35}S in barley excised roots. They observed that the total sulphate absorbed can be separated in two parts (i) Diffusible sulphate (ii) Actively absorbed SO_4 .

Saturation effects

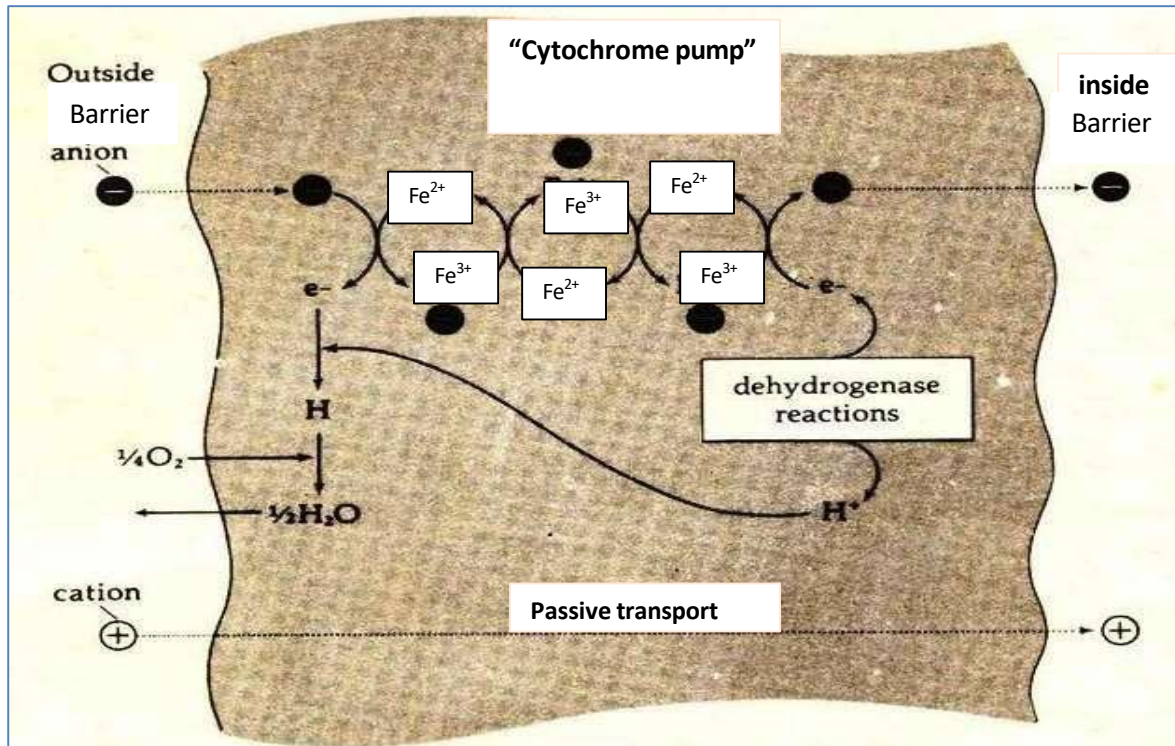
The concept presence of carriers in cell wall can also be demonstrated by experiments when there is a high salt concentration, absorption rate decreases due to engagement of all active sites or carriers.

Specificity

Roots absorb ions selectively. There is a specific carrier for specific ions. Epstein and Hogen (1952) have shown that monovalent cations like Potassium, Cesium and Rubidium compete with each other for the same binding site. Absorption of one can be lowered by addition of K^+ or Cesium to the nutrient solution that can only be overcome by addition of rubidium.

Ion pump mechanism: (Lundegardh and Burstrom 1933)

Ion absorption takes place through oxidation and reduction processes. Cation absorption occurs through passive process, whereas anion absorption takes place through cytochrome system.



Lundegardh and Burstrom (1933) claimed that there is close relationship between anion absorption and respiration. They observed that the rate of respiration increases when plant is transferred from water to salt solution. The increase of respiration rate due to transfer of plant tissue from water to salt solution is called salt respiration.

Later on Lundegardh (1950, 54) concluded:

1. Anion absorption is independent of cation absorption and takes place by different mechanism.
2. An oxygen gradient exists from the outer surface to the inner surface of the membrane which favours oxidation at the outer surface and reduction at the inner surface.

3. Actual transport of anion occurs through a cytochrome system.

According to Lundegardh's theory dehydrogenase reactions on the inner surface of the barrier or membrane produce protons (H^+) and electrons (e^-). The electrons move outward through cytochrome chain and anions move inward. At the outer surface of the barrier the reduced iron of the cytochrome is oxidized losing an electron and picking up an anion. The released electron unites with a proton and O_2 to form water. At the inner barrier surface the oxidised iron of cytochrome becomes reduced in dehydrogenase reactions. The anion is released on the inside of the barrier in the last reaction. Cations are absorbed passively to balance the potential difference caused by the accumulation of anions on the inner barrier surface.

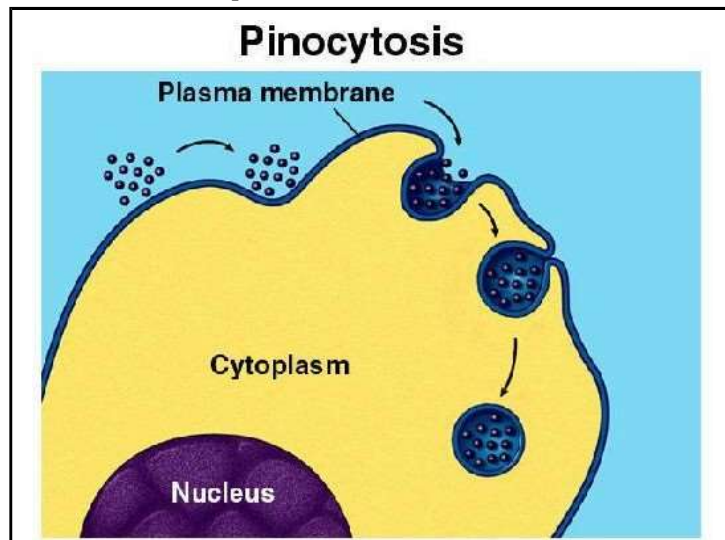
ATP Carrier Mechanism

Findings of Roberts, Wilkins and Weeks (1951) suggested that 2-4 dinitro phenol inhibited ATP formation resulting in decreased salt absorption. It clearly indicates participation of ATP in salt absorption.

Pinocytosis: It is the phenomenon which accounts for transport of larger molecules across the membrane like proteins, viruses etc. The plasma membrane is not smooth.

The larger molecules first adhere to its surface. At this point the membrane invaginates and surrounds the particles on all sides forming a tiny vesicle or vacuole like structure around the particle. The vesicle is then pinched off from the membrane and molecules are released into

the cytoplasm. Here the vesicle membrane dissolves releasing the particle into the cytoplasm.



12. Membrane transporters, aquaporins, mechanism of ion or nutrient uptake and transport in plants, factors affecting nutrient uptake.

Membrane transporters

There are several transmembrane proteins facilitates the transport of molecules or ions across membrane as mentioned below:

Channels

Transmembrane proteins that function as selective pores, through which molecules or ions can diffuse across membrane. Channels only permit passive absorption and limited mainly to ions or water. They have structures called gates which open and close the doors in response to external signals like voltage change, hormone binding, light etc.

Pumps

Membrane proteins that carry out primary active transport across a biological membrane. Most pumps transport ions, such as H^+ or Ca^{2+} . ATP releases the energy when its terminal phosphate is hydrolysed. Reaction is catalyzed by ATP phosphohydrolase which is one of the transport proteins. This energy is used to transport protons (H^+) from one side of the membrane to other side against electrochemical gradient. This transport of H^+ provides energy that is used to transport essential mineral salts.

Carriers

Proteins present in the membrane. During transport, the substances being transported is initially bound to a specific site on the carrier protein which was released free on the inner side of membrane enzymatically.

Symporters

An integral membrane protein involved in movement of two or more different molecules or ions across a phospholipid membrane against the concentration gradient

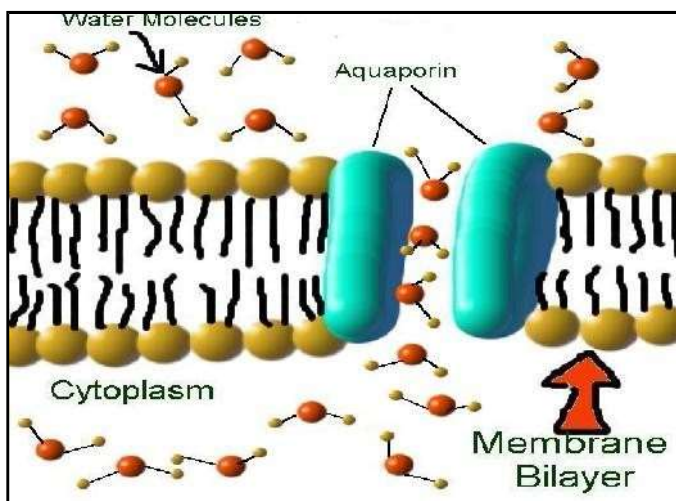
in the same direction. The phenomenon is called symport and proteins are called symporters.

Antiporter

Coupled transport in which the downhill movement of protons drives the active (uphill) transport of a solute in the opposite direction. The phenomenon is called antiport and the protein involved in the process is called antiporter.

Aquaporins

These are class of proteins relatively abundant in plant membranes. Aquaporins reveal no currents when expressed in oocytes, but when the osmolarity of the external medium is reduced, expression of these proteins result in swelling and bursting of oocytes due to rapid influx of water across oocyte plasma membrane which normally has a low water permeability. Aquaporins form water channels in the membranes and the activity appears to be regulated by phosphorylation in response to water availability (Tyreman et al. 2002).



Factor affecting salt absorption

Temperature

An increase in temperature increases the salt absorption. However, beyond 40°C temperature there was a decrease in salt absorption which was mainly due to denaturation of enzymes involved in salt absorption. Temperature changes affect both passive and active absorption processes. The rate of free diffusion depends on kinetic energy of diffusing molecules which is dependant on temperature. Low temperature also reduces rate of biochemical reactions required for active transport.

pH of soil: The availability of ions in the soil solution is greatly affected by hydrogen ion concentration or pH of the soil. For example mono valent phosphate H_2PO_4^- which is

readily taken up by the plants is common in acidic soils. However, as soil approaches towards alkaline medium HPO_4^{-2} and PO_4^{-3} forms are available. H_2PO_4^- form is easily taken up by the plants, HPO_4^{-2} form is not easily taken up by the plant and PO_4^{-3} form is not absorbed by the plants. Hence soils having low pH values are associated with higher absorption of phosphorus.

Light

The effects of light on opening and closing of stomata and on photosynthesis indirectly affects salt uptake. Opened stomata increases mass flow of water which also accelerates salt absorption due to transpiration stream. The energy obtained from photosynthesis provides energy for active salt absorption and oxygen given off also improves conditions for active absorption of ions.

O₂ tension

The salt absorption is retarded in absence of O₂ due to decrease in ion pump mechanism and oxidation reduction processes.

Interaction of ions

Absorption of one element is affected by presence of others. Viets (1944) found that the K⁺ absorption is affected by presence of Ca⁺⁺, Mg⁺⁺ and other polyvalent cations in external medium. He found that uptake of K⁺ and bromine is less in absence of Ca⁺⁺, but it further decreases after the calcium concentration is increased past a maximum point. Olesen (1942) found that the absorption of Mg⁺⁺ is affected by presence of Ca⁺⁺. Competition for binding sites also affects salt absorption. Potassium, Rubidium and Cesium compete one another for mutual binding sites.

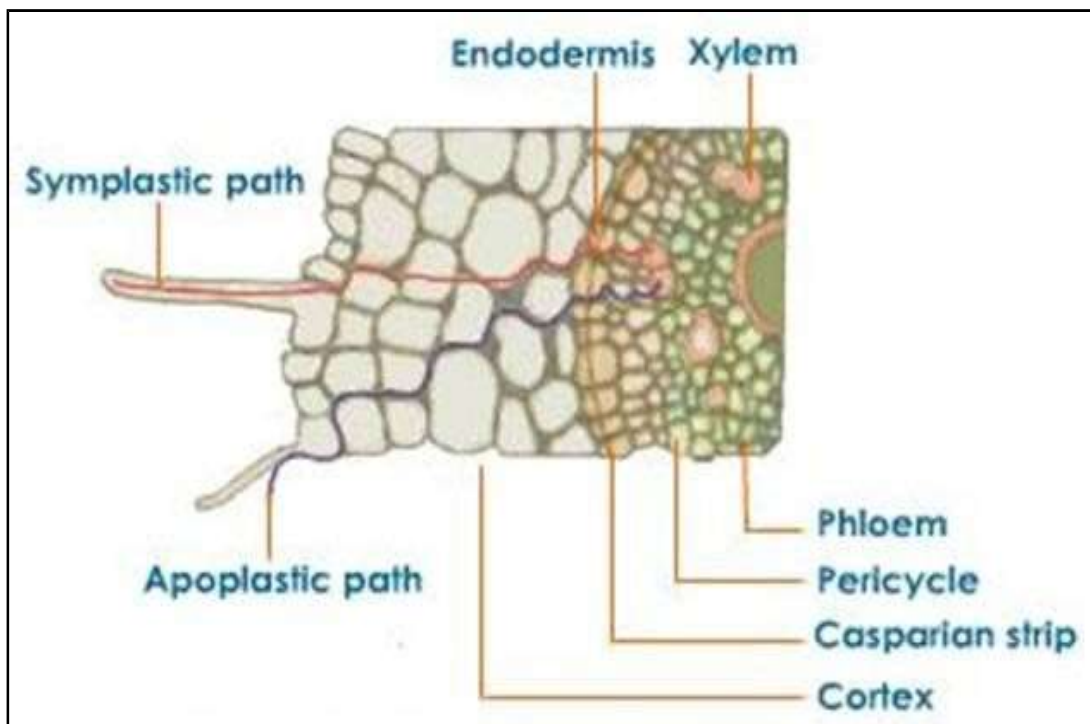
Growth

The growth of plant increases surface area, number of cells, synthesis of new binding sites or carriers, factors etc. thereby increasing salt absorption. The increased volume of water taken up by a cell as it matures may dilute the internal concentration of salt and thus increase absorption activity. Stage of plant tissue also influences the salt absorption. Example – in roots with the age advancement suberin deposits over the roots which restricts further salt absorption. Rapid vegetative development demands

elements and water which increases water movement and salt absorption through passive absorption.

Mechanism of ion uptake

The actual absorption of salts by roots is both passive and active. The movement of salts into apparent free space is passive allowing for free diffusion of ions. Apparent free space may be confined to cell walls and part of cytoplasm. The absorbed ions move

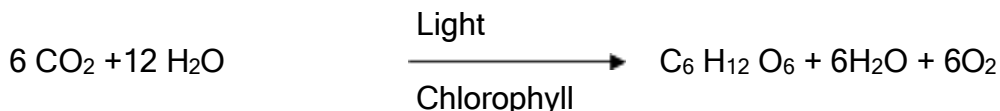


freely up to endodermis where further penetration is retarded by casparian strip. Diffusing ions move unhindered through wet cell wall (apoplast) and plasmodesmata (symplast) of the cortex cells to the endodermis. Scientists have proposed various theories how passage of salts across endodermis takes place into xylem. Most accepted theory is a gradient of decreasing O_2 from cortex to stele (Crafts and Broyer 1938). The living cells in the immediate area of xylem possess a low level of metabolic activity. Since energy is required to accumulate salt against a concentration gradient and to hold this salt, innermost cells favour the loss of salts. Thus it is thought that carrier system operates from cortex towards stele (Crafts 1951). Since diffusion back through the casparian strip is impossible there is unidirectional loss of salts into lumina of xylem vessels.

13. Mechanism of photosynthesis: light reaction, photolysis of water, quantum requirements and pigment systems, photophosphorylation (cycle and non cyclic).

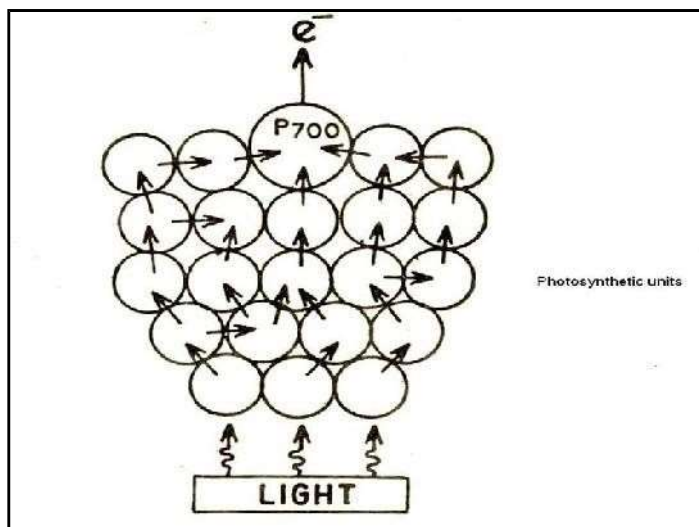
PHOTOSYNTHESIS

- Process in which light energy is used to reduce CO_2 to organic compounds; occurs in chloroplasts in higher plants and algae.
- The conversion of light energy to chemical energy by photosynthetic pigments using water and CO_2 and producing carbohydrates.
- Process by which green plants manufacture complex carbonaceous substances from CO_2 and water in presence of solar energy and chlorophyll. O_2 being the end product.



Photosynthetic units or quantasomes

Smallest group of coordinating pigment molecules necessary to affect photochemical act i.e. absorption and transportation of light quantum to trapping centre where it causes excitation and release of an electron. They contain chlorophylls, carotenoids, quinones, lipids, glycerides and sterols.



Emerson and Arnold (1932) observed in chlorella that 2500 molecules of chlorophyll pigment are necessary to fix one molecule of CO_2 . They termed this number 2500 as photosynthetic unit. The quantasomes are $1800 \text{ \AA}$ broad and $1000 \text{ \AA}$ thick ($18 \times 16 \times 10 \text{ nm}$). Each quantasome has 230 molecules of chlorophyll (160 chlorophyll a and 70 chlorophyll b), 48 carotenoids, 46 quinone compounds, 116 phospholipids, 144

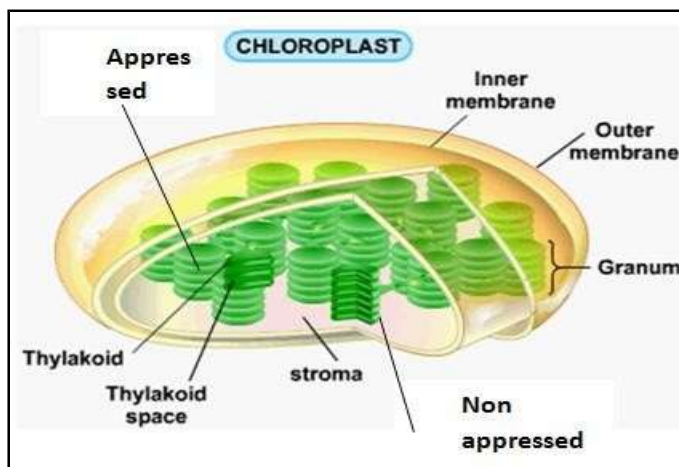
digalactosyl diglycerides, 346 monogalactosyl glycerides, 48 sulpholipids, unidentified lipids and many sterols. Quantasomes have a molecular weight of about two million. The middle region of quantasome is called reaction centre.

Structure of chloroplast

They are generally spherical in shape, 1µ thick and 4-6 µm in length. No. varies from cell to cell. Chlamydomonas contains only one chloroplast. They contain carbohydrates, proteins, lipids, chlorophylls and carotenoids etc. It is covered with two membranes which are smooth and semipermeable. Inside is a clear structureless stroma contains enzymes that convert CO_2 into carbohydrates (dark reaction), starch grains and osmophilic droplets.

Internal structure of a chloroplast granum

Inside stroma system of membranes exist which run parallel to one another along the length of chloroplasts. They are called lamellae. They occur in pairs. Comparatively thin membranes are called stroma lamellae. In certain places 10-100 paired lamellae form disc type of structures and arranged



one above the other like stack of coins called grana lamellae. The complete structure may be called as grana, whereas individual lamellae is called granum. They are also called granum thylakoid (Thylakoid- Greek word Thylacos (sac or pouch) as the ends or margins of paired lamellae are joined together to form closed disc shaped sacs called thylakoids. Chloroplast pigments occur in grana. In thylakoids light energy is used to oxidize water and form ATP and NADPH. The region where one granum thylakoid contacts another is called appressed region. Other region is called nonappressed region. They carry out different photochemical reactions. Lamellae composed of proteins and phospholipids. Chloroplast consists of 40-50% proteins, 23-25%

phospholipids, chlorophylls 5-10%, carotenoids 1-2%, RNA 5%, DNA in small amount. PS I is located towards stroma side of thylakoid. PS II and cyto $b_6 f$ complex are found in appressed region, whereas PS I and cyto f are found in nonappressed region. Cyto b_6 is also called b_{563} . Cyto f contains iron.

Mechanism of photosynthesis

It is an oxidation and reduction process in which water is oxidized to H^+ and OH^- and CO_2 is reduced to carbohydrate with water and O_2 being by products. During light reaction the energy necessary for reduction of CO_2 is produced, while in dark CO_2 is reduced to carbohydrates utilizing the energy produced in light reaction. Light reaction is also called photochemical decomposition of water and dark reaction is called thermochemical reduction of CO_2 .

Light reaction

Light reaction takes place in grana and takes about 10^{-9} Seconds, within such a short period of time there will be synthesis of molecules of ATP and NADPH which are utilized for dark fixation of CO_2 .

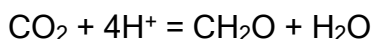
The following steps are covered in light reaction:

Photolysis of water or hill reaction

Splitting of water in presence of light to produce H^+ and OH^- ions is called photolysis of water. H^+ ions are used to reduce CO_2 and OH^- ions recombine to form water along with release O_2 and e^- .



These $4H^+$ are used to reduce CO_2 .



Source of O_2

The work of Ruben, Kamen and Randall (1941) using isotope of O_2 (O^{18}) clearly showed that O_2 comes from water not from CO_2 . When experimental material was

supplied with labelled water (H_2O^{18}), the released O_2 was of O^{18} type and when plant was supplied with labelled CO_2^{18} , the O_2 released was of normal type.

Bacteria use H_2S as hydrogen donor in place of water.



Arnons work

Arnon showed that CO_2 fixation takes place in stroma while ATP and NADPH are produced in grana.

Quantum requirements

4 light quanta are required to fix one molecule of CO_2 and release of one mole of O_2 . Every CO_2 molecules requires $2\text{H}_2\text{O}$ molecules for reduction i.e. 4 hydrogen atoms. Two light quanta are required for transfer of every atom of hydrogen during photosynthesis. Quantum yield may be explained on the basis of number of light quanta required to reduce one molecule of CO_2 and release of one molecule of O_2 .

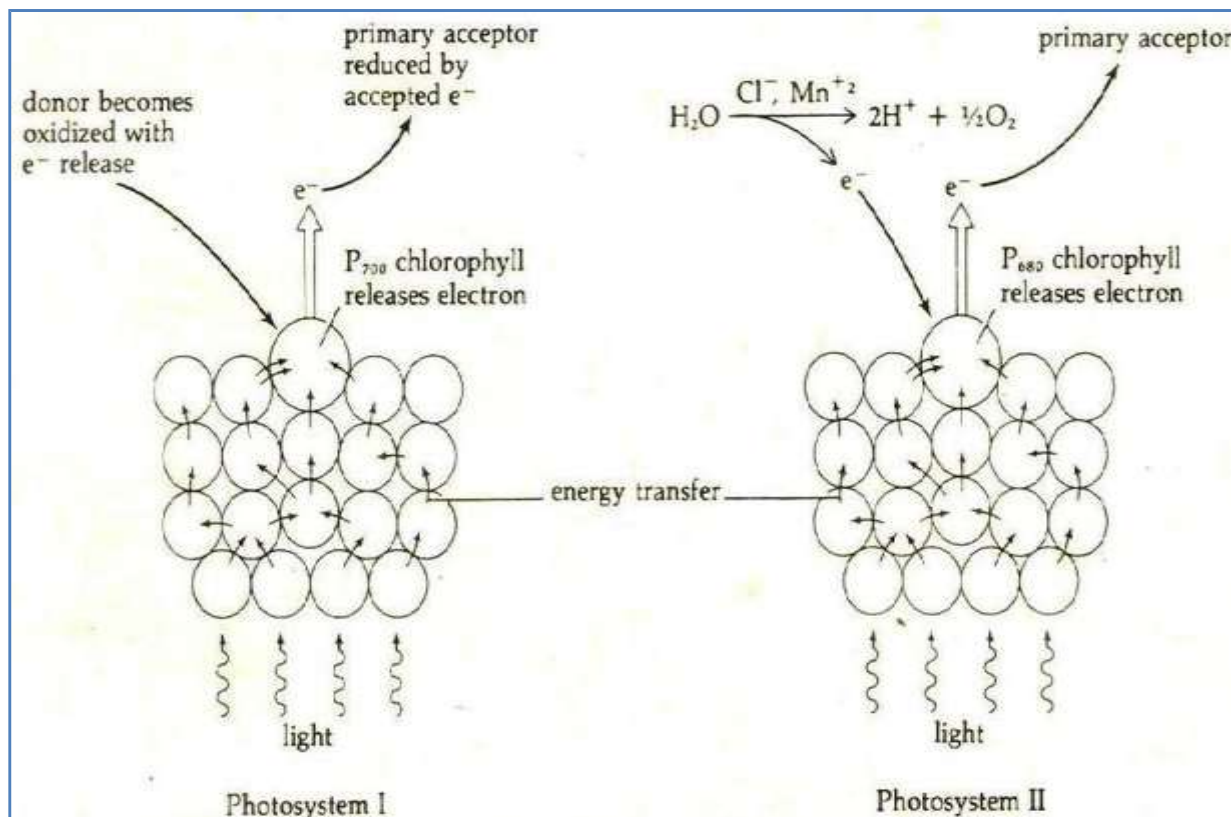
Quantum yield

According to Emerson Lewis (1943) 8 quanta of light are required for reduction of one molecule of CO_2 and release of one molecule of O_2 . Therefore, the quantum yield is $1/8$ or 12%. Two light quanta are required to excite one electron. It may also be explained as moles of CO_2 fixed or O_2 evolved in photosynthesis, or electrons transported in the photosynthetic membrane, per mole of quanta absorbed, in the context of gas exchange often restricted to the linear, light-limited part of the photosynthesis-irradiance curve, when measuring chlorophyll fluorescence, it refers to the full range of photosynthetic irradiance. It has been observed that the quantum yield is dropped near the far red region of spectrum. Dropping of quantum yield near far red region of the spectrum which begins at wavelengths greater than 680 nm in green plants and 650 nm in red algae is called **Red drop phenomenon**. This rate of drop in photosynthesis can be rectified by providing light of shorter wave length. This

Enhancement of photosynthetic rate under influence of two wavelengths (long and short) is called **Emerson effect**.

Two pigment systems

In the late 1950s and 1960s, the Emerson effect received a great deal of attention. It became apparent that photosynthesis requires two functioning pigments termed photosystems. Photosystem I is rich in chlorophyll a and contains carotenoids



and less chlorophyll b than does photosystem II. In both the photosystems most of the pigments operate to harvest light energy and transfer it, possibly by resonance, to chlorophyll a molecules located at photochemically active reactive centre termed traps. The active centre pigment for photosystem I consists of chlorophyll a, which absorbs at 703 nm and is called P700. The chlorophyll a collecting pigment at the reactive centre of photosystem II exhibits an absorption peak at 682 nm and is termed P680. The chlorophyll a molecules (donor molecules) reduce specific electron acceptors (A) and become oxidized themselves. The electron carriers that are thus reduced initiate electron flow and the conversion of light energy to chemical energy (transduction).

Light harvesting complexes

Besides PS I and PS II two other green bands are also present. Each band contains chlo.a + chlo.b + little amount of β carotene. All these pigments are protein bound. One band functions with PS I and another with PS II. Function of these bands is to absorb light energy and transfer it to the appropriate pigment system.

Coordination between PS I and PS II

According to Jung (1982) each granum has about 200 units of PS I and PS II which functions jointly to transfer electrons from water to NADP. Since they are located a quite apart, certain intermediates are required to carry electrons from PS II to PS I. They are of two types (a) A copper containing proteins called plastocyanin bound loosely to the inside of thylakoid membranes (b) A group of quinones called plastoquinones (PQ). Besides, certain other carriers are also involved.

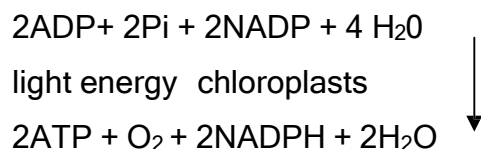
Photophosphorylation

- The addition of phosphate group to ADP under influence of light energy to form ATP.
- The formation of ATP from ADP and inorganic phosphate (Pi) using light energy stored in the proton gradient across the thylakoid membrane.

Arnon and others (1954) demonstrated that isolated chloroplasts produce ATP in the presence of light. It was termed as photosynthetic phosphorylation or photophosphorylation. It was shown that the mitochondria are not the alone cytoplasmic organelles involved in the ATP formation. The formation of most ATP in mitochondria takes place by means of process known as oxidative phosphorylation.

Also, ATP formation in chloroplasts differs from that it is independent of respiratory oxidants. In chloroplast light energy is used in the formation of ATP i.e., light energy is converted to chemical energy. ATP is one of the only requirements for carbohydrate production. A reductant must be formed in photosynthesis that will provide the hydrogens or electrons. As back as 1951, Arnon demonstrated that isolated chloroplasts are capable of reducing pyridine nucleotides when these chloroplasts are

exposed to light. The photochemical reaction has to be coupled with an enzyme system capable of utilizing the reduced pyridine nucleotide as quickly as it is formed. It was reported that NADPH is the reduced pyridine nucleotide active in photosynthesis. In the presence of H₂O, ADP and orthophosphate (Pi) substrate amounts of NADP were reduced accompanied by the evolution of oxygen as follows:



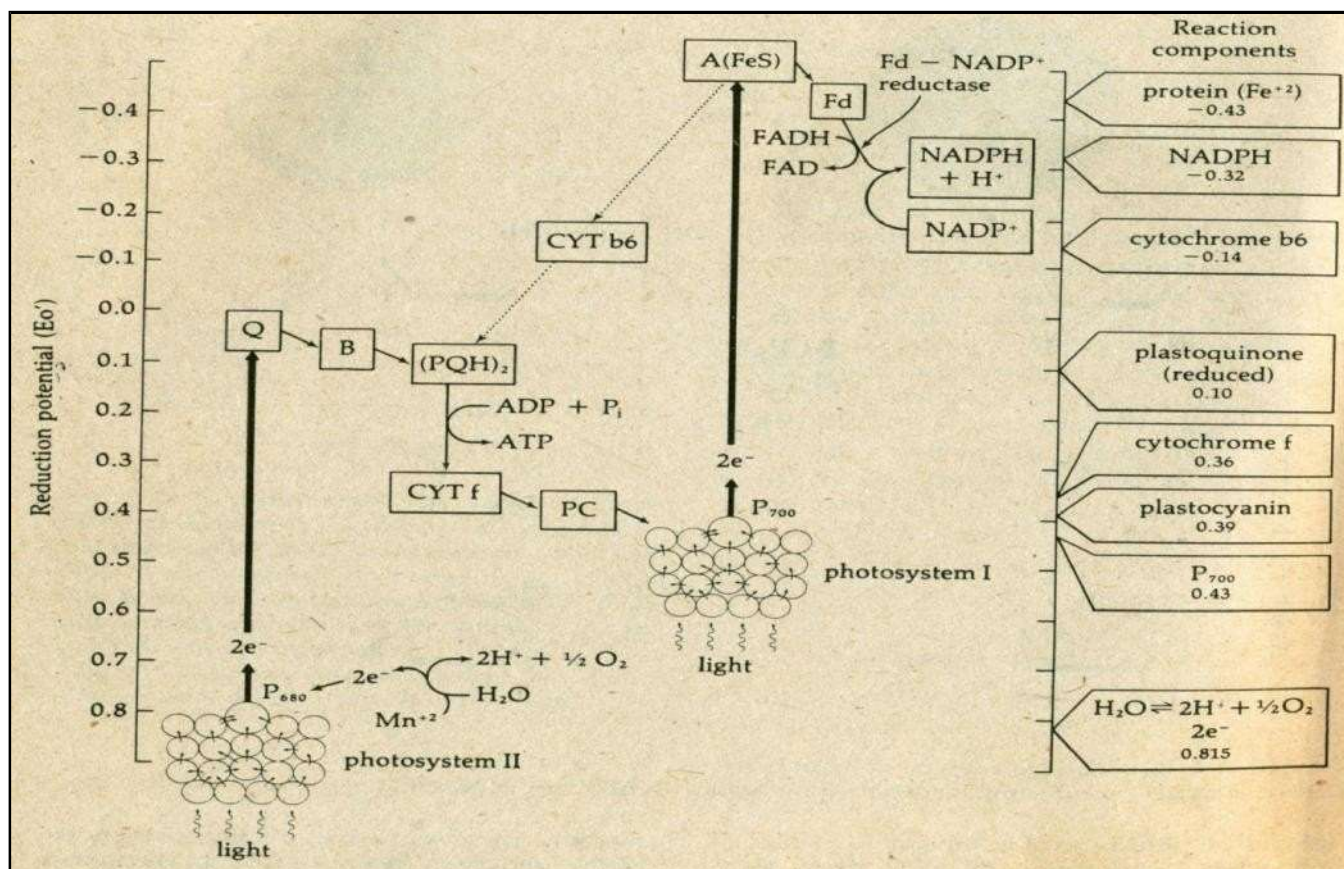
It shows that evolution of one mole of O₂ is accompanied by the reduction of the 2 moles of orthophosphate. Together ATP and NADPH provide the energy and reducing power for CO₂ fixation and reduction. In bacterial photosynthesis NADP is utilized instead of NADPH.

Z-Scheme: Electron Transport and Photophosphorylation (Noncyclic Photophosphorylation)

The Z- scheme illustrates electron transport and the production of NADPH and ATP in chloroplasts. It is called Z scheme due to zig zag pattern of electron flow. The primary flow of electrons within a given granum thylakoid may be initiated almost simultaneously for each photosystem through integrated (coupled) reactions and photolysis of water, which provides the necessary electron flow to produce ATP and NADPH. This integration of two photosystems is most commonly referred to as noncyclic photophosphorylation to describe one means of ATP production in chloroplasts. It is also termed noncyclic electron transport to refer to the manner of electron flow during the process.

In the process after excitation of P₇₀₀ the trap chlorophyll of photosystem I, the electrons are passed on to an unknown primary electron acceptor, believed to be an iron-sulphur protein and designated A (FeS). The electrons are then passed to ferredoxin and ultimately to NADP⁺, with the formation of NADPH⁺. Normally the reduced form of NADP is written as NADPH. In fact it should be written like NADPH + H⁺. The transfer of electrons to NADP⁺ creates an electron debit referred to as a hole in photosystem I. However, this deficit is made up by the excitation of P₆₈₀ of photosystem

II, subsequently photoejection of electrons and their transport through a system of carriers QB, plastoquinone (PQ), cytochrome f (CYT f), and plastocyanin (PC). At this point Q and B are unidentified compounds. Figure illustrates that plastoquinone shuttles protons and passes electrons to cytochrome f. At this point ATP is produced. The hole created in photosystem II is filled by electrons that are derived from the splitting (photolysis) of water. Thus the passage of electrons is not in a cyclic manner.

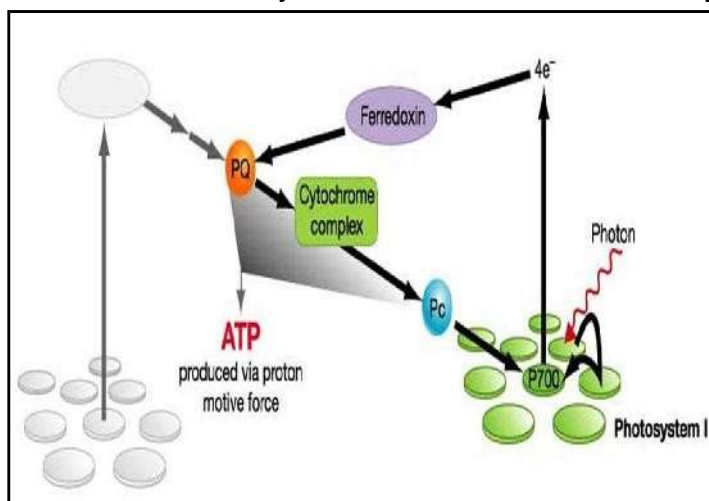


Cyclic photophosphorylation

One way of excluding noncyclic photophosphorylation is to illuminate chloroplasts with wavelengths of light greater than 680 nm. Under these conditions only photosystem I is activated and electrons are not removed from H₂O, as illustrated by the lack of oxygen evolution under these circumstances. When the flow of electrons from H₂O is stopped, noncyclic photophosphorylation is also stopped and as a consequence CO₂ assimilation is retarded as a result of which oxidized NADP is no longer available as an electron acceptor. Activation of photosystem I by wavelengths of light greater than

680 nm causes electrons to flow from P_{700} to A (FeS). When electrons are not passed to $NADP^+$, they may be lost to cytochrome b_6 which in turn passes electrons back to P_{700} via cytochrome f and plastocyanin. There is some evidence that plastoquinone instead of cytochrome b_6 may act as the primary acceptor of electrons from A (FeS). This possibility is quite likely because plastoquinone is necessary for proton transport across the thylakoid membrane for the generation of ATP.

There is possibility of production of two ATPs, one between (FeS) and cytochrome b_6 and one between cytochrome b_6 and cytochrome f which is not very likely without plastoquinone mediation. Nevertheless, the term photophosphorylation is used to denote the cycling of electrons from the donor (excited P_{700} system) to an acceptor (possibly FeS) and back to the P_{700} trap with some generation of ATP. If cyclic photophosphorylation does not indeed operate appreciably in certain organisms, it only produces limited ATP.



Primary electron acceptors and donors

In the late 1950s scientists thought that the reduction of $NADP^+$ was associated with a soluble protein factor found in chloroplasts. Arnon and his colleagues (1957) observed that this protein preferentially reduced with the evolution of stoichiometric amounts of oxygen. They called it the NADP - reducing factor which was purified and named photosynthetic pyridine nucleotide reductase (PPNR), since its catalytic activity was only apparent when chloroplasts were illuminated. Tagawa and Arnon (1962) recognized that PPNR is one of a family of nonheme, nonflavin, iron - containing proteins that is universally present in chloroplasts. Generally generic term **ferridoxin** is used to describe these proteins. Scientists have isolated proteins of the ferridoxin family from the chloroplasts of a variety of plants and have assigned them various functions.

What we now call methaemoglobin-reducing factor, photosynthetic pyridine nucleotide reductase (PPNR), heme-reducing factor and red enzyme.

Before the discovery of ferridoxin, NADP^+ was thought to be the initial electron acceptor of the photosynthetic light reaction. However, neither NADP^+ nor ferridoxin is believed to be the primary acceptor of electrons from P_{700} . There is evidence that suggests the existence of an intermediate of an iron-sulphur protein acceptor, A (FeS) between ferridoxin and photosystem I.

In earlier Z- schemes, plastoquinone was designated as the primary electron acceptor from P_{680} . In the figure Q stands for the unknown primary acceptor that quenches the fluorescence of chlorophyll a. Plastoquinone is reduced by transfer of electrons from Q through B, the latter being a secondary unidentified acceptor that is associated with a photosystem II membrane protein.

The reduced plastoquinone is oxidized by the transfer of an electron to cytochrome f. Either cytochrome f or plastocyanin (Cu containing protein) is the immediate electron donor to photooxidized P_{700} . Both compounds are found associated with the photosynthetic tissues of algae and higher plants and both compounds have redox potentials close to that of P_{700} (about 0.43). However, there is some indication that plastocyanin is located closer than cytochrome f to the photoreaction centre P_{700} of photosystem I. Therefore, plastocyanin is considered the immediate electron donor to photooxidized P_{700} . Cytochrome f, in this case, would transfer electrons to plastocyanin.

Proposed mechanisms of ATP formation

Electron flow and the photophosphorylation of ADP to ATP and H_2O are distinct processes that are coupled, or have energy transferred from one to the other, by some common reactant. Evidence for this coupling is based on several observations. 1. In the presence of coupling agents, ATP formation can be inhibited but electron transport continues and often shows an increase in rate. When the coupler is removed, ATP formation resumes in pace with electron transport. 2. When electron transport is impeded or blocked by certain herbicides, such as the triazins, triazinones,

biscarbamates and (3-[3,4 - dichlorophenyl [-1,1-dimethylurea), phosphorylation is also inhibited. 3. Scientists have commonly observed simultaneous oxidation of NADPH (NADH in respiration) and FADH in ATP formation.

Scientists have proposed three mechanisms of ATP production.

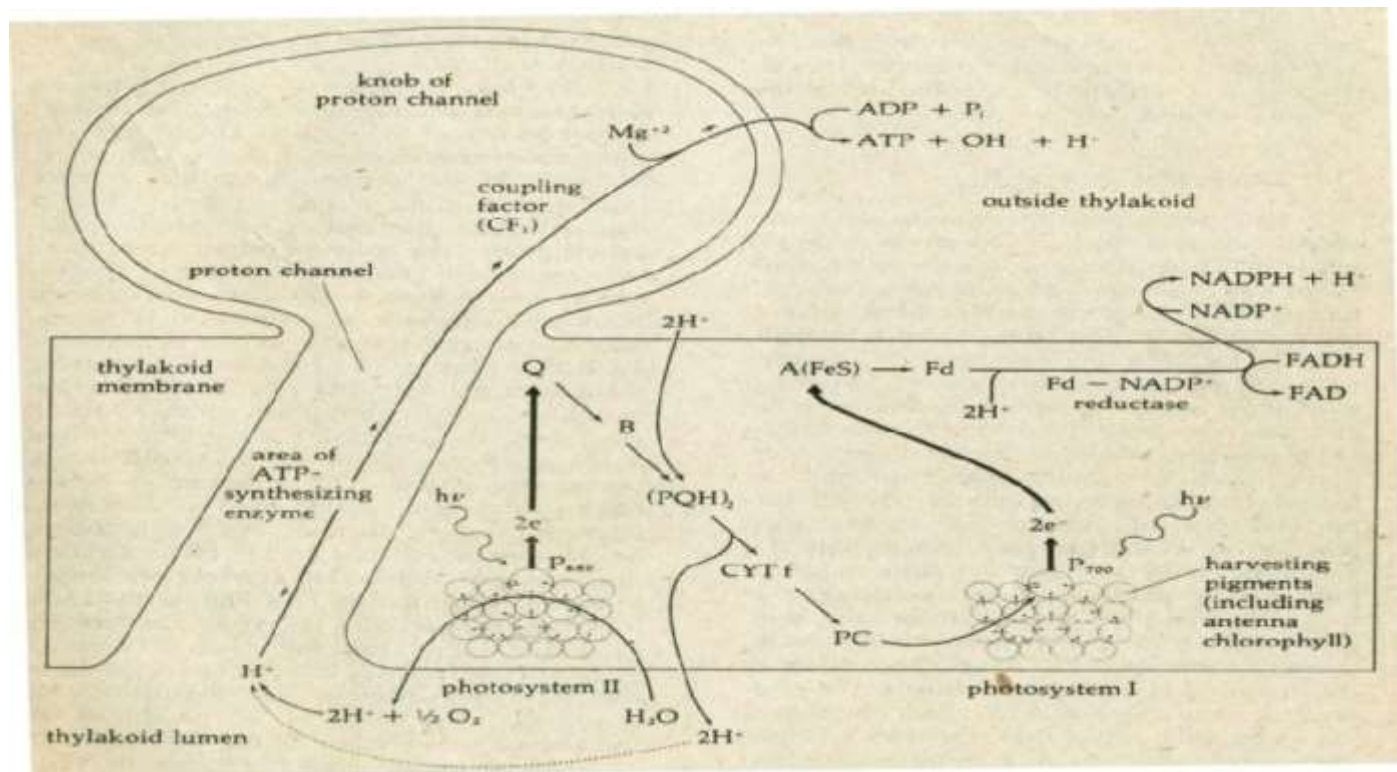


Fig- Granum thylakoid membrane illustrating location of photophosphorylation and coupling of electron flow to ATP production

Conformational coupling

It is promised by the idea that the membranes of the mitochondria or chloroplast thylakoids undergo structural changes and that these changes presumably induce high energy states, or conformations, that favour the release of energy for the ATPase catalyzed production of ATP. Although ATPase normally catalyses ATP decomposition to ADP and inorganic phosphate (Pi). It will work in the reverse direction when sufficient

energy is available. Electron micrographs illustrate differences in structure of membranes (mostly mitochondrial membranes) during organelle activity.

Chemical coupling

Another hypothesis, developed in the 1960s suggests that an unknown coupling protein might act as an energy transfer agent between electron transport and ATP formation. According to this idea, a coupling factor (CF) believed to be a protein, initially forms a high energy CF complex with one of the electron carriers, a participant at the site of phosphorylation along the electron transport chain. The formation of CF-carrier-complex involves an endergonic reaction provided with energy released during electron transfer. The CF-carrier-complex then enters into an exchange reaction in which inorganic phosphate (Pi) exchanges with electron carrier to form a high energy phosphorylated coupling factor (CF-P) complex which releases the high energy phosphate to ADP, thereby forming the ATP. Thus, according to the chemical coupling hypothesis, the endergonic formation of ATP is accomplished via a coupling factor that transfers electron energy promoted by light (photosynthesis) or oxidation of organic chemicals (respiration).

Chemiosmotic coupling

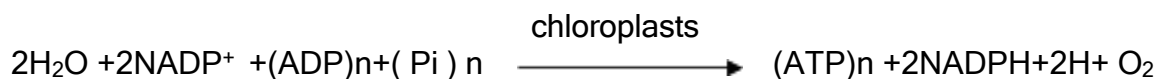
This hypothesis is the most widely accepted explanation for oxidative phosphorylation in mitochondria and has recently gained importance as an explanation for phosphorylation in thylakoid membranes. In 1961, after observing that hydrogen ions are actively released from respiring mitochondria at the expense of energy received from the electron transport process. Mitchell (1961, 1978) proposed the idea of chemiosmotic coupling. He suggested that a concentration gradient of protons is established across the mitochondrial membrane because there is an accumulation of hydrogen on one side of the mitochondrial membrane. The proton accumulation is necessary for energy transfer to the endergonic ADP phosphorylation process. Jagendorf (1975) demonstrated that a pH gradient across the thylakoid membrane stimulated ATP production when chloroplasts were maintained in darkness. He also

demonstrated that under normal light conditions an H^+ concentration gradient is established in actively photosynthesizing chloroplasts. As figure illustrates, the electron carriers are located in the granum membrane. ATP and NADPH are produced on the stroma side surface of the thylakoid. An important aspect of the model is the mobility of plastoquinone. This carrier presumably transfers electrons to cytochrome f and in addition pick up H^+ ions on the outside and releases protons to the thylakoid channel. The transfer of protons to the inside and the production of protons from the photolysis of water incurs buildup of protons inside and a p^H gradient across the thylakoid membrane to the outside (stroma side), where the hydrogen concentration is relatively low. The membrane itself is not permeable to protons concentrated on the channel side, which represents a source of energy. It is believed that protons flow from the inside of the stroma side of the membrane through special pathways of CF (stalks) that terminate as knobs at the outer (stroma side) surface. These stalks and knobs are the sites of photophosphorylation. The proton flow along the gradient provides the necessary energy for the following reaction:



The proton flow and phosphorylation are thought to be brought together (coupled) by the activity of the enzyme ATPase (also called coupling factor) which is associated with the destruction of ATP, but it will operate in the reverse situation as long as sufficient energy is supplied (in this case from the proton flow). For every two electrons passing through the transport system, two protons are transported by the reduced plastoquinone, a water molecule is photolyzed. Theoretically, one molecule of ATP is produced for every three protons passing through the CF.

The light reaction phase of photosynthesis may be summarized by the following equation, which represents the photochemical, photophosphorylation, photoreduction and photooxidation (splitting of water) events:



The summary equation also indicates that the stoichiometry of the overall equation is not exact, particularly for ATP production and the quanta required. We do not know the number of ATP molecules produced per oxygen molecule liberated. Some investigators claim 2 molecules of ATP are produced for every oxygen molecule liberated, and others maintain 4. According to plant scientists 8 to 12 quanta (photons) appear to be necessary to produce NADPH and ATP sufficient for CO₂ fixation. Approximately 2NADPH and 3ATP molecules are required to incorporate 1 molecule of CO₂ into sugar phosphate.

Difference between cyclic and non-cyclic photophosphorylation

S. No	Cyclic	Non-cyclic
1.	Only PS I participates	PS I and PS II participate.
2.	PS I gets back electron in a cyclic fashion.	PS I gets back electron from PS II and PS II from water.
3.	Water does not participate hence No O ₂ evolution.	O ₂ evolved as water participates.
4.	Found in bacteria.	Found in green plants.

Summary of light reaction

- Eight light photons are required to oxidize 4H₂O.
- 2 e⁻ and 1 H⁺ are required to reduce 1 molecule of NADP to NADPH₂.
1H⁺ remains in the medium.
- Transfer of 1 electron from H₂O to NADP requires 2 photons as excitation of both the photosystems is necessary.
- Photolysis of 4H₂O produces 2H₂O, O₂, 4 e⁻ and 4H⁺.
- Reduction of 1 molecule of CO₂ requires 2NADPH₂ and 3ATP.
- 4 molecules of water after photolysis forms 3ATP and 2NADPH₂ and cycle repeats 6 times producing 18 ATP and 12 NADPH₂ during light reaction of photosynthesis.

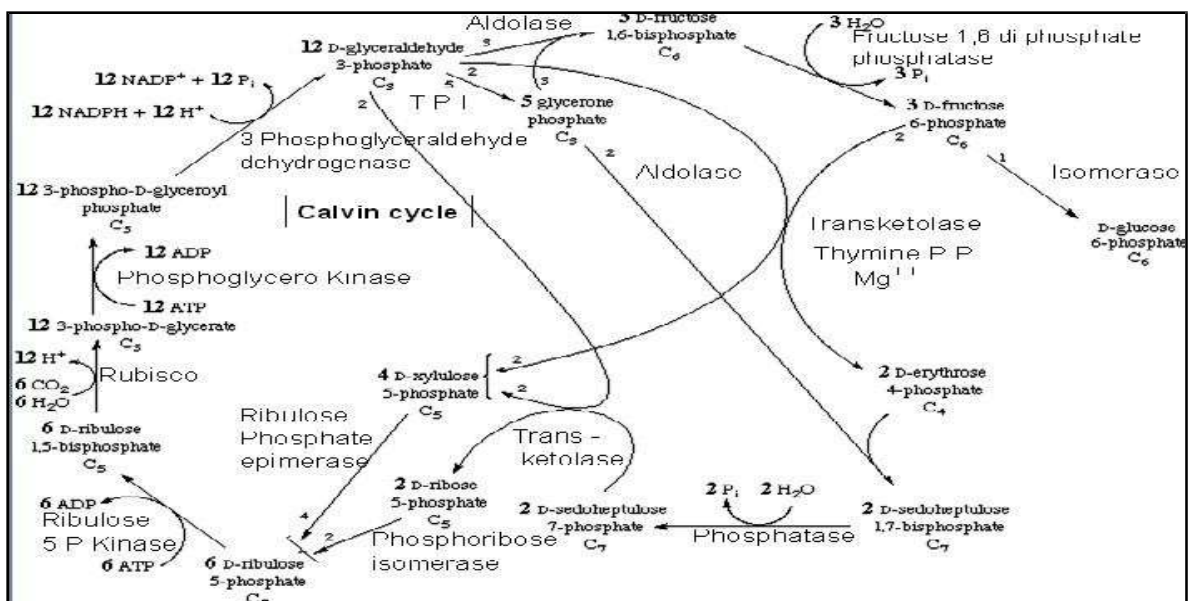
14. Calvin cycle, Hatch and Slack pathway

Dark reaction

CO₂ is fixed in one of the following pathways in green plants. 1. C₃ pathway or Calvin cycle 2. C₄ pathway or Hatch and Slack cycle. 3. C₂ or Glycolate pathway. 4. Crassulacean acid metabolism (CAM plants) .

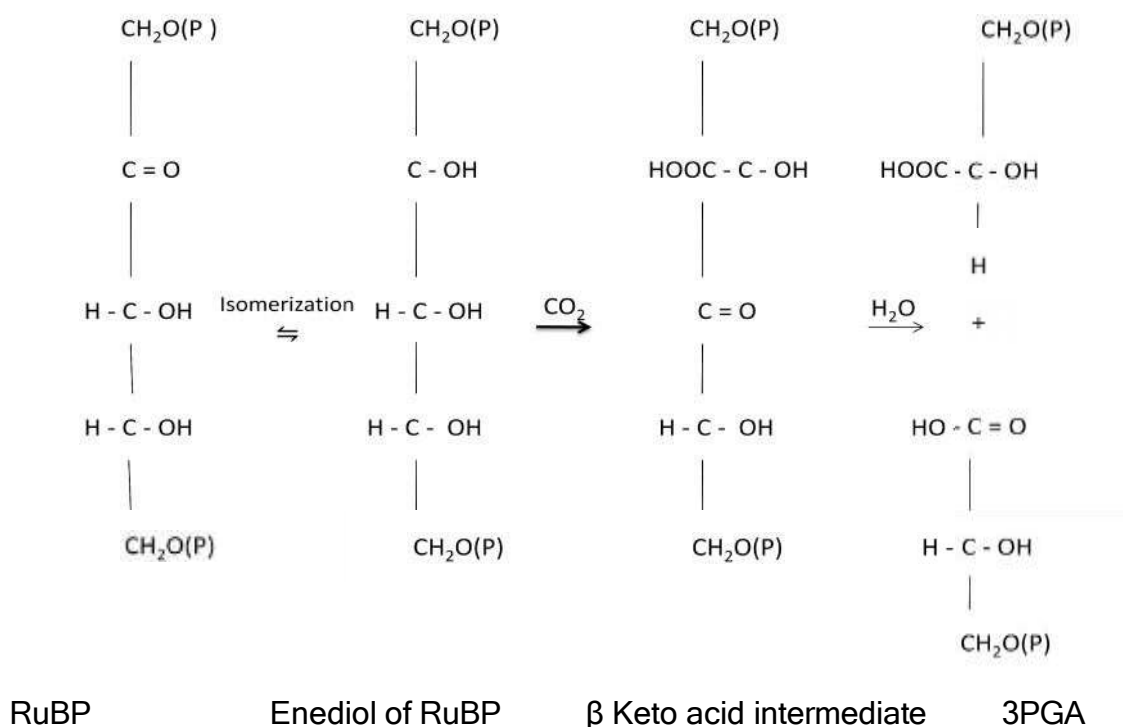
C₃ pathway or Calvin cycle

- First stable compound formed after the entry of CO₂ in plant system is triose sugar viz; Phosphoglyceric acid (PGA) (Calvin 1961).
- Pathway of photosynthetic CO₂ assimilation beginning with carboxylation of RuBP by Rubisco.
- The biochemical pathway for the reduction of CO₂ to carbohydrate. The cycle involves three phases, the carboxylation of ribulose-1,5-bisphosphate with atmospheric CO₂ catalyzed by rubisco, the reduction of the formed 3-phosphoglycerate to triose phosphate by 3-phosphoglycerate kinase and NADP-glyceraldehyde -3-phosphate dehydrogenase, and the regeneration of ribulose-1,5-bisphosphate through the concerted action of ten enzymatic reactions.



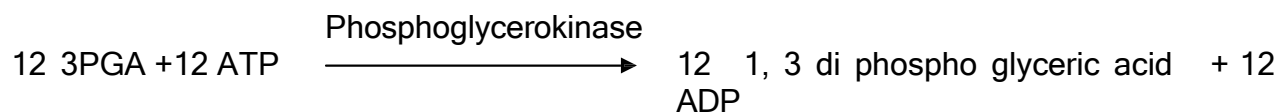
It is called C₃ pathway because the first stable compound formed after the entry of CO₂ in plant system is triose sugar i.e. PGA. Calvin by using isotope of carbon C¹⁴ could identify various products formed during reduction of CO₂ by paper chromatography and radio autography. Calvin (1961) later on got noble prize for this work. Chromatography is a technique for separation of compounds present in a small mixture. On the other hand radioautography is a technique to find out isotopes in a particular source. Different radioactive isotopes emit different types of radiation. C¹⁴ emits β rays. Calvin worked on these aspects in blue green algae chlorella.

Six molecules of CO₂ react with 6 molecules of Ribulose 1, 5 biphosphate or bisphosphate to form 12 molecules of 3PGA. The reaction is catalysed by RuBP carboxylase . First CO₂ is added to a 5C sugar to form 6C sugar which then splits into two molecules of 3 carbon compound.

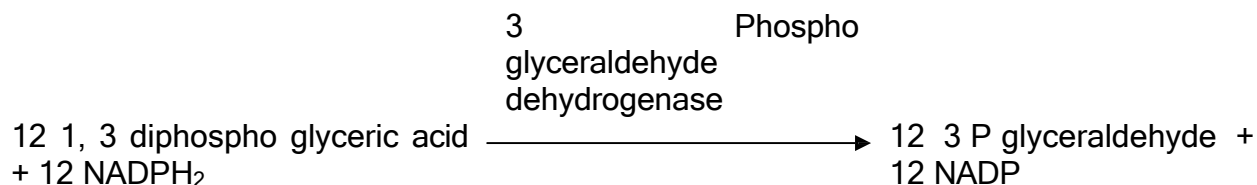


The cleavage of β Keto acid intermediate takes place by water between C_2 and C_3 of ribulose chain.

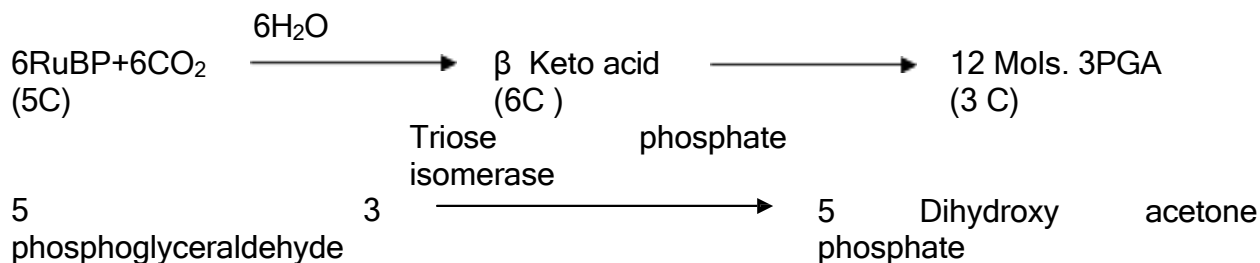
In next step 12 molecules of 3PGA are phosphorylated to 12 molecules of 1-3 di phospho glyceric acid. The reaction is catalyzed by phospho glycerokinase



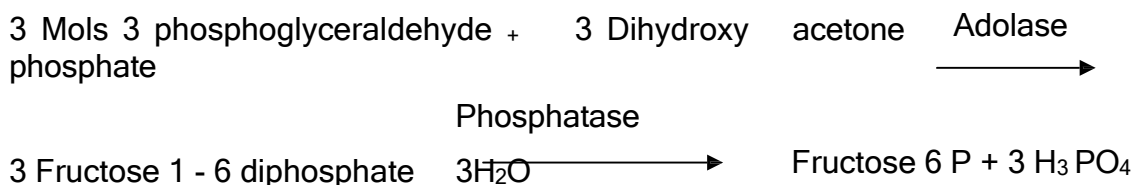
In the next step 12 molecules of 1, 3 di phospho glyceric acid reduced to 12 molecules of 3 phospho glyceraldehyde by 12 molecules of NADPH produced in the light phase of photosynthesis.



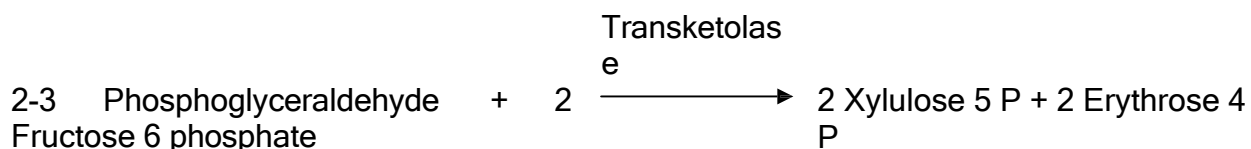
Five molecules of 3 phospho glyceraldehyde are isomerized to five molecules of dihydroxy acetone phosphate (5 molecules) in presence of triose phosphate isomerase.



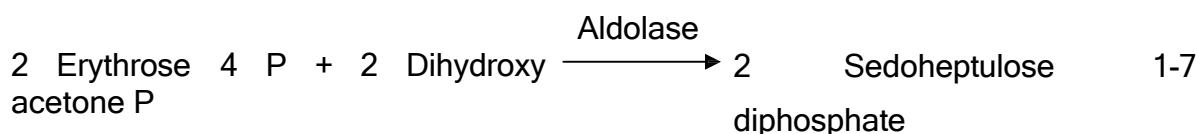
The 3 molecules of 3 phosphoglyceraldehyde condensed with 3 dihydroxy acetone phosphate to form 3 molecules of fructose 1, 6 diphosphate in presence of aldolase which are then dephosphorylated to 3 mol. of fructose 6 P in presence of phosphatase.



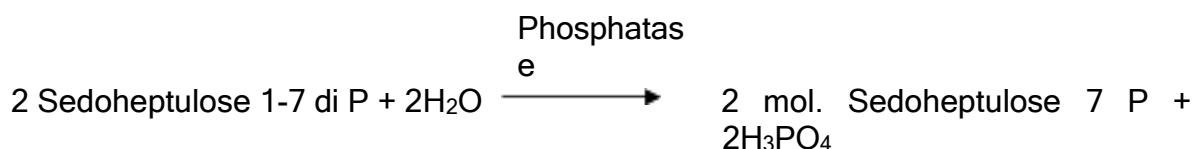
Two molecules of 3 phosphoglyceraldehyde reacts with 2 mol. of fructose 6 phosphate to form 2 mol. of xylulose 5 P and 2 mol. of erythrose 4 P in presence of transketolase and thymine pyrophosphate



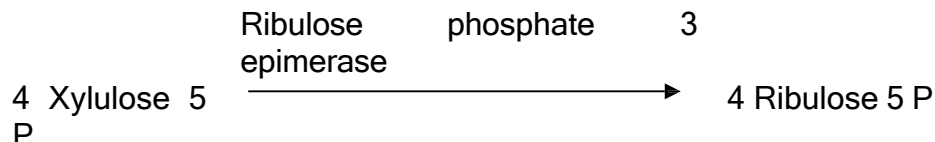
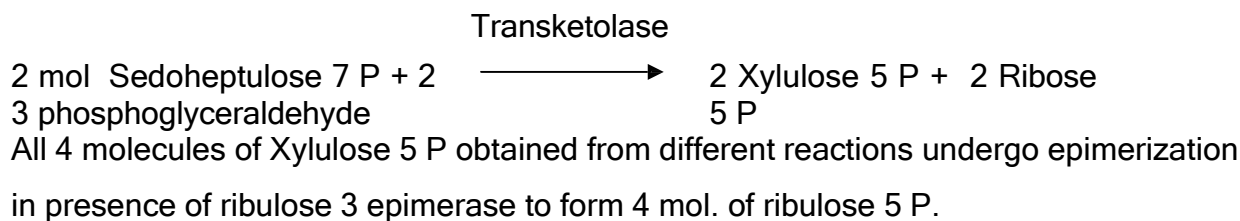
Two mol. of erythrose 4 P reacts with two mol. of 3 phosphoglyceraldehyde to form two sedoheptulose 1-7 diphosphate in presence of aldolase.



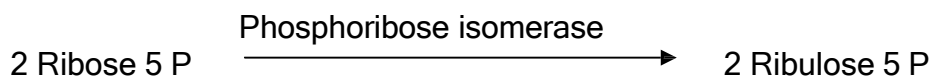
Two molecules of sedoheptulose 1-7 diphosphate then dephosphorylated to two molecules of sedoheptulose 7 P in presence of phosphatase.



Two mol. of sedoheptulose 7 P condensed with the remaining two mol. of 3 P Glyceraldehyde in presence of enzyme transketolase to produce two mol. of Xylulose 5 P and two molecules of ribose 5 P.

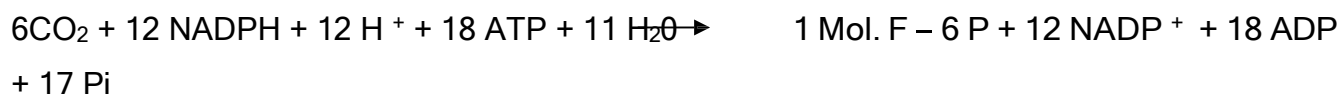


Two mol. of ribose 5 P undergo isomerization in presence of phosphoribose isomerase to form 2 mol. of ribulose 5 P.



Six molecules of Ribulose 5 P are formed up to this stage. In the final step all 6 molecules of ribulose 5 phosphate are phosphorylated at the expense of 6 mol. of ATP in presence of enzyme phosphoribulo kinase to form 6 mol. of carbon acceptor ribulose 1-5 diphosphate. Then all 6 the molecules of ribulose 1-5 diphosphate are regenerated reenter into cycle.

Whole reaction can be written as:

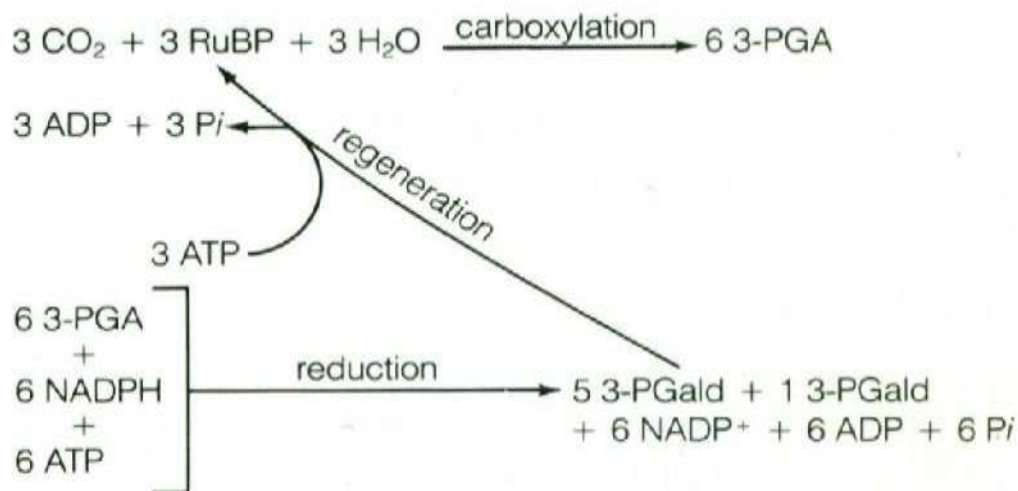


Synthesis of carbohydrate

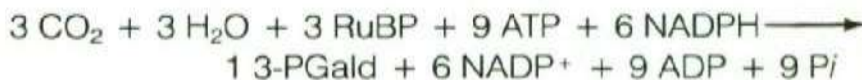
Out of 3 mol. of fructose 6 phosphate one molecule is isomerised under the influence of enzyme isomerase to form glucose 6 phosphate. Dephosphorylation of glucose or fructose 6 P in the influence of phosphatase enzyme forms glucose or fructose which further combines to form sugar or starch.

Calvin cycle occurs in three main parts:

1. **Carboxylation** - Which involves addition of CO_2 and H_2O to form two molecules of 3 PGA.
2. **Reduction** - In which COOH group in 3 PGA is reduced to an aldehyde group (3 P Galdehyde) .
3. **Regeneration of RuBP** – Out of the entry for every 6CO_2 molecules the output is one mole of glucose and rest go to the regeneration of RuBP.



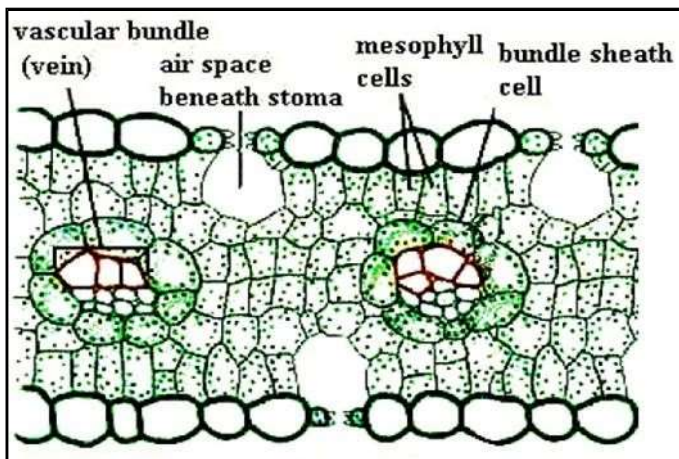
summary:



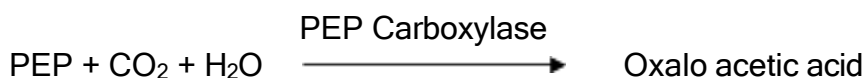
Hatch and Slack pathway (C₄ cycle)

- The photosynthetic carbon metabolism of certain plants in which the initial fixation of CO₂ and its subsequent reduction takes place in different cells, the mesophyll and bundle sheath cells, respectively. The initial carboxylation is catalyzed by phosphoenolpyruvate carboxylase, (not by rubisco as in C₃ plants), producing a four-carbon compound (oxaloacetate), which is immediately converted to malate or aspartate.

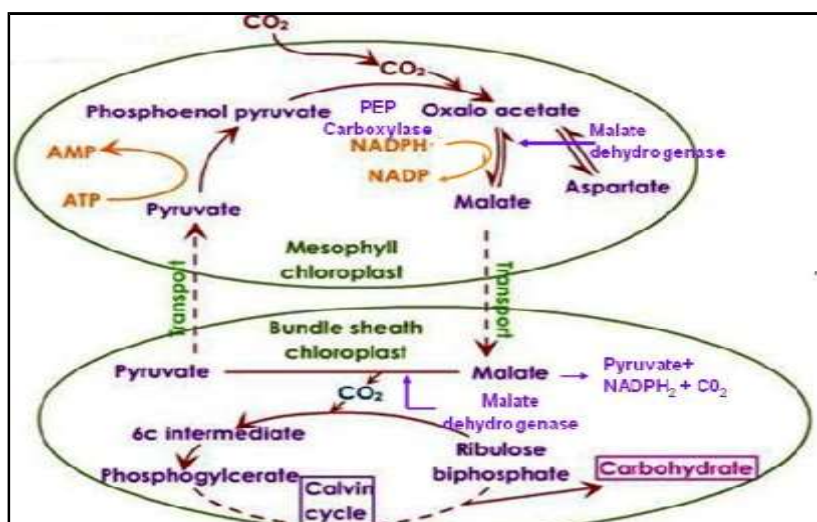
Hatch and Slack (1966) found that the first product of this cycle is 4 carbon acid. This cycle occurs mainly in sugarcane, maize, grasses, atriplex etc. C₄ plants possess Kranz anatomy. In this case the mesophyll cells are not differentiated into palisade and spongy parenchyma. Vascular bundles are surrounded by



layers of radially arranged parenchymatous cells. The sheath appears like a wreath, hence called Kranz (wreath) anatomy. Phospho enol pyruvate is the initial acceptor of CO_2 .



Initially CO_2 is accepted by Phospho enol pyruvate (PEP) under influence of PEP carboxylase enzyme forming aspartate. Malate is produced by activity of malic dehydrogenase in presence of NADPH_2 . Malate is transferred to chloroplast of bundle



sheath. Here malate is decarboxylated under influence of malate dehydrogenase to produce pyruvate, CO_2 and NADPH_2 . $\text{NADPH} + \text{H}^+$ travels back to mesophyll cells to regenerate malate, while pyruvate also travels back to mesophyll cells, where it utilizes the light generated ATP to produce PEP again. CO_2 released by decarboxylation of malate is fixed in the bundle sheath cells by C_3 pathway (accepted by RuBP).

In C_4 plants there are two carboxylations, one by atmospheric CO_2 which forms dicarboxylic acids and second by internally generated CO_2 entering the RuBP. In this case C_3 and C_4 both pathways operate. C_3 and C_4 pathways are delimited to bundle sheath cells and mesophyll cells, respectively. Because there is carboxylations at two sites, the pathway is also known as dicarboxylation pathway.

Chollet and Orgen (1975) found three categories of C_4 plants.

1. Fixation of CO_2 by PEP which forms oxalo acetate then malate Ex. maize, sugarcane.
2. Oxalo acetate gets converted into aspartate in mesophyll cells and it is transported to bundle sheath. In bundle sheath cells aspartate is reconverted to

oxalo acetate which is then converted to pyruvate and CO₂ Ex. *Panicum maximum*, *Chloris guyana*.

3. Aspartate produced in mesophyll cells is transported to bundle sheath cells where it gets transaminated to oxalo acetate first and then gets reduced to malate in mitochondria using NADH. The malate is decarboxylated to produce pyruvate and CO₂ Ex. *Atriplex spongiosa*.

Significance of C₄ pathway

1. They possess higher rates of photosynthesis due to higher affinity of PEP carboxylase to CO₂.
2. They can carry on photosynthesis even under low CO₂ concentrations (10ppm).
3. Even under almost closed conditions of stomata C₄ plants can continue to photosynthesize.
4. There is almost negligible photorespiration.

It is not necessary that C₄ pathway is always more efficient than C₃ pathway but most of the time C₄ pathway leads to better utilization of available CO₂ in C₃ fixation . C₄ pathway itself does not produce carbohydrates. It is only contributory pathway for C₄ cycle.