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Research
University of Baghdad
College of Science
Department of Biology



Plant Physiology

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المرحلة الثالثة - الدراسات الصباحية والمسائية
الفصل الدراسي الاول

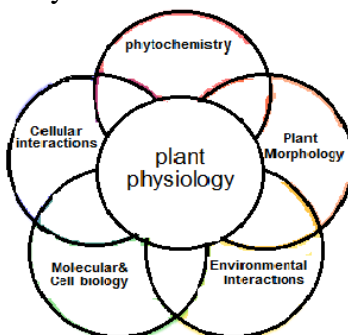
تدريس المائدة

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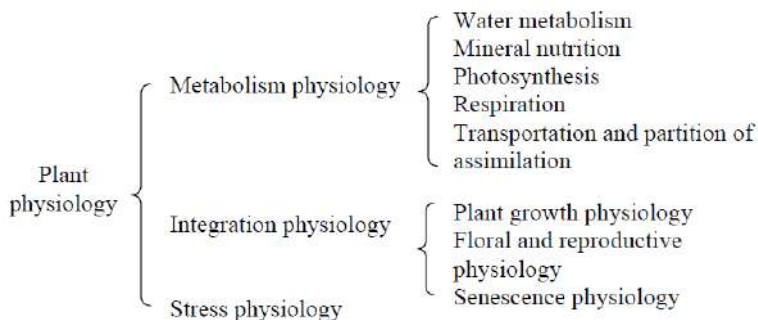
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Plant physiology

In this course, will emphasize the physiological and biochemical functions of plants, but it is important to recognize that these functions depend on structures, whether the process is gas exchange in the leaf, water conduction in the xylem, photosynthesis in the chloroplast, or ion transport across the plasma membrane. At every level, structure and function represent different frames of reference of a biological unity.



Plant physiology considered under the following main headings .:



During plant anatomy course, we examined the structure of the “higher” plant body, with occasional references to the functions of plant systems. In the next few days we shall look at how plants:

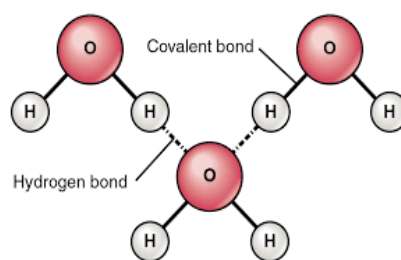
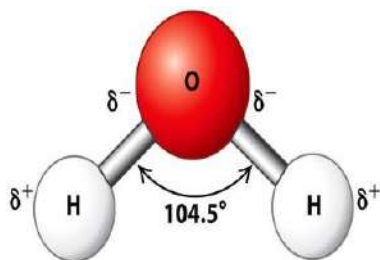
1. Maintain water balance and transport water throughout the plant.
2. Transport nutrients and solutes to cells and tissues.
3. Obtain nutrients for growth and survival from their substrate and from the atmosphere.
4. Regulate growth and developmental activities.

The structure and properties of water

Water has special properties that enable it to act as a solvent and to be readily transported through the body of the plant. These properties drive primarily from the polar structure of the water molecule. Water is important in the life of plants because it makes up the matrix and medium in which most biochemical processes essential for life take place. The structure and properties of water strongly influence the structure and properties of proteins, membranes, nucleic acids and other cell constituents.

Water is normal oxide of hydrogen in which the two hydrogen atoms are joined to oxygen

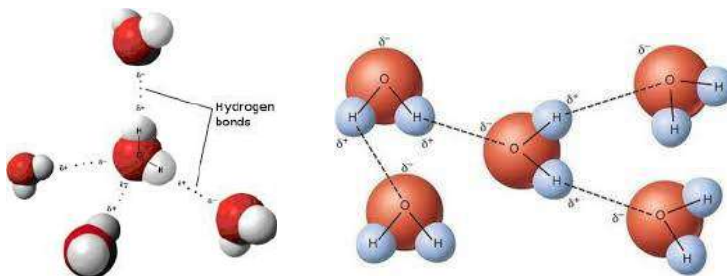
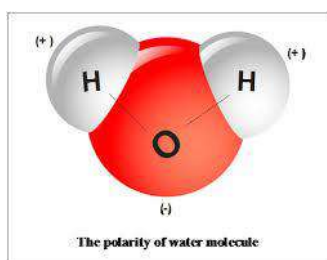
atom by **covalent bond** forming an angle of 105° , since oxygen atom is more electronegative than hydrogen atom, the electrons of the covalent bonds tend to be attracted towards oxygen atom. This result in **partial negative charge (δ^-)** on oxygen and equal **partial positive charges (δ^+)** on each hydrogen in water molecule. Because the partial negative and positive charges are equal, water molecule carries **no net charge** and is **neutral**. However, partial negative and positive charges on two sides of water molecule make it a polar molecule with the result that positive side of one water molecule is attracted towards negative side of another water molecule forming a **weak electrostatic chemical bond** between the polar water molecules which is called as a **hydrogen bond** and is represented by **dotted line**. The hydrogen bonds present in between the water molecules provide water with **unique physical properties**.



Hydrogen bond is a weak electrostatic chemical bond formed between covalently bonded hydrogen atom and a strongly electronegative atom with a lone pair of electrons such as nitrogen or oxygen and is represented by dotted line. In plants, hydrogen bonds may also be formed between water and other substances especially those which contain electronegative O or N atom with lone pair of electrons. The hydrogen bonds are tremendous biological importance, especially the N-H....N bond that enables complex proteins and nucleic acids to build up.

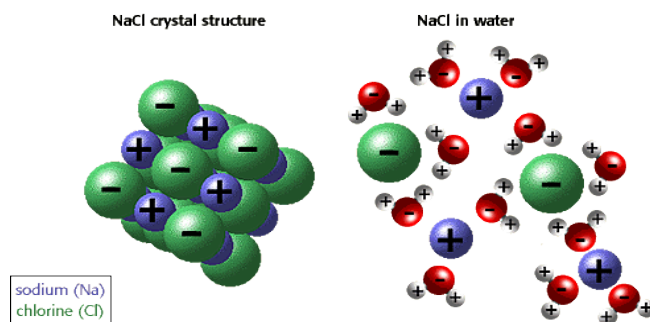
Water polarity

When the two hydrogen atoms bond with the oxygen, they attach to the top of the molecule rather like Mickey Mouse ears. This molecular structure gives the water molecule polarity, or a lopsided electrical charge that attracts other atoms. The end of the molecule with the two hydrogen atoms is positively charged. The other end, with the oxygen, is negatively charged. Just like in a magnet, where north poles are attracted to south poles ('opposites attract'), the positive end of the water molecule will connect with the negative end of other molecules.



The polarity of water makes it an excellent solvent

Water's polarity allows it to dissolve other polar substances very easily. The dissolving power of water is very important for life on Earth. Wherever water goes, it carries dissolved chemicals, minerals, and nutrients that are used to support living things. When a polar substance is put in water, the positive ends of its molecules are attracted to the negative ends of the water molecules, and vice versa. When an ionic or polar compound enters water, it is surrounded by water molecules. **The relatively small size of water molecules typically allows many water molecules to surround one molecule of solute.** The partially negative dipoles of the water are attracted to positively charged components of the solute, and vice versa for the positive dipoles. An example of an ionic solute is table salt. The dipole forces of water can disrupt the attractive forces that hold the sodium and chloride in the salt molecule together and, thus, dissolve it. Liquid water has a partially ordered structure in which hydrogen bonds are constantly being formed and breaking up. Water dissolves greater amounts and wide variety of substances than any other solvent. Water is especially powerful solvent for electrolytes and other substances such as sugars, proteins, etc... Which have polar -OH or -NH₂ groups.



DIFFUSION, OSMOSIS AND IMBIBITION

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

Diffusion

If a small bottle filled with some gas or vapors is opened at a certain place in the room, very soon its molecules become evenly distributed throughout the available space in that room. Similarly, if a solute is placed in its solvent, it is dissolved and its particles move so that they are evenly distributed throughout the container. **The movement of particles or molecules from a region of higher concentration to a region of lower concentration is called as diffusion without losing any energy.** The rate of diffusion of gases is faster than liquids or solutes. The **diffusing particles** have a certain **pressure** called as the **diffusion pressure** which is directly proportional to the **number or concentration of the diffusing particles**. Therefore, the diffusion takes place always from a region of higher diffusion pressure to a region of lower diffusion pressure i.e., along a diffusion pressure gradient.

Diffusion depends on many factors that increase the rate of diffusion if:

1. Temperature is increased - If the temperature increase then the kinetic energy will also increase and if kinetic energy increase then the velocity will also increase. This high velocity leads to high rate of diffusion.

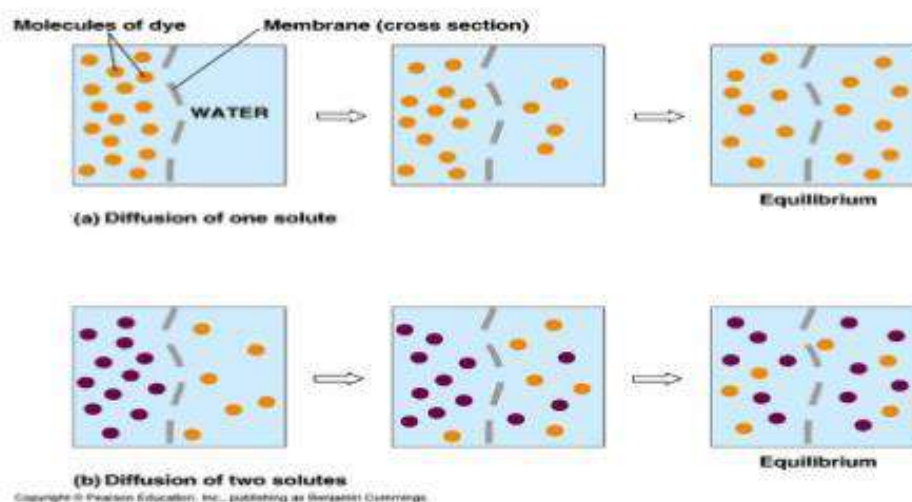
2. Medium through which diffusion occurs is less concentrated - The particles present in the medium act as barrier to diffusion. This means that larger the molecules lower the rate of diffusion and greater number of molecules reduce the rate of diffusion.

3. The density of the diffusing particles is lesser than solvent density - As the density of a **solvent** increases, the rate of diffusion decreases. The molecules slow down because they have a more difficult time getting through the denser medium. If the medium is less dense, diffusion increases. Because cells primarily use diffusion to move materials within the cytoplasm, any increase in the cytoplasm's density will inhibit the movement of the materials.

4. The diffusion pressure gradient or concentration gradient is steeper - Concentration is defined as the number of **solute molecules** that can be found within a given volume. Volume of high concentration gradient has a large difference in the concentration leads to a greater probability of molecular collisions over the region and therefore increases the rate of diffusion. Generally the greater the concentration gradient, the greater the rate of diffusion. While the closer the distribution of the material gets to equilibrium, the slower the rate of diffusion becomes.

Solute polarity: *Non-polar* or *lipid-soluble* materials more easily pass through plasma membranes than do polar materials, i.e., materials that have asymmetrical charge distributions across molecules with no net electrical charge.

Mass of the molecules diffusing: Heavier molecules move more slowly; therefore, they diffuse more slowly. The reverse is true for lighter molecules.



Diffusion of more than one substance at the same time and place may be at different rates and in different directions but is independent of each other. A very common example of this is the gaseous exchange in plants. Simple diffusion also plays a very important role in the life of the plants: -

- It is an essential step in the **exchange of gases** during respiration and photosynthesis.
- During passive salt uptake, the **ions are absorbed** by simple process of diffusion.

- Last step in **stomatal transpiration** is the diffusion of water vapors from the intercellular spaces into the outer atmosphere through open stomata.

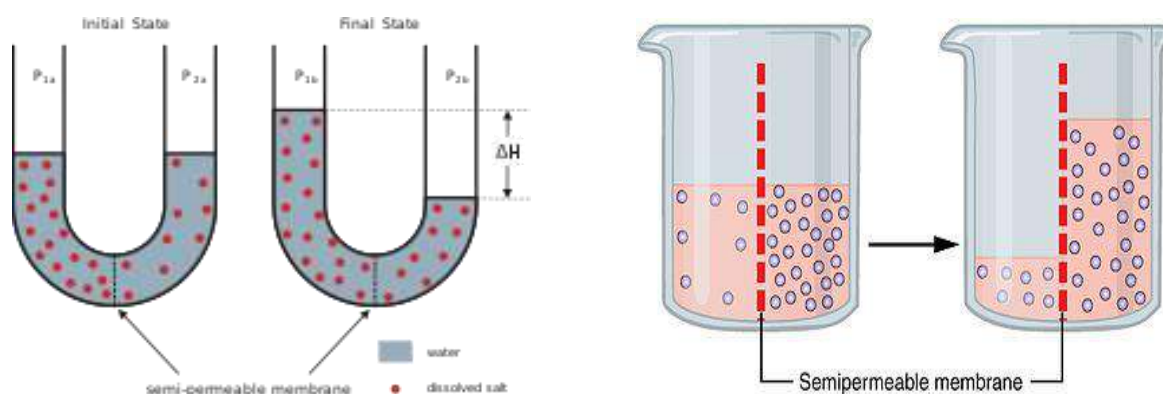
Osmosis

If a solution and its pure solvent are separated by a **semi permeable membrane** (which allows only **solvent** and not the solute to pass through it), the solvent molecules diffuse into the solution. This diffusion of solvent molecules into the solution through a semipermeable membrane is call as osmosis (sometimes called as **Osmotic Diffusion**).

Is the spontaneous net movement of solvent molecules through a selectively permeable membrane into a region of higher solute concentration, in the direction that tends to equalize the solute concentrations on the two sides.

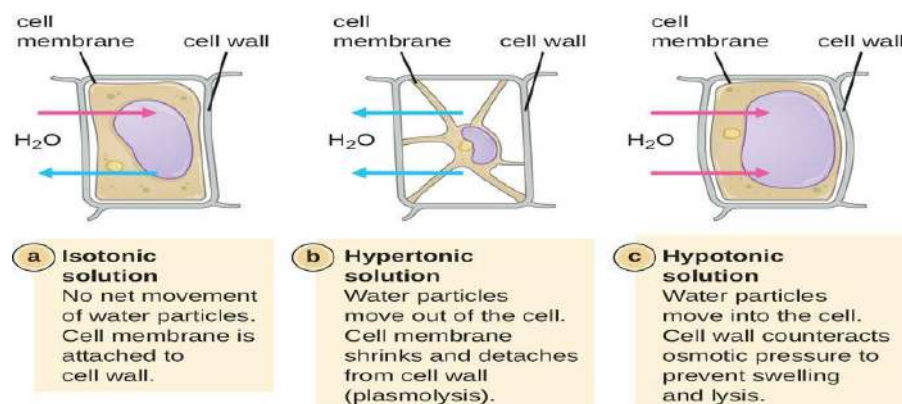
In case, there are two solutions of different concentrations separated by the semi permeable membrane, the diffusion of solvent will take place from the less concentrated solution into the more concentrated solution till both the solutions reach equal concentration.

Osmotic Pressure. As a result of the separation of solution from its solvent or the two solutions by the semipermeable membrane, a pressure is developed in solution due to the presence of dissolved solutes in it. Meaning that the osmotic pressure depends on the molar concentration of the solute but not on its identity. This is called as **Osmotic Pressure (O.P.)**.



1. Osmotic pressure is measured in terms of atmospheres.
2. Osmotic pressure is directly proportional to the concentration of dissolved solutes in the solution. More concentration solution has higher osmotic pressure.
3. Osmotic pressure of solution is always higher than its pure solvent.
4. Osmotic pressure does not increase by the addition of **insoluble solute** in the solution.
5. Osmotic diffusion of solvent molecules will not take place if the two solutions separated by the semi-permeable membrane are of equal concentrations having equal osmotic pressures (*i.e.*, they are isotonic).

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

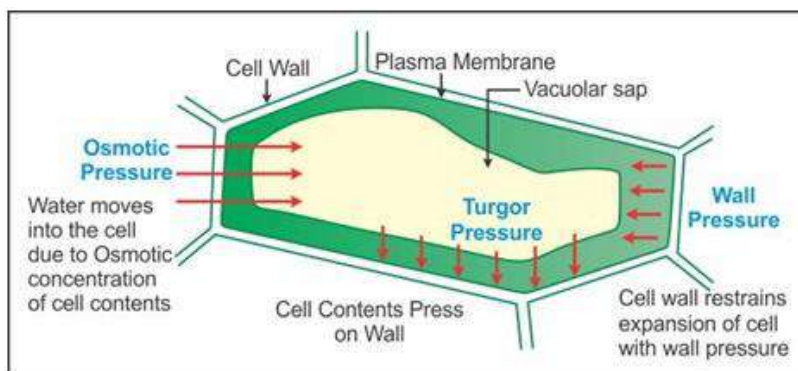


Plant Cells as Osmotic Systems.

Living cells in plants form osmotic systems due to the presence of semi-permeable plasma membrane and the cell sap having a certain osmotic pressure. The tonoplast or the vacuolar membrane also possesses the same nature. The solvent in case of plants is always water. The cell wall is permeable.

* If a living plant cell or tissue is placed in water or hypotonic solution (whose O.P. is lower than that of cell sap), water enters into the cell sap by osmosis. This process is called as **endosmosis**. As a result of entry of the water into the cell sap, a pressure is developed which presses the protoplasm against the cell wall and the cell becomes turgid. This pressure is called as turgor pressure. Consequence of the turgor pressure is the wall pressure, which is exerted by the elastic cell wall against the expanding protoplasm. At a given time, turgor pressure (T.P.) equals the wall pressure (W.P.).

$$\text{Turgor Pressure (T.P.)} = \text{Wall Pressure (W.P.)}$$



* If the plant cell or the tissue is placed in **hypertonic solution** (whose O.P. is higher than that of cell sap) the water comes out of the cell sap into the outer solution and the cell becomes flaccid. This process is known as **exosmosis**.

* Cell or tissue will remain as such in **isotonic** solution.

Significance of osmosis in plants:

(1) Large quantities of water are absorbed by roots from the soil by osmosis.

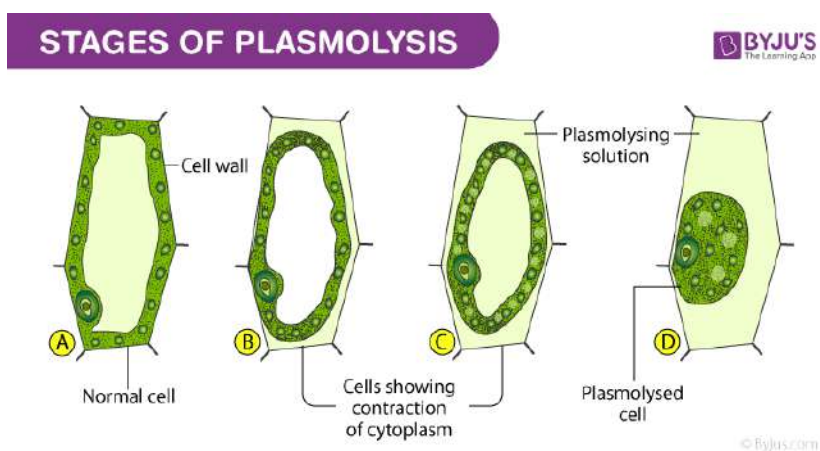
- (2) Cell to cell movement of water and other substances dissolved in it involves this process.
- (3) Opening and closing of stomata depend upon the turgor pressure of the guard cells.
- (4) Due to osmosis the turgidity of the cells and hence the shape of their organs is maintained.
- (5) The resistance of plants to drought and frost increases with increase in osmotic pressure of their cells.
- (6) Turgidity of the cells of the young seedlings allows them to come out of the soil.

Plasmolysis

If this plant cell or tissue is placed in a hypertonic solution, water comes out from the cell sap into the outer solution due to exosmosis and the protoplasm begins to contract from the cell wall. This is called as **Incipient plasmolysis**.

If the outer hypertonic solution is very much concentrated in comparison to the cell sap, the process of exosmosis and contraction or shrinkage of protoplasm continues and ultimately the protoplasm separates from the cell wall and assumes a spherical form. This phenomenon is called as plasmolysis and the cell or the tissue is said to be plasmolysed. Because of the permeable cell wall the space in between the cell wall and plasma-membrane in plasmolysed cells is filled with outer hypertonic solution.

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



Advantages of plasmolysis

1. It indicates the semi-permeable nature of the plasma-membrane.
2. This phenomenon is utilized in salting of meat and fishes and addition of concentrated sugar solution to jams and jellies to check the growth of fungi and bacteria which become plasmolysed in concentrated, solution.
3. It is also used in determining the O.P. (osmotic pressure) of the cell sap.

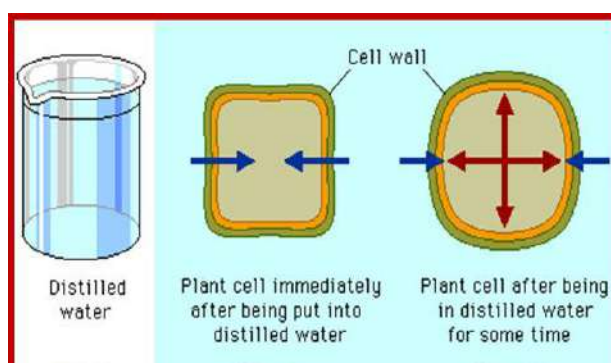
Diffusion pressure deficit (D.P.D.) (suction pressure)

Diffusion pressure is the potential ability of substances to diffuse. Diffusion pressure of a

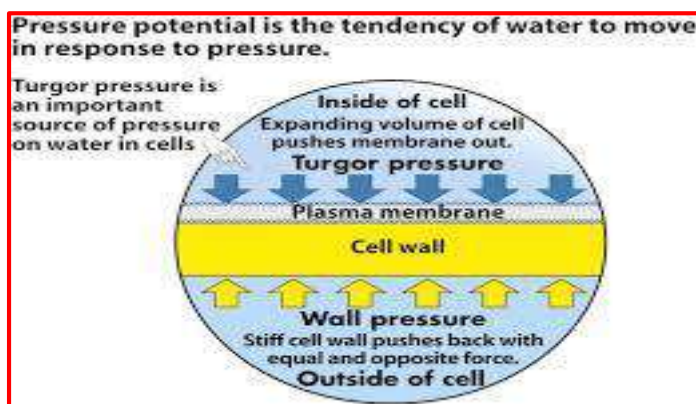
solution is always lower than its pure solvent. The difference between the diffusion pressure and its solvent at a particular temperature is called as (D.P.D.). D.P.D. is the amount by which the diffusion pressure of solution is less than its pure solvent or water. If the solution is more concentrated, the D.P.D. increases, and it decreases with the dilution of the solution.

D.P.D. \propto Conc. of the solution

In case of plants the cell sap is a watery solution of many inorganic and organic substances; i.e., its pure solvent is water. If these cells are placed in pure water the water will enter into the cells due to higher D.P.D. of the cell sap or water deficit.



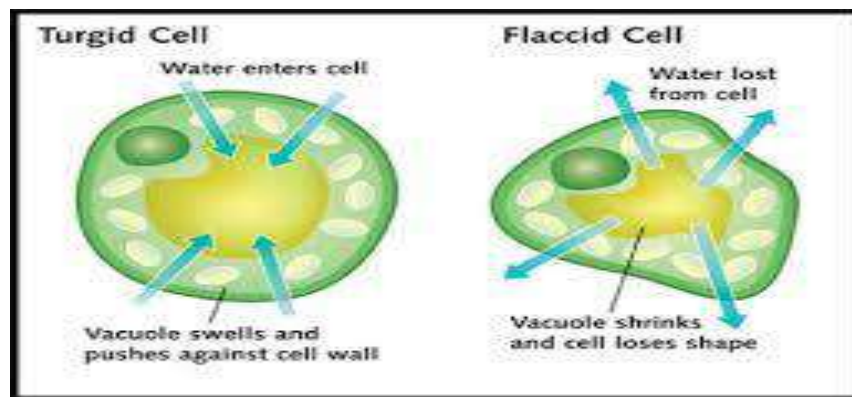
In other words, the D.P.D. of the cell sap or the cells is a measure of the ability of the cells to absorb water and hence it is often called as the Suction Pressure (S.P.). It is related with osmotic pressure (O.P.) and turgor pressure (T.P.) of cell sap and the wall pressure (W.P.)



as follows:

$$\text{D.P.D (S.P.)} = \text{osmotic pressure (O.P.)} - \text{wall pressure (W.P.)}$$

But wall pressure (W.P.) = Turgor pressure (T.P.)
 therefore, $D.P.D. = O.P. - T.P.$



Due to the entry of the water, the osmotic pressure of the cell sap decreases while its turgor pressure is increased so much so that in a **fully turgid cell** **turgor pressure** equals the osmotic pressure:

$$O.P. = T.P. \quad (\text{in fully turgid cell})$$

and hence, $D.P.D. (S.P.) = 0 \text{ (zero)}$

On the other hand, the removal of water from the cell sap (exosmosis) results in an increase of its O.P. and decrease of the turgor pressure so much so that in **fully plasmolysed cells** the value of turgor pressure becomes zero.

$$T.P. = 0 \quad (\text{in fully plasmolysed cell})$$

and hence, $S.P. = O.P.$

	$DPD = OP - TP \text{ (WP)}$
Fully turgid cell	
	$DPD = 0$
	$OP = TP$
fully flaccid cell	
	$TP = 0$
	$DPD = OP$

In case, the cell is placed in a **hypotonic solution** instead of pure water, the suction pressure of the cell sap will be:

$$S.P. = (O.P. - O.P._1) - T.P.$$

where $O.P._1$ is the osmotic pressure of outer hypotonic solution. That Thus it is obvious that the D.P.D. or S.P. in case of plant cells is not directly proportional to their osmotic pressure or the concentration of the cell sap, but depend both on O.P. and T.P.

Concept of water potential and osmotic relations of plant cells:

Water potential is : is a measure of the potential energy of water per unit volume relative to pure water which is known as **chemical potential** or the difference in potential energy between a

given water sample and pure water (at atmospheric pressure and ambient temperature). Water potential quantifies the tendency of water to move from one area to another due to [osmosis](#), [gravity](#), mechanical [pressure](#), or matrix effects such as [capillary action](#) (which is caused by [surface tension](#)). The concept of water potential has proved useful in understanding and computing water movement within [plants](#) and [soil](#).

Using only the basic laws of physics and the simple manipulation of energy, plants can move water to the top of a 116-meter-tall tree. Water potential is critical for moving water to leaves so that photosynthesis can take place.

Water potential is denoted by the Greek letter ψ (psi) and is expressed in units of pressure (pressure is a form of energy) called mega pascals (MPa). One mega pascal is equal to 10 bars.

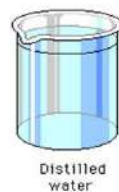
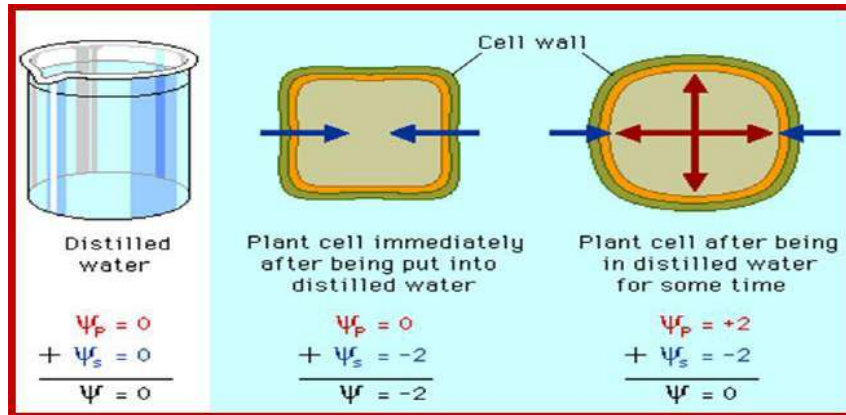
The potential of pure water (Ψ_w pure H_2O) is designated a value of zero (even though pure water contains plenty of potential energy, that energy is ignored). Water potential values for the water in a plant root, stem, or leaf are, therefore, expressed in relation to Ψ_w pure H_2O .

Water potential is lowered by the addition of solutes and because water potential value is zero for pure water, all other water potential values will be negative. In other word, the movement of water will take place in osmotic or other systems from a region of higher water potential (less negative) to a region of lower water potential (more negative).

Osmotic pressure (O.P.) in a solution results due to the presence of solutes and the latter lower the water potential. Therefore, osmotic pressure is a quantitative index of the lowering of water potential in the solution and called as osmotic potential (Ψ_s). Osmotic pressure and osmotic potential values are numerically equal but the former has positive sign, the latter carries a negative sign (If OP= 20 atms, Ψ_s = - 20 atms).

In an **open osmotic system**, the water potential and the osmotic potential values are numerically similar and also have same sign i.e., negative (similar will be the case in plasmolysed cells). On the other hand, in a **closed osmotic system** e.g., in plant cells a pressure is imposed on water which increases the water potential. In plants this pressure is called as turgor pressure. This is the actual pressure with positive sign and ranges between zero and numerical osmotic potential value. The potential created by such pressures is called as hydrostatic pressure or **pressure potential** (Ψ_p), Thus in such cases water potential is equal to osmotic potential plus pressure potential.

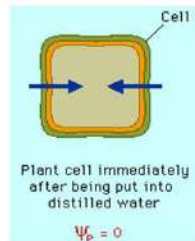
$$(\Psi_w = \Psi_s + \Psi_p)$$



Distilled water

$$\Psi_p = 0$$

In open system (like beaker)
pressure potential $\Psi_p = 0$



$$\Psi_p = 0$$

In closed system
(like plant cell with rigid cell wall)
pressure potential Ψ_p can be a positive or negative number, or zero.

Summarizing, it is more appropriate to say that osmotic entry of water into a cell depends, on its lower water potential than the outer solution or other cell, instead of saying that it is due to its higher D.P.D, (diffusion pressure deficit). Water potential values of plant cells under different osmotic conditions are as follows,

$\Psi_w = \Psi_s$ (as $\Psi_p = \text{nil}$).....in plasmolysed or flaccid cell.(lowest)

$\Psi_w = \Psi_s + \Psi_p$ in partially turgid cell.(higher)

$\Psi_w = \text{zero}$ (as Ψ_p numericallyin fully turgid cell.(highest) equals Ψ_s but both having opposite signs).

Imbibition

Certain substances if placed in a particular liquid absorb it and swell up. For example, when some pieces of a piece or dry wood or dry seeds are placed in water they **absorb** the water quickly and swell up considerably so that their volume is increased. These substances are called as imbibes and the phenomenon as imbibition. In this process **the liquid molecules move from the region of the high partial pressure to its lower partial pressure.**

- ❖ The solid particles which absorb water or any other liquid are called **imbibants**
- ❖ The liquid which is to be absorbed is known as **imbibate**.
- ❖ The pressure that an imbibant will develop if submerged in pure imbibing liquid, is called **imbibition pressure**, which replaced now by the name **matric pressure**.

Main conditions of the imbibition

- **There exists certain force of attraction in between the imbibant and the imbibed substances.** In plants, this is because of the presence of a large number of hydrophilic colloids both in living and dead cells in the form of proteins, carbohydrates such as starch, cellulose, pectic substances etc. which have strong attraction toward water.
- **Water moves by imbibition into a substance only when its water potential exceeds that of the imbibant.**

Imbibition plays a very important role in the life of the plants: —

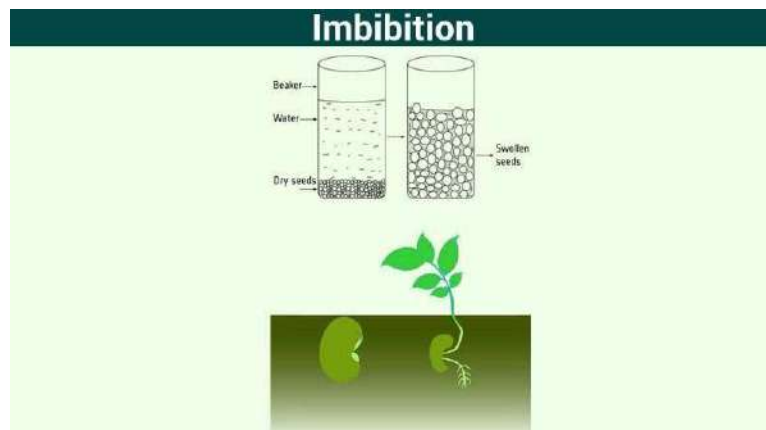
1. The first step in the absorption of water by the roots of higher plants is the imbibition of water by the cell walls of the root hairs.
2. Imbibition of water is very essential for dry seeds before they start germination.

The water potential of an imbibant is equal to its matric potential (always negative) plus any turgor or other pressure (pressure potential) which may be imposed upon the imbibant :

$$\text{(Water potential) } \Psi_w = \text{(Matric pressure) } \Psi_m + \text{(Turgor or Pressure potential) } \Psi_p$$

If the imbibant is unconfined, no turgor or such pressure is involved and hence the above equation simplifies to:

$$\Psi_w = \Psi_m$$



ABSORPTION OF WATER

MECHANISM OF ABSORPTION OF WATER

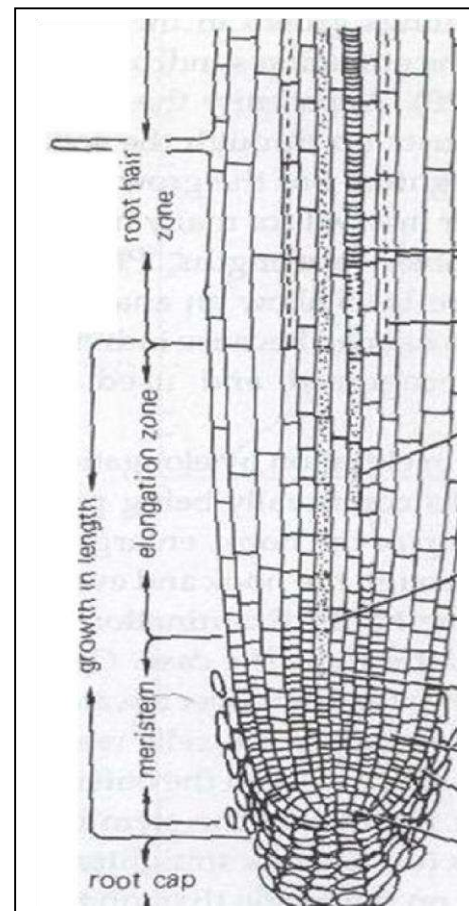
In higher plants water is absorbed through root hairs, which are in contact with soil water and form a root hair zone a little behind the root tips. When roots elongate, the older hairs die and new root hairs are developed so that they are in contact with fresh supplies of water in the soil.

Mechanism of water absorption is of two types:

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

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

[2] **Passive Absorption** of Water. It is mainly due to transpiration; the root cells do not play active role and remain passive.



(1/a) Active Osmotic Absorption of water

First step in the osmotic absorption of water is the Imbibition of soil water by the hydrophilic cell walls of root hairs.

Osmotic Pressure (O.P.) of the cell sap of root hairs is usually higher than the O.P. of the soil water. There for the Diffusion Pressure Deficit (D.P. D) in the root hairs become higher and water from the cell walls enters into them through plasma membrane by osmotic diffusion. In the same way, the water by cell to cell osmotic diffusion gradually reaches the innermost 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 their walls which are impervious to water.

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*.

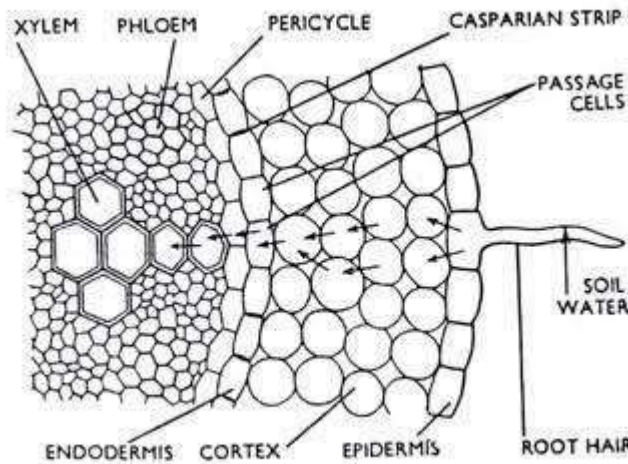


Fig. 4.2. A part of T.S. of typical dicot root. The arrows indicate the path of water.

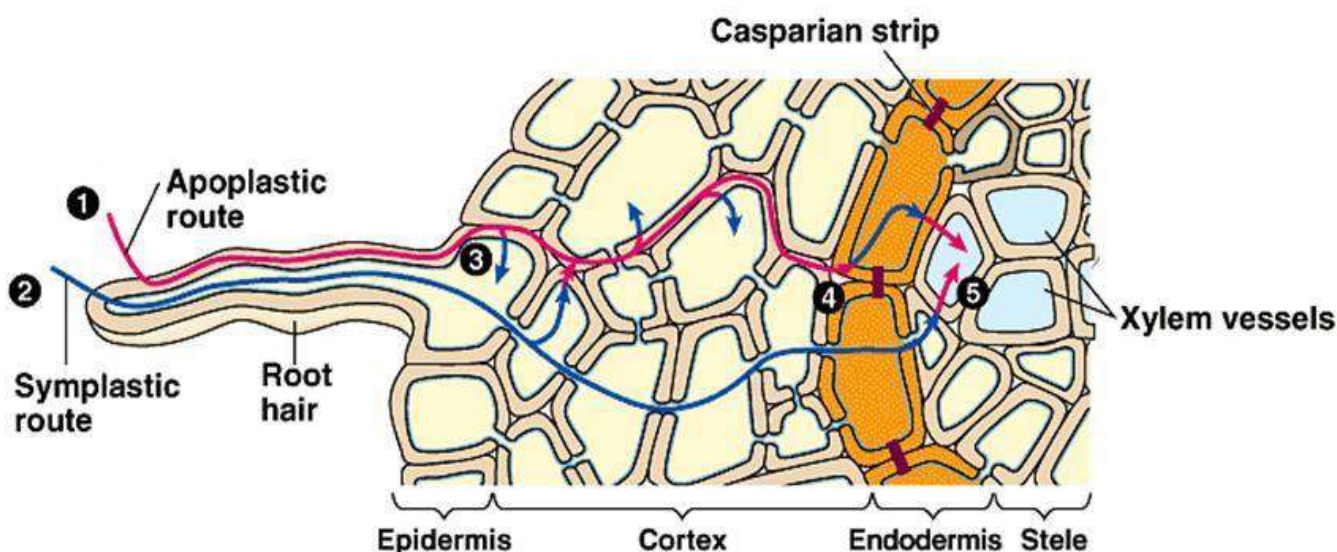
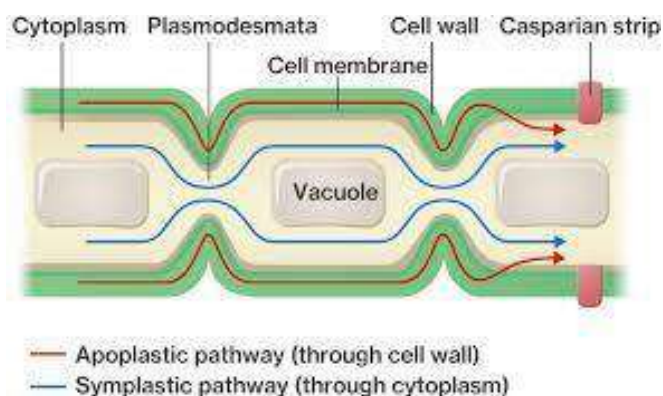
(I/b) Active non-Osmotic Absorption of water

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

[2] Passive Absorption of water

Passive absorption of water takes place when rate of transpiration is usually high. Rapid evaporation of water from the leaves during transpiration creates a tension in water in the xylem of the leaves. This tension is transmitted to water in xylem of roots through the xylem of stem and the water rises upward to reach the transpiring surfaces. As a result, soil water enters into the cortical cells through root hairs to reach the xylem of roots to maintain the supply of water. The force for this entry of water is created in leaves due to rapid transpiration and hence, the root cells remain passive during this process. During absorption of water by roots, the flow of water from epidermis to endodermis may take place through, three different pathways:

- (1) apoplastic pathway (cell walls and intercellular spaces).
- (2) transmembrane pathway (by crossing the plasma membranes) .
- (3) symplastic pathway (through Plasmodesmata).



External factors affecting absorption of water:

1. Available Soil Water:

Increase in the water content of the soil increases water absorption. But this increase is to a certain limit. Over irrigation of soil decreased soil aeration. Thus it decreases the water absorption.

2. Concentration of the Soil Solution:

Increased concentration of soil solution (due to presence of more salts in the soil) results in higher O.P. If O.P. of soil solution will become higher than the O.P. of cell sap in root cells, the water absorption particularly will be greatly suppressed. eg. Poor absorption of water in alkaline and saline soils.

3. Soil Air:

Water absorption also needs energy. This energy is released in the process of respiration. Oxygen is required for respiration. This oxygen is provided by soil air.

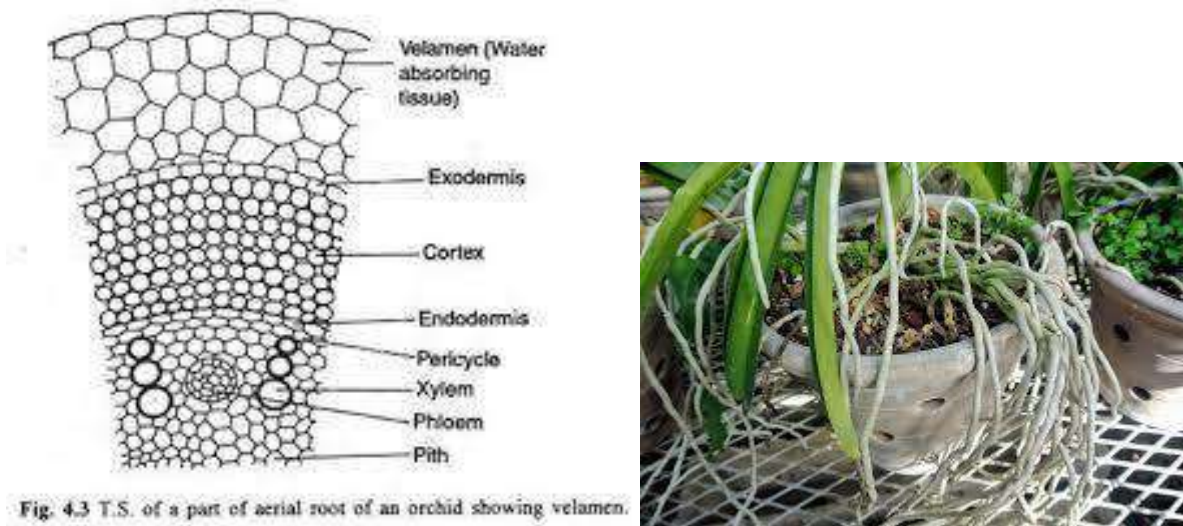
Therefore, water absorption depends on availability of soil air. Water logged soils have little air. So the plants wilt in this soil.

4. Soil Temperature

Increase in soil temperature up to about 30°C favors water absorption. At higher temperatures water absorption is decreased. At low temp, also water absorption decreases so much.

Velamen

Many epiphytic orchids develop special aerial adventitious roots, which can absorb moisture from the atmosphere. For this purpose, a special water absorbing tissue is present around the cortex of such roots, which is called as velamen. It consists of thin walled parenchyma, and the moisture absorbed by transferred to the root xylem through exodermises, cortex, endodermis and the pericycle. It is many cell layers thick and capable of absorbing atmospheric moisture and nutrients. However, its main function may lie in protecting the underlying cells against damaging UV rays.



Transpiration and guttation

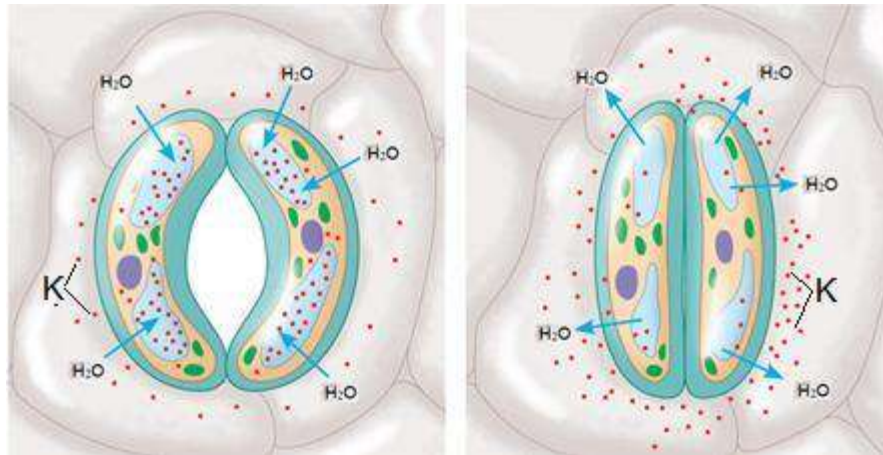
Transpiration differs from evaporation. Transpiration is a vital physiological process in plants in which water is lost from their aerial parts in the form of water vapours and for which living tissues are essential. Evaporation on the other hand,
is a purely physical process in which there is conversion of any liquid into

vapours without necessarily reaching the boiling point. Living tissues are not essential for evaporation.

Kinds of Transpiration:

It is of 3 types:

- (1) Stomatal Transpiration. Most of the transpiration takes place through stomata. Stomata are usually confined in more numbers on the lower sides of the leaves. In monocots e.g. grasses they are equally distributed on all sides. While in aquatic plants with floating leaves they are present on the upper surface.
 - (2) Cuticular Transpiration. (Per-stomatal transpiration) Although cuticle is impervious to water still some water may be test through it. It may contribute a maximum of about 10% of the total transpiration.
 - (3) Lenticular Transpiration. Some water may be lost by woody stems through lenticels which is called as Lenticular transpiration.
- (Transpiration from leaves is called as foliar transpiration)



Mechanism of stomatal transpiration

The mechanism of stomatal transpiration which takes place during the day time can be studied in 3 steps:

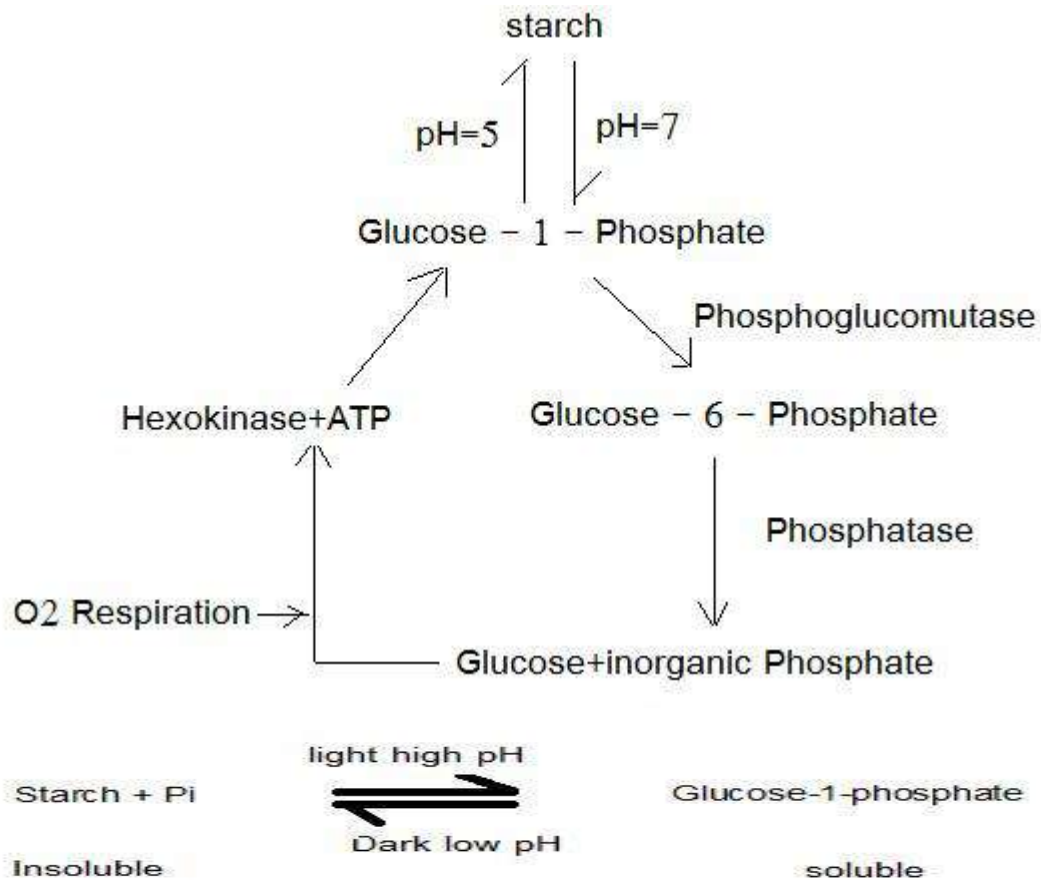
- (1) Osmotic diffusion of water in the leaf from xylem to intercellular spaces above the stomata through the mesophyll cells.
- (2) Opening and closing of stomata (stomatal movement).
- (3) Simple diffusion of water vapours from intercellular spaces to outer atmosphere through open stomata.

There may be several different agents or mechanisms which create osmotic potential in the guard cells and control stomata movements such as hydrolysis

of starch into sugars in guard cells, synthesis of sugars or organic acids in them and the active pumping of K⁺ ions in the guard cells which is accompanied by Cl⁻ or organic acids counter ions.

a- Starch-Sugar Interconversion Theory.

This classical theory is based on the effect of pH on Starch phosphorylase enzyme which reversibly catalyses the conversion of starch + inorganic phosphate into glucose-1-phosphate. **During the day**, pH in guard cells is high. This favors hydrolysis of starch (which is insoluble) into glucose-1-phosphate (which is soluble) so that osmotic potential becomes higher in the guard cells. Consequently, water enters into the guard cells by osmotic diffusion from the surrounding epidermal and mesophyll cells. Guard cells become turgid and the stomata open. **During dark** reverse process occurs. Glucose-1-phosphate is converted back into starch in the guard cells thereby increasing the osmotic potential. The guard cells release water, become flaccid and the stomata become closed.



b- Synthesis of Sugars or Organic Acids in Guard Cells.

During day, light photosynthesis occurs in guard cells as they contain chloroplasts. The soluble sugars formed in this process may contribute in decreasing the water potential of guard cells and hence resulting in stomata opening. As a result of photosynthesis, CO₂ concentration in guard cells decreases that leads to increased pH in them during daylight. There may be some buildup of organic acids, chiefly malic acid during this period in guard cells (HCO₃⁻ - combining with phosphoenol pyruvate (PEP) to form malic acid in the presence of the enzyme PEP-Carboxylase).

c- ATP-Driven Proton (H⁺) - K⁺ Exchange Pump Mechanism in Guard Cells.

The concept of K⁺ ion transport was given by Fujino. It was supported and elaborated by Levitt & Rashke in 1975. It appears to be an active mechanism which needs ATP. It is based on recent observations and (explains the mechanism as follows:

A. Opening of Stomata during Daytime (in presence of light):

Opening of stomata depends upon following

conditions: (a) Presence of light.

(b) Decrease in starch contents of guard cells.

(c) Increased concentration of malic acid in guard cells.

(d) Influx of K⁺ ions in guard cells.

(e) Efflux of H⁺ ions from guard cells.

(f) Intake of Cl⁻ ions by guard cells.

(g) Low CO₂ concentration in an around guard cells.

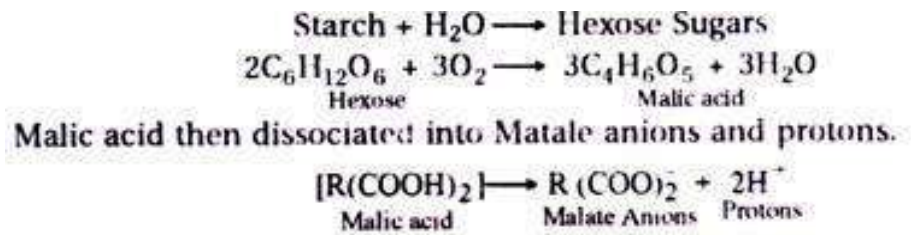
(h) High pH (more than 7) in guard cells (hence, alkaline medium of the cell sap in guard cells).

- (i) High T.P. in guard cells due to endosmosis, (turgidity of cells).
- (j) TP more towards thin wall of guard cell & stomata open.

Explanation of Levitt Concept:

This is explained as follows:

In the guard cells, starch is converted into malic acid in presence of light (during day time).



Protons (H^+) thus formed are used by the guard cells for the uptake of K^+ ions (in exchange for the protons H^+). This is an active ionic exchange and requires ATP energy and (a plant hormone). In this way, the concentration of K^+ ions increases in guard cells. At the same time, the concentration of H^+ ions decreases in guard cells. The pH of the cell sap in guard cells also increases simultaneously (pH becomes more than 7 and the medium becomes alkaline). There is also an increased uptake of Cl^- (anions) by the guard cells to maintain the electrical and ionic balance inside and outside the guard cells. The malate anions formed in the guard cells are neutralized by the K^+ ions. This results in the formation of potassium malate.

Malate anions + $\text{K}^+ \rightarrow$ Potassium malate:

Potassium malate enters the cell sap of the guard cells thereby reducing the water potential while increasing the osmotic concentration (and the O.P.) of the cell sap. Hence, endosmosis occurs, guard cells become turgid and kidney-shaped and the stomata open.

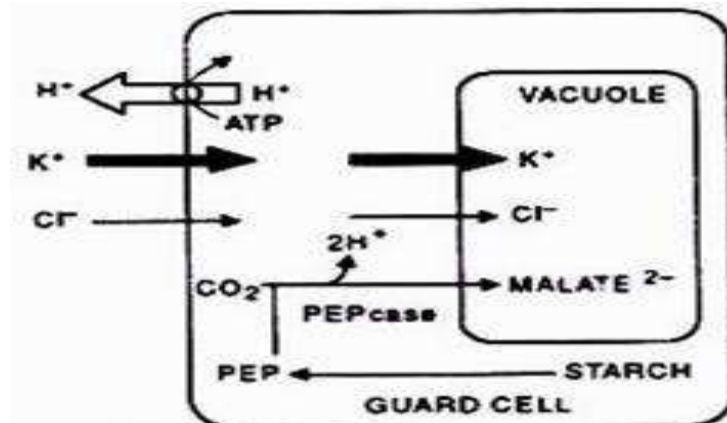


Fig. 4.7 Role of K^+ , Cl^- and malate in increasing osmotic concentration (decreasing water potential) of guard cells.

It is also observed that the CO_2 concentration is low in and around guard cells during day time. This is due to high photosynthetic utilization of CO_2 . It helps in opening of stomata.

B. Closing of Stomata in Absence of Light (Darkness/Night Time): Closing of stomata depends on following conditions:

- (a) Absence of light.
- (b) Decreased concentration of malic acid in guard cells.
- (c) Efflux of K^+ ions from guard cells.
- (d) Influx of H^+ ions in guard cells.
- (e) Acidic medium of the cell sap in guard cells.
- (f) Loss of Cl^- ions from guard cells.
- (g) Increases CO_2 concentration in and around guard cell due to release of CO_2 in respiration combined with the absence of photosynthetic activity in dark.
- (h) Presence of plant growth inhibiting hormone abscissic acid (ABA), (i) Loss of turgidity and loss of kidney-shape by guard cells.

All these conditions represent the reversal of the daytime events. Under these conditions, the guard cells lose water by exosmosis and become flaccid. This causes closing of the stomata.

Significance of transpiration

Transpiration has a role in the movement of water, absorption and translocation of minerals, and in the regulation of temperature.

Plants waste much of their energy in absorbing large quantities of water, most of which is ultimately to be lost through transpiration. While some people think transpiration as advantageous to plant which in fact is not true, others regard it as an unavoidable process which is rather harmful.

(B) Transpiration as unnecessary.

It is quite obvious from the above discussion that transpiration is not of much importance to plants in special plants.

- (1) Many **xerophytes** have to develop structural modifications and adaptations to check transpiration.
- (2) **Deciduous** trees have to shed their leaves during autumn to check loss of water.

But, in spite of the various disadvantages the plants cannot avoid transpiration due to their peculiar internal structure particularly those of leaves. Their internal structure although basically meant for gaseous exchange for respiration, photosynthesis etc. is such that it cannot check the evaporation of water.

Difference between Transpiration and Guttation Transpiration

Definition: The loss of water in the form of vapours from the living tissues of aerial parts of the plant is termed as transpiration.

1. Water loss in the form of vapour
2. Occurs through stomata, cuticle or lenticels
3. Usually occurs during day time
4. Water vapours are pure and free of dissolved substances
5. It is a controlled phenomenon
6. Universal phenomenon and occurs in plant
7. The stomata of leaves become closed or open according to the need, usually they remain open during day and closed during night.
8. It is controlled by the guard cells
9. Root pressure is not involved in transpiration

Guttation:

Loss of water in the form of liquid from the uninjured margins of the leaves is called guttation.

1. Water loss in the form of water drops
2. Occurs through hydathodes*
3. Usually occurs during night
4. Guttation water has many dissolved substances
5. It is an uncontrolled phenomenon
6. It occurs only in some plants like grasses, Colocasia, tomato etc
7. The hydathodes remain open whole day and night

8. It is not controlled by guard cells
9. Guttation takes place due to the development of root pressure

of stomata called water stomata through which guttation takes place. * Hydathodes are special type

ASCENT OF SAP

The water after being absorbed by the roots is distributed to all parts of the plant (excess of which is lost through transpiration), in order to reach the top most all parts of the plant, the water has to move upward through the stem. **This upward movement of water from roots to aerial parts through xylem is called as Ascent of Sap.** Ascent of sap can be studied under the following two heads:

- (A) Path of Ascent of Sap.
- (B) Mechanism of Ascent of Sap.

Although the mechanism of ascent of sap is not well understood, a number of theories have been put forward to explain it.

(A) VITAL THEORIES

Supporters of vital theories think that the ascent of sap is under the control of vital activities in the stem. Two such theories are common but they are not very convincing:-

- (1) According to **Godlewski** (1884) ascent of sap takes place due to the **pumping activity** of the **cells of xylem parenchyma** which are living. The cells of the **medullary rays** which are also living, in some way change their **O.P.** When their **O.P.** becomes high they draw water from the **lower vessel** and their **O.P.** becomes low. Now due to the low **O.P.** water from the cells of xylem parenchyma is **pumped** into the **above vessel**. This process is repeated again and again and water rises upward in the xylem. This theory *seemed* only hypothetical, and was further discarded by the experiments of **Strasburger** who demonstrated that ascent of sap continues even in the stems in which living cells have been killed by the uptake of poisons.
- (2) According to **Bose** (1923) upward translocation of water takes place due to the **pulsatory activity** of living cells of inner most cortical layer just outside the endodermis. This theory was also rejected because many workers could not repeat his experiment and many others found no correlation between pulsatory activity and the ascent of sap.

(B) ROOT PRESSURE THEORY

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

- (1) Magnitude of root pressure is very low (about 2 atms).
- (2) Even in the absence of root pressure, ascent of sap continues. For example, when a leafy twig is cut under water and placed in a beaker full of water. It remains fresh and green for sufficient long time.

(3) In gymnosperms root pressure has rarely been observed.

(C) PHYSICAL FORCE THEORIES

Various physical forces may be involved in the ascent of sap:—

(1) Atmospheric Pressure

This does not seem to be convincing because: (i) it cannot act on water present in xylem in roots, (ii) in case it is working, then also it will not be able to raise water beyond 34'.

(2) Imbibition

Sachs (1878) supported the view that ascent of sap could take place by imbibition through the walls of xylem. Now it is well known that **imbibitional force** is insignificant in the ascent of sap because it takes place through the **lumen** of xylem elements and not through walls. (A leafy twig is cut under water and the cut end is dipped in melted paraffin wax for some time. A thin section of stem near cut end is removed to expose the cell walls. The twig is transferred to a beaker containing water. The twig soon wilts because the lumens of xylem elements have been plugged by wax).

(3) Capillary force

In plants the xylem vessels are placed one above the other forming a sort of continuous channel which can be compared with long capillary tubes and it was thought that as water rises in capillary tube due to capillary force, in the same manner ascent of sap takes place in xylem. There are many objections to this theory:

- (1) For capillarity a free surface is required.
- (2) The magnitude of capillary force is low.
- (3) In spring when there is more requirement of water due to the development of new leaves, the wood consists of **broad elements**. While in autumn, when water supply decreases, the wood consists of **narrow elements**. This is against capillarity.
- (4) In Gymnosperms usually the vessels are absent. Other xylem elements do not form continuous channels.

(D) TRANSPIRATION PULL AND COHESION OF WATER THEORY

This theory is very convincing and has now been widely supported by many workers. It is based on the following features:—

- (1) **Cohesive and Adhesive** properties of water molecules to form a continuous water column in the xylem
- (2) **Transpiration pull** exerted on this water column. Water molecules remain joined to each other due to the presence of **H-bonds** between them molecules.

Although H-bond is very weak (containing about 5k. cal. energy) but when they are present in enormous numbers as in case of water, a very strong mutual force of attraction or **cohesive force** develops between water molecules and hence they remain in the form of a **continuous water column** in the xylem. The magnitude of this force is very high (sometimes up to 350 atmos.), therefore the continuous water column in the xylem cannot be broken easily due to the force of gravity or other obstructions offered by the internal tissues in the upward movement of water.

The **adhesive properties** of water i.e. the attraction between the water molecules and the container's walls (here the walls of xylem) further ensure the continuity of water column in xylem.

ABSORPTION OF MINERAL SALTS

MECHANISM OF MINERAL SALT ABSORPTION

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

Mineral salts are absorbed from the soil solution in the form of ions. They are chiefly absorbed through the meristematic regions of the roots near the tips. However, some mineral salts may also be absorbed at other locations on the root surface or over the entire root surface including zone of elongation and root hairs that depends upon the high availability of such minerals around them and/or strong tissue demand at such locations. Some mineral soils can also be absorbed by leaves of the plants during foliar application of chemical fertilizers on them. Plasma membrane of the root cells is not permeable to all the ions. It is **selectively permeable**. All the ions of the same salt are not absorbed at equal rate.

The further process of the absorption of mineral salts may be of two types :

(1) Passive and (2) Active.

(1) PASSIVE ABSORPTION OF MINERAL SALTS

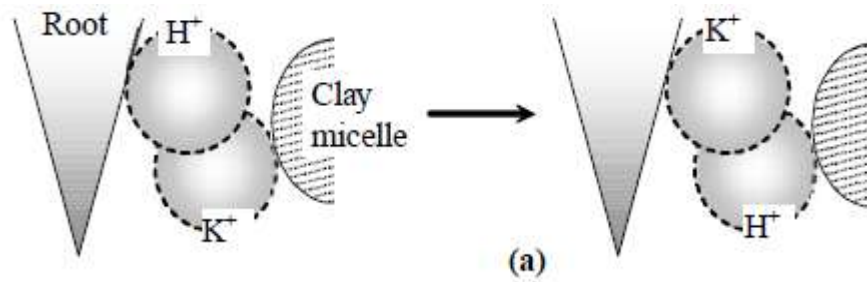
- (a) First step in the passive absorption of mineral salts is the process of **Ion-Exchange** which **does not require** metabolically energy but greatly facilitates mineral salt absorption.

ION-EXCHANGE

The ions **adsorbed** on the surface of the walls or membranes of root cells may be **exchanged with the ions of same sign from external solution**. For example, the cation **K⁺** of the external soil solution may be exchanged with H⁺ ion adsorbed on the surface of the root cells. Similarly, an anion may be exchanged with **OH⁻ ion**. There are two theories regarding the mechanism of ion exchange :

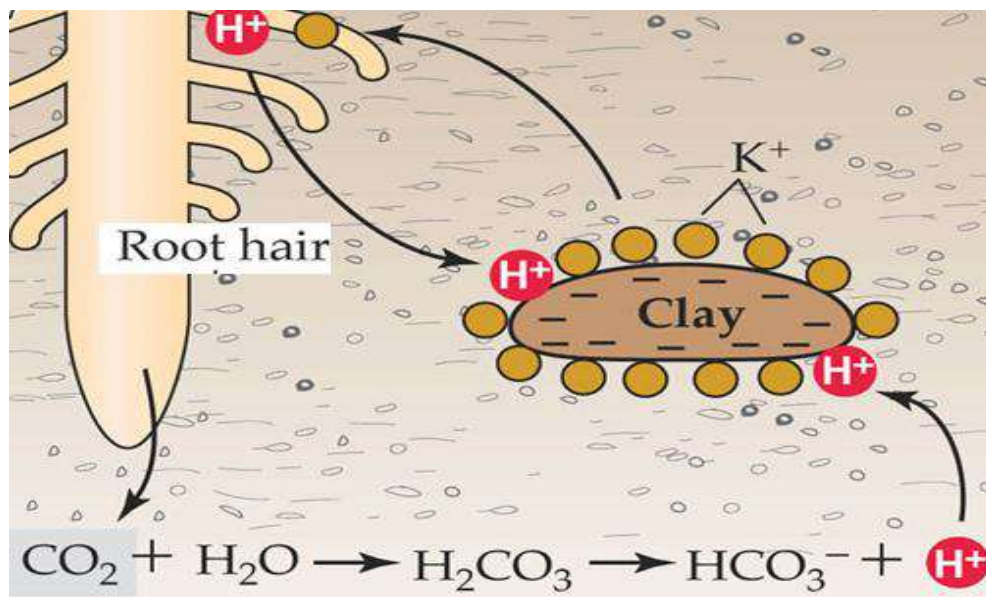
(A) Contact Exchange Theory

According to this theory, the **ions adsorbed on the surface of root cells and clay particles** (or clay micelles) **are not held tightly but oscillate within small volume of space**, and then exchange without first being dissolved in soil solution.



(B) Carbonic Acid Exchange Theory

According to this theory, the CO_2 released during respiration of root cells combines with water to form carbonic acid (H_2CO_3). Carbonic acid dissociates into H^+ and an anion HCO_3^- in soil solution. These H^+ ions may be exchanged for cations adsorbed on clay particles. The cations thus released into the soil solution from the clay particles, may be adsorbed on root cells in exchange for H^+ ions or as ion pairs with bicarbonate. Thus, soil solution plays an important role in carbonic acid exchange theory.



(b) Donnan's equilibrium

This theory explains the accumulation of some mineral ions inside the cells against the concentration gradient without expenditure of the metabolic energy, can be explained by Donnan's equilibrium theory (also known as the **Donnan effect**, **Donnan law**, **Donnan equilibrium**, or **Gibbs–Donnan equilibrium**)

According to this theory, there are certain pre-existing ions inside the cell which cannot diffuse outside through membrane. Such ions are called as **indiffusible or fixed ions** which may be in the form of charged carboxyl (**Anions**, - COO^-) and amino (**Cations**, - NH_4^+) groups of proteins or charged groups of other macromolecules in the cell. However, the membrane is **permeable** to both

anions and cations of the outer solution.

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

(c) Diffusion

When the concentration of mineral salts is higher in the outer solution than in the cell sap of the root cells, the **mineral** salts are absorbed according to the **concentration** gradient by simple process of **diffusion**. This is called as **passive absorption** because it does not require expenditure of metabolic energy. It is now known that during passive absorption; the mineral salts may diffuse through cell membranes directly through lipid-layer.

(2) ACTIVE ABSORPTION OF MINERAL SALTS

The absorption and accumulation of mineral salts against the concentration gradient is an **active process** which involves the **expenditure of metabolic energy** through **respiration**.

It has often been observed that the cell sap in plants accumulates large-quantities of mineral salts ions **against the concentration gradient**. For example, in alga *Nitella* the cell accumulated **K⁺** and **phosphate** ions to such an extent that their concentrations were hundreds times greater than in the lake water in which the plant was growing. The movement of mineral salts is mainly through mechanisms:

THE CARRIER CONCEPT

According to this theory, the plasma membrane is impermeable to free ions. But some compound present in it acts as carrier and combines with ions to form carrier-ion-complex which can move across the membrane. On the inner surface of the membrane, this complex breaks releasing ions into the cell while the carrier goes back to the outer surface to pick up fresh ions.

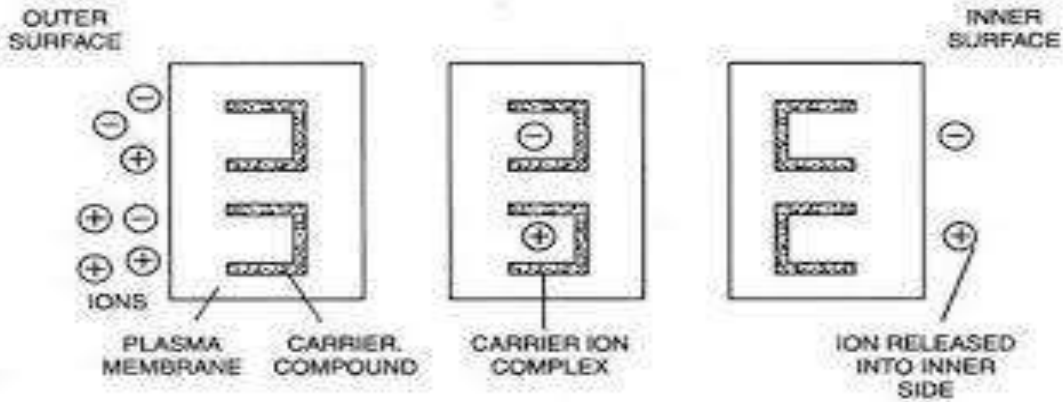


Fig. 7.2. Diagrammatic representation of a model illustrating the carrier concept.

(1) Lundegardh's Cytochrome Pump Theory:

Lundegardh and Burstrom (1933) believed that there was a definite correlation between respiration and anion absorption. Thus when a plant is transferred from water to a salt solution the rate of respiration increases. This increase in rate of respiration over the normal respiration has been called as **anion respiration** or **salt respiration**. Lundegardh (1950, 54) proposed cytochrome pump theory which is based on the following assumptions :

- (i) The mechanism of anion and cation **absorption is different**.
- (ii) Cations are **absorbed passively**.
- (iii) Anions are absorbed through **cytochrome chain** by an active process.

According to this theory .:

- (1) dehydrogenase reactions on inner side of the membrane give rise to protons (H^+) and electrons (e^-).
- (2) The electron travels over the cytochrome chain towards outside the membrane, so that the Fe of the cytochrome becomes reduced (Fe^{++}) on the outer surface and oxidised (Fe^{+++}) on the inner surface.
- (3) On the **outer surface**, the reduced cytochrome is oxidised by oxygen releasing the electron (e^-) and taking an anion (A^-).
- (4) The electron thus released unites with H^+ and oxygen to form water.
- (5) The anion (A^-) travels over the cytochrome chain towards inside.

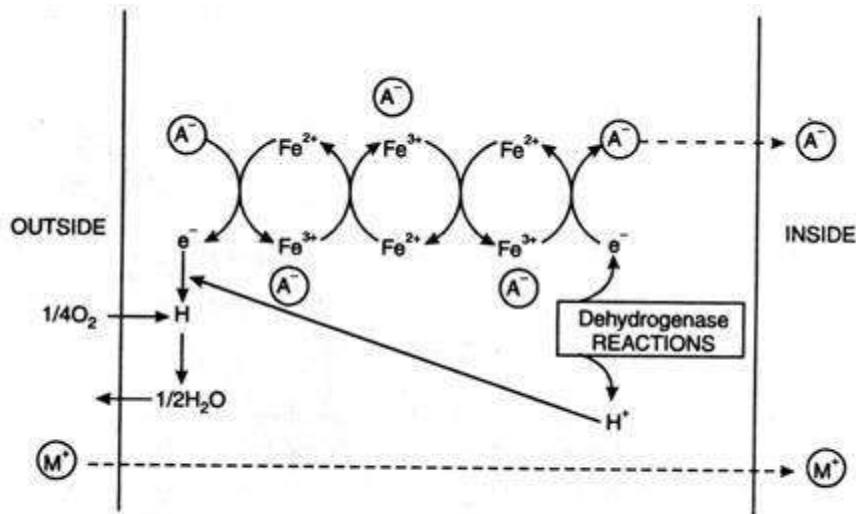


Fig. 7.3 Diagrammatic representation of the Lundegardh's cytochrome pump theory.

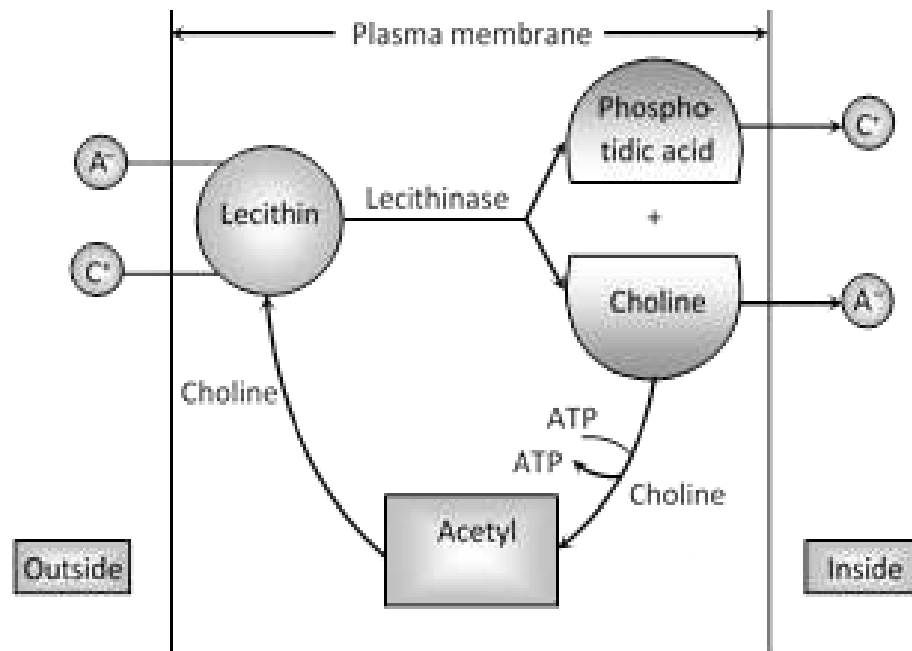
- (6) On the inner surface the oxidised cytochrome becomes reduced by taking an electrons produced through the dehydrogenase reactions, and the anion (A⁻) is released.
- (7) As a result of anion absorption, a cation (M⁺) moves passively from outside to inside to balance the anion.

(2) Bennet-Clark's Protein-Lecithin Theory

In 1956, Bennet-Clark suggested that because the cell membranes chiefly consist of phospholipids and proteins and certain enzymes seem to be located on them, the carrier could be a protein associated with the phosphatide called as lecithin. He also assumed the presence of different phosphatides to correspond with the number of known competitive groups of cations and anions (which will be taken inside the cell).

According to this theory:

- (i) The phosphate group in the phosphatide is regarded as the active center binding the cations, and the basic choline group as the anion-binding center.
- (ii) The ions are liberated on the inner surface of the membrane by decomposition of the lecithin by the enzyme lecithinase.
- (iii) The regeneration of the carrier lecithin from phosphatidic acid and choline takes place in the presence of the certain enzymes and ATP which acts as a source of energy.



The protein-lecithin carrier concept

TRANSPORT OF SOLUTES ACROSS MEMBRANE IN PLANTS

In recent years, much work has been done on permeability of cell membranes especially plasma membrane and tonoplast (vacuolar membrane), and various trails membrane transporters **(proteins)** have been identified in them which enhance movement of solutes across such membranes. These transporter proteins are highly specific with complex structure and different models have been given by scientists to explain their functioning. These membrane transporter proteins can be grouped in three categories, (i) ion-channels (ii) carriers (iii) pumps.

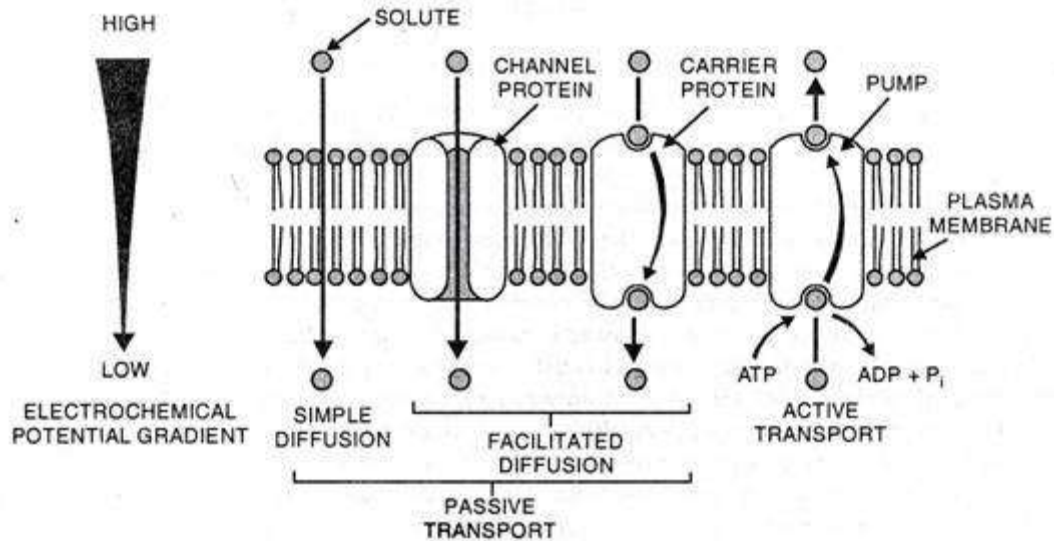


Fig. 7.6. Diagrammatic representation of passive and active transport of solutes across the membrane.

1. Ion-Channels:

- Ion-channels are **transmembrane proteins** which function as **selective pores** through which ions can diffuse easily across the membrane.
- Ion-channels are usually highly specific for one or limited number of ion species. The specificity depends upon the size of the pore and density of surface electric charges on its interior lining more than on selective binding of ions.
- Transport of ions through channels is always passive. The channels are not open all the time but are 'gated'

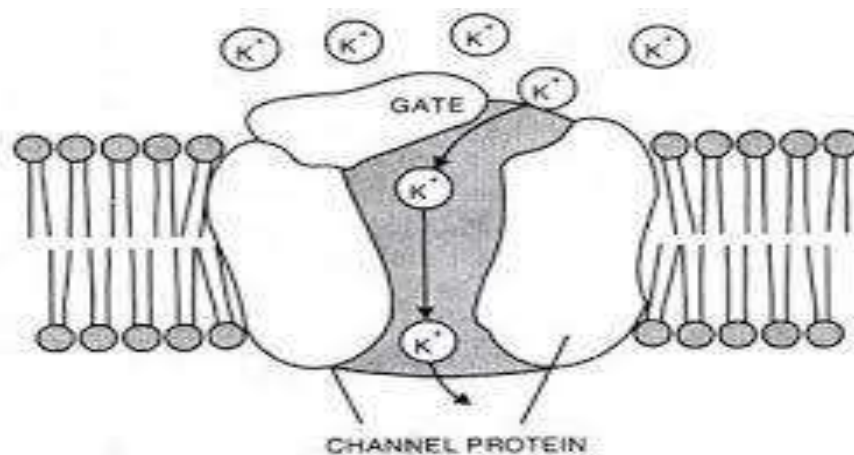


Fig. 7.7. A model showing gated ion-channel

- Ions can diffuse through an open channel

- Those channels which allow inward transport of ions (*i.e.*, towards cytosol side is called as inward rectifying or inward channels, and those which allows outward diffusion (from cytosol to other side) are called as outward rectifying or outward channels.
- Ca²⁺ channels are inward rectifying while anion channels are always outward, for transport of such ions in reverse direction, active transport mechanisms are required)
- K⁺ is exceptional. It can diffuse inward or outward across the membrane through channels depending upon more negative or more positive membrane potential, respectively.

3. Carriers:

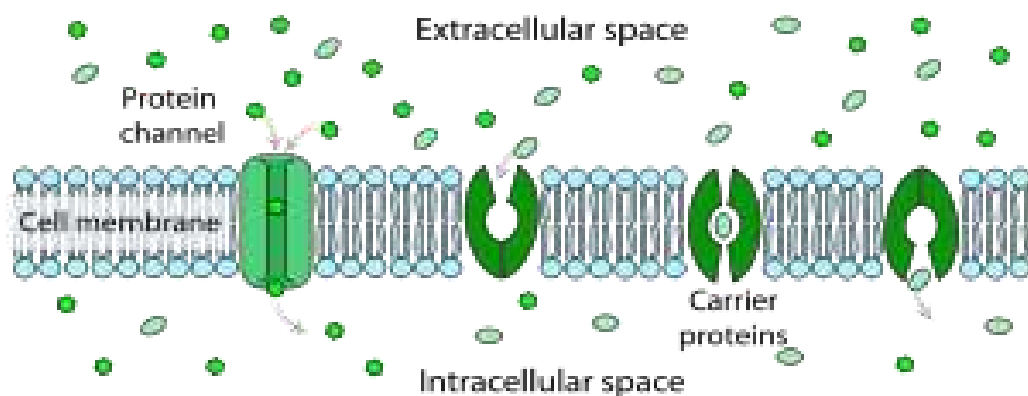
- These transmembrane transporter proteins **do not form pores in membrane**, instead they selectively bind the solute to be transported to a specific site on them. This causes **conformational change in carrier protein** which exposes the solute to other side of the membrane. After the solute is released from the binding site, the carrier protein reverts back to its original conformation to pick up a fresh solute molecule or ion. Thus, binding and releases solute through carrier is similar to an enzyme catalysed reaction.
- Carrier mediated transport of solutes enables transport of much wider range of solute, but is slower as compared to channel mediated solute transport.
- Carrier mediated solute transport may be of two types.

(A) Passive Transport

- Carrier mediated passive transport of solutes **occurs along the electrochemical potential gradient** and **does not require expenditure of energy**. This has also been called as facilitated diffusion. According to some scientists, **both carrier mediated passive transport and channel mediated transport** should come under the purview of facilitated diffusion.

(B) Active Transport

- Carrier mediated active transport of solutes takes place against the electrochemical potential gradient and **requires additional input of energy that chiefly comes from hydrolysis of ATP**. In such cases, the carrier proteins are called as 'pumps' and the transport of solutes is called as **primary active transport** because it directly utilizes energy from hydrolysis of ATP.



Sl. No.	Simple Diffusion	Facilitated Diffusion
1	Simple diffusion does not require the assistance of a carrier molecule.	Facilitated diffusion can occur only with the assistance from a carrier molecule.
2	The speed of simple diffusion is relatively slow.	The speed of facilitated diffusion is comparatively fast.
3	Simple diffusion is not solute specific.	Facilitated diffusion is always solute specific.
4	The simple diffusion process cannot be inhibited by an inhibitor molecule.	The facilitated diffusion can be inhibited by specific inhibitor which binds to the carrier molecules.
5	Simple diffusion is always a passive process.	Facilitated diffusion may be an active or passive process.
6	Simple diffusion does not require energy from ATP.	Facilitated diffusion may or may not require energy from ATP.
7	In simple diffusion, the molecules can pass only in the direction of concentration gradient.	In facilitated diffusion, the molecules can pass both in direction and opposite of the concentration gradient.
8	Simple diffusion permits the passage of only small and nonpolar molecules across the plasma membrane.	Facilitated diffusion permits the passage of large and polar molecules across the plasma membrane.

3. Pumps

As mentioned earlier, the membrane transporter proteins involved in primary active transport of solute are called as pumps. Most of the pumps transport ions such as H^+ and Ca^{2+} across the membrane and are known as ion-pumps. Some pumps (such as those of ABC transporters category) may also transport large organic solutes across the membranes.

some of the most common pumps in plant cells is

(1) Proton-ATPase Pumps (H^+ -ATPases) (2) Calcium Pumping ATPases (Ca^{2+} -ATPases)

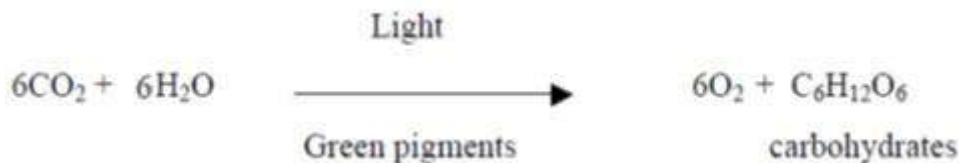
Photosynthesis

Introduction:

Photosynthesis is considered the most important process for all living organisms except anaerobic bacteria, which can fix CO₂ without using hydrogen of H₂O as a source of protons. In addition, the process is essential for life in terms of energy transfer.

The photosynthetic organisms produce about 50 million ton of sugar annually by this process and this amount of sugar require transfer of 10²¹ calories of sun energy to chemical energy.

Photosynthesis is sometimes called as carbon assimilation (assimilation: absorption into the system). This is represented by the following traditional equation.



During the process of photosynthesis, the light energy is converted into chemical energy and is stored in the organic matter, which is usually the carbohydrate. One molecule of glucose for instance, contains about 686 K Calories energy. CO₂ and water constitute the raw material for this process and oxygen and water are formed as the byproduct during photosynthesis.

Photosynthesis needs the following essential requirements:

1. Source of light energy
2. Source of energy capture
3. Means for energy storage

Photosynthesis pigments:

Studied conducted by plant physiologists indicated that the pigments absorbed light energy and transfer it to chemical energy are the chlorophylls which occurred in the plastids. The pigments are classified as follow:

Chlorophyll

The quantity of chlorophyll is 10 times more than carotenoids and it includes the following types:

- a. Chlorophyll a: occurred in all photosynthetic plants and maximum absorption of light appeared at 430 and 660 nm wavelengths.

- b. Chlorophyll b: occurred in all higher plants and green algae and maximum absorption of light appeared at 450 and 640 nm wavelengths.
- c. Chlorophyll c: Occurred in brown algae
- d. Chlorophyll d: Occurred in red algae
- e. Chlorophyll e: Occurred in some plant species

The chemical structure of chlorophyll a and chlorophyll b are well established. The molecular formula for chlorophyll a: $C_{55}H_{72}O_5N_4Mg$ and chlorophyll b: $C_{55}H_{70}O_6N_4Mg$. Both of them consist of Mg porphyrin head, which is hydrophilic, and a phytol tail, which is lipophilic. The two chlorophylls differ because in chlorophyll b there is a $-CHO$ group instead of CH_3 group at the 3rd C atom in pyrrole ring II.



Figure 1. Chlorophyll structure

2. Other pigments: It Consists of two groups:

a. Carotenoids

Carotenoids are lipid compounds that are distributed widely in both animals and plants and range in color from yellow to purple. Carotenoids are present in variable concentrations in nearly all higher plants and in many microorganisms (e.g., red and brown algae, photosynthetic bacteria and fungi). It includes α -carotene, β -carotene and lycopene.

b- Xanthophylls

The xanthophylls are more abundant in nature than carotenes and located in chloroplast.

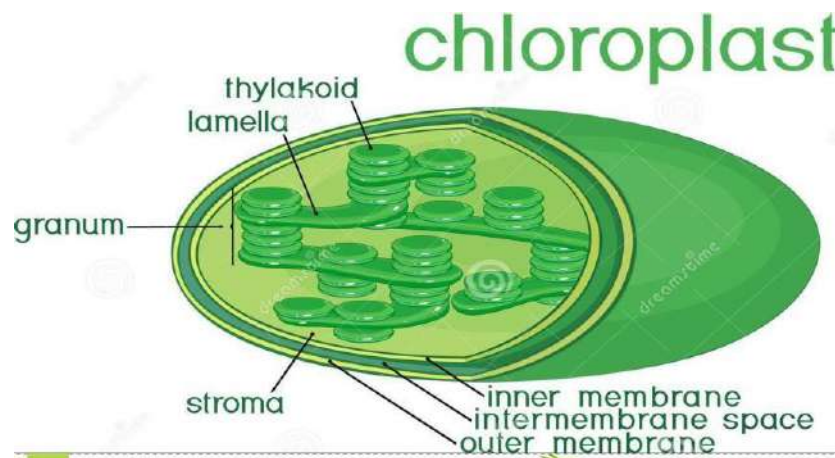
Xanthophylls are carotenes containing oxygen.

Functions of Carotenoids:

- a. Protect chlorophylls against photooxidation in excessive light.
- b. Absorption of light and transfer it to chlorophylls.
- c. Causes phototropism in plants.

Location of photosynthetic pigments in chloroplast

The photosynthetic pigments are located in grana portions of the chloroplast. They are present in the thylakoid membrane or membrane of grana lamella. The membrane of thylakoid is made up of proteins and lipids or the membrane consists of both lipid layer and protein layer. The hydrophilic heads of the chlorophyll molecules remain embedded in the protein layer while lipophilic phytol tail in the lipid layer. The other pigments are thought to be present along with chlorophyll molecules.

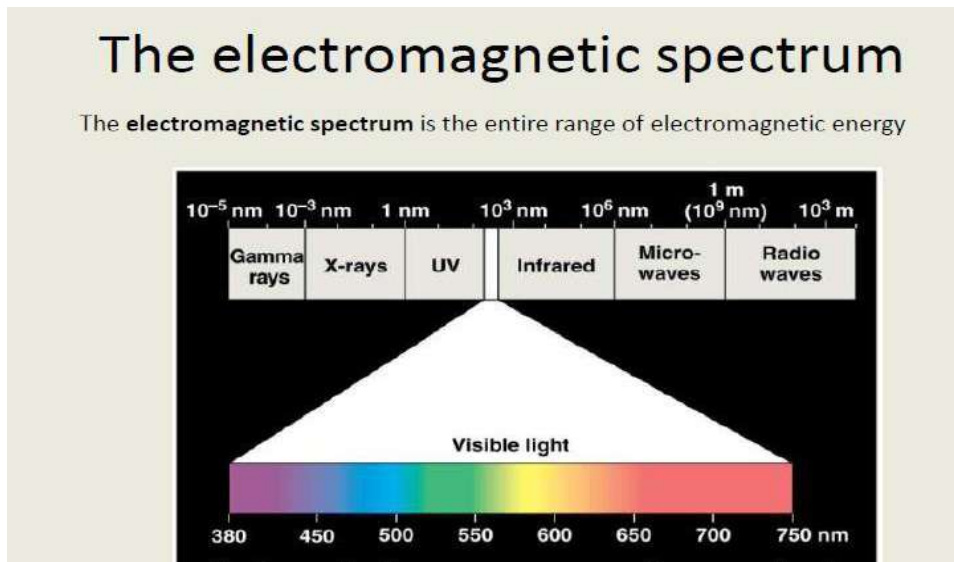


Grana portions of the chloroplast Light

The chief source of light energy for photosynthesis is sun. The solar radiation or solar energy passes through the space and reaches the earth in the form of *electromagnetic radiation* with waves of varying lengths. The various portions of electromagnetic spectrum are gamma rays, ultraviolet rays, visible rays and infrared rays. The wavelength of these rays ranges from 280 nm to 1000 nm.

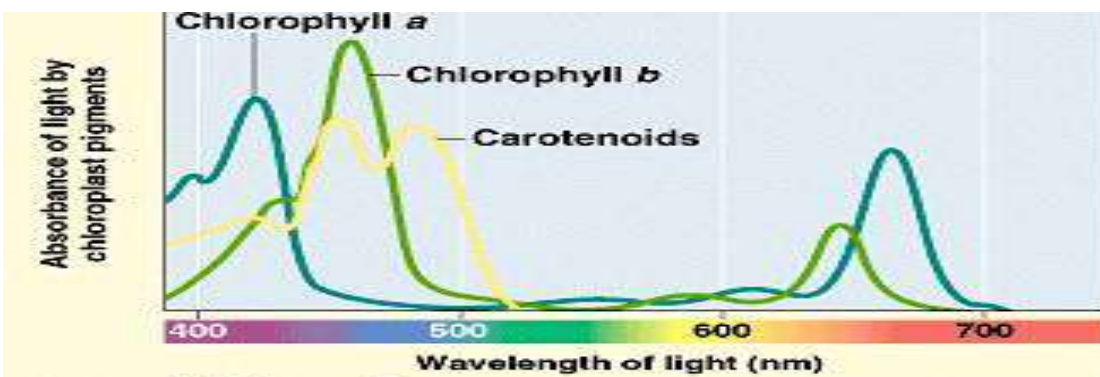
Photosynthetic pigments absorb light energy only in the visible part of the spectrum. As a result of the response of these pigments to light, the plant can carry out the photosynthesis process. Light rays consist of tiny particles called photons and the energy carried by a photon is called quantum. When these particles collide with the chlorophyll, their light

energy is transferred to the chlorophyll electrons, thus pushing the chlorophyll particles to cause the photochemical reaction.



Absorption spectra of chlorophyll

Chlorophyll is affected and excited if exposed to visible light as it occupies a very small area, ranging from 430-660 nm. While the wavelengths greater than that or smaller they are electromagnetic radiation that does not benefit the plant. The absorption of different wavelengths of light by a particular pigment is called absorption spectrum, and the chlorophyll molecules are excited by absorbing light energy. Chlorophylls absorb maximum light in the violet blue and red part of the spectrum. The absorption peaks of chlorophyll a are 430 and 660; for chlorophyll b 450 and 640. Carotenoids absorb light energy in blue and blue green part of the spectrum.



Mechanism of Photosynthesis Photo systems (Two pigment systems)

The process of photosynthesis takes place through two reactions:

1- Light reaction which produces energy in the form of NADPH and ATP

2- Dark reaction which fix CO₂

The light reaction is driven by two photochemical processes. These processes are associated with two groups of photosynthetic pigments called **as (P₇₀₀) pigment system I or photosystem I and as (P₆₈₀) pigment system II or photosystem II**. Wavelength of light shorter than 680 nm affect both the pigments systems while wavelength longer than 680 nm affect only pigment system I. The two pigment systems I and II are interconnected by a protein complex called cytochrome b₆-f complex. The other intermediate components of electron transport chain viz., **plastoquinone (PQ)** and **plastocyanin (PC)** act as mobile electron carriers between the complex and either of the two pigment systems.

Electrons transfer and photophosphorylation (Z Scheme)

*When photosystems I and II exposed to light, both of them are excited.

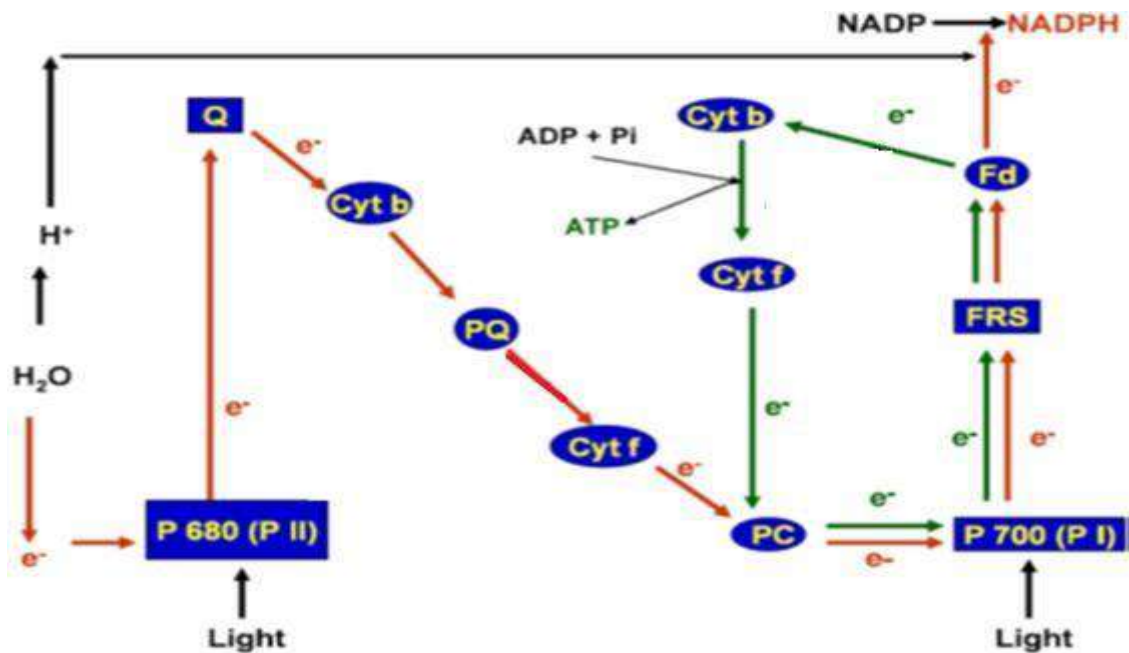
*Photosystem II starts dissociating H₂O and liberating the electrons. The electrons are first captured by P₆₈₀ in photosystem II, which transfer them to **primary electron acceptor** called **Q** which changes to reduced form.

*The reduced form transfers the electron to another carrier called B, which transfers the electron to plastoquinone.

*Then the electron reaches the active site of photosystem I (P₇₀₀) through **three** carriers namely cytochrome b₆, cytochrome f and plastocyanin respectively. ***Both cytochromes and plastocyanin are located in photosystem I.***

*The electrons are transferred from photosystem I to another electron acceptor called **ferredoxin (Fe-S)** and change it to reduced form. The reduced form of ferredoxin reduced NADP⁺ to NADPH by the enzyme **ferredoxin - NADP⁺ reductase**.

*The NADPH is used to fix CO₂ in the dark reactions. Therefore we can conclude that ferredoxin is the terminal electron acceptor in the photosynthetic light reaction. The light reaction can be diagramed as Z Scheme.



Z scheme

Photophosphorylation:

The process by which the plant can produce ATP in the presence of light. It happens in the plastids and through the light reactions. There are two types of Photophosphorylation:

A. Non cyclic Photophosphorylation

The ATP is produced when the electron transfers from **cytochrome b** to **cytochrome f**. The process requires the contribution of both photosystems in order to ensure the flow of the electrons through the carrier system.

Significance of non cyclic Photophosphorylation

1. It involves PS I and PSII
2. The electron expelled from P680 of PSII is transferred to PS I and hence it is a non cyclic electron transport.
3. In non cyclic electron transport, photolysis of water (Hill's reaction and evolution of O₂) takes place.
4. Phosphorylation (synthesis of ATP molecules) takes place at only one place.
5. The electron released during photolysis of water is transferred to PS II.

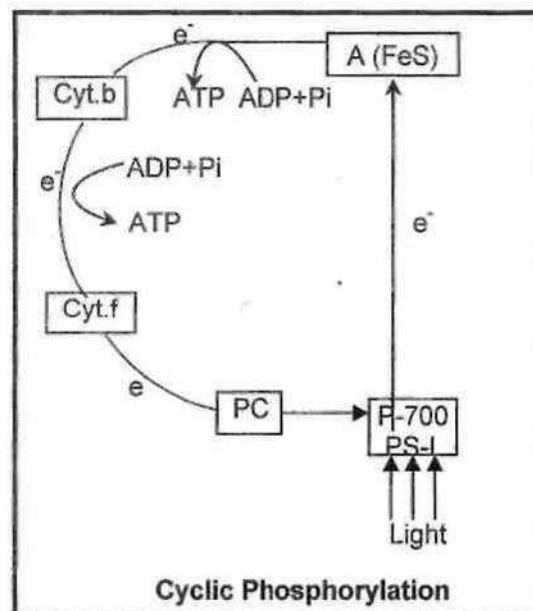
6. The hydrogen ions (H^+) released from water are accepted by NADP and it becomes NADPH.
7. At the end of non cyclic electron transport, energy rich ATP, assimilatory power NADPH and oxygen from photolysis of water are observed.
8. The ATP and NADPH are essential for the dark reaction wherein, reduction of CO_2 to carbohydrate takes place.

B. Cyclic Photophosphorylation

This process happens when the plastids exposed to wave length of light more than 680 nm causing P700 excited only.

Significance of cyclic photophosphorylation

1. During cyclic electron transport and phosphorylation, photolysis of water, O_2 evolution and reduction of NADP **do not take place**. The electron returns or cycles back to original position in the P700 form of chlorophyll a. Here, chlorophyll molecule serves both as donor and acceptor of the electron.
2. CO_2 fixation is retarded.
3. Non cyclic photophosphorylation is stopped.



The different between Photosystem I and Photosystem II (PS I vs PS II)

Photosystem I (PS I)	Photosystem II (PS II)
PS I is located at the outer surface of the grana thylakoid membrane (non-appressed granal regions and stroma lamella).	PS II is located at the inner surface of the grana thylakoid membrane (appressed granal region).
The photocentre or reaction centre is P700.	The photocentre is P680.
PS I has an iron-sulphur (FeS) type reaction centre (or type I)	PS II has a quinone type reaction centre (also known as Q-Type or type II)
The core complex of PSI is composed by a smaller number of proteins (~15 subunits) than PSII	The core of PSII is a multi-subunit complex composed of about ~25-30 subunits.
Pigments absorb longer wavelengths of light (>680nm).	Pigments absorb shorter wavelengths of light (<680nm).
Rich in chlorophyll a than chlorophyll-b	Rich in chlorophyll b than chlorophyll-a
Participates in cyclic as well as <i>non cyclic photophosphorylation</i> .	Participates only in non-cyclic photophosphorylation.
It is not associated with photolysis of water.	It is associated with photolysis of water.
PS I generates a strong reducing agent (reducing NADP^+ to NADPH) and a weak oxidant	PS II is the strong oxidant (capable of oxidizing H_2O) and a weak reductant
Main function is ATP synthesis in cyclic photophosphorylation and NADPH synthesis in non-cyclic photosynthesis.	Main functions are ATP synthesis and hydrolysis of water.

The different between cyclic and non-cyclic photophosphorylation

Non-cyclic photophosphorylation	Cyclic photophosphorylation
Electrons do not come back to the same molecule.	Electrons come back to the same molecule.
First electron donor is water	First electron donor is P_{700} (PSI)
Involves both PSI & PSII	Involves PSI only
Last electron acceptor is NADP	Last electron acceptor is P_{700} (PSI)
The net products are ATP, NADPH and O_2	The product is ATP only

Photosynthesis

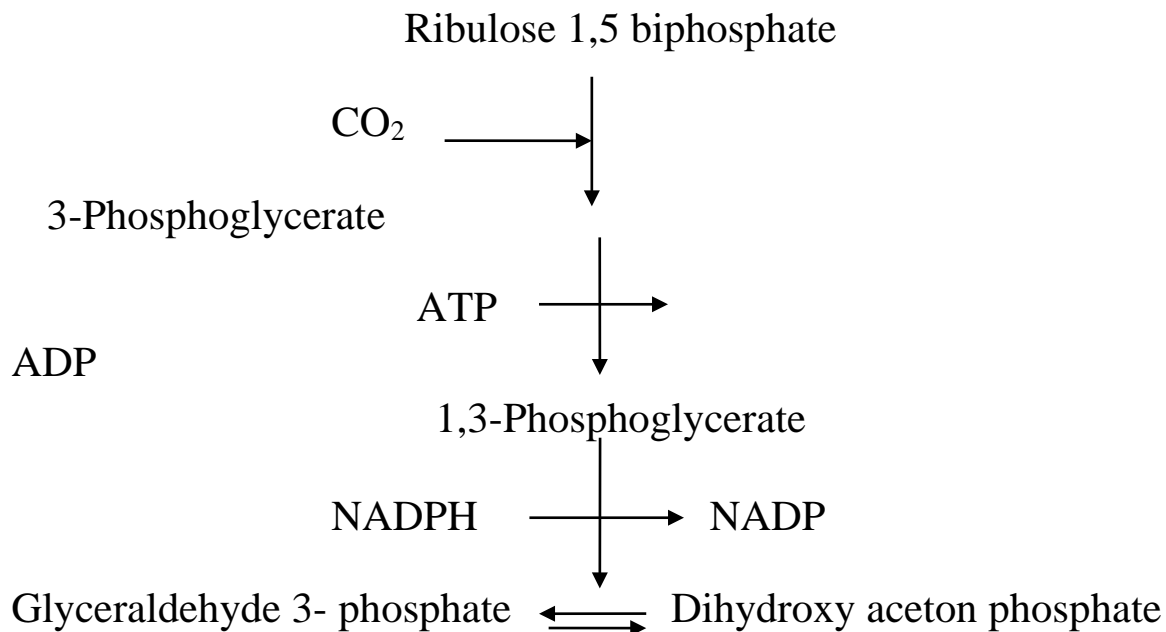
Dark reactions:

Several studies were conducted to determine the compound produced from fixation of CO₂.

- * Some studies suggested that formaldehyde was the first compound; others mentioned that pyruvate was the compound produced from CO₂ fixation. All these thoughts were uncorrected.
- * The discovery of labeled isotope and paper chromatography technique at that time provides a good means for separation and identification of the first compounds produced after CO₂ fixation and the other intermediate compounds produced in dark reactions.
- * The famous plant biochemist Dr Calvin from university of California used the above techniques and found that Phosphoglyceric acid (PGA) was the first compound produced from the fixation of CO₂ in to 5 carbon compound named Ribulose diphosphate according to the following reaction:



- * The enzyme catalyzed this reaction is **Ribulose diphosphate carboxylase** which is proved to be biosynthesized in chloroplast.
- * The dark reactions which known to be a cyclic have collectively named the **Calvin cycle**. Calvin was awarded a **Nobel Prize** for this work in 1961.
- * ATP and NADPH₂ produced by the light reactions are utilized in the following reactions in Calvin cycle:
- * The chemical compounds produced in Calvin cycle are 3C, 4C, 5C, 6C and 7C compounds.
- * The primary functions of Calvin cycle are listed as follow:
 1. The cycle is essential for CO₂ fixation and keeps its ratio stable in the air.
 2. The cycle is very necessary to produce different types of carbohydrates which involve in biosynthesis of essential compounds in the cell such as lipids, proteins, vitamins, nucleic acids, cellulose, lignin and cell wall.
- * It has been shown that one mole of CO₂ requires 3 moles of ATP and 2 moles of NADPH.



Methods of CO₂ fixation:

Studies revealed the presence of 3 kinds of CO₂ fixation in plants. Accordingly, the plants were divided in to 3 groups:

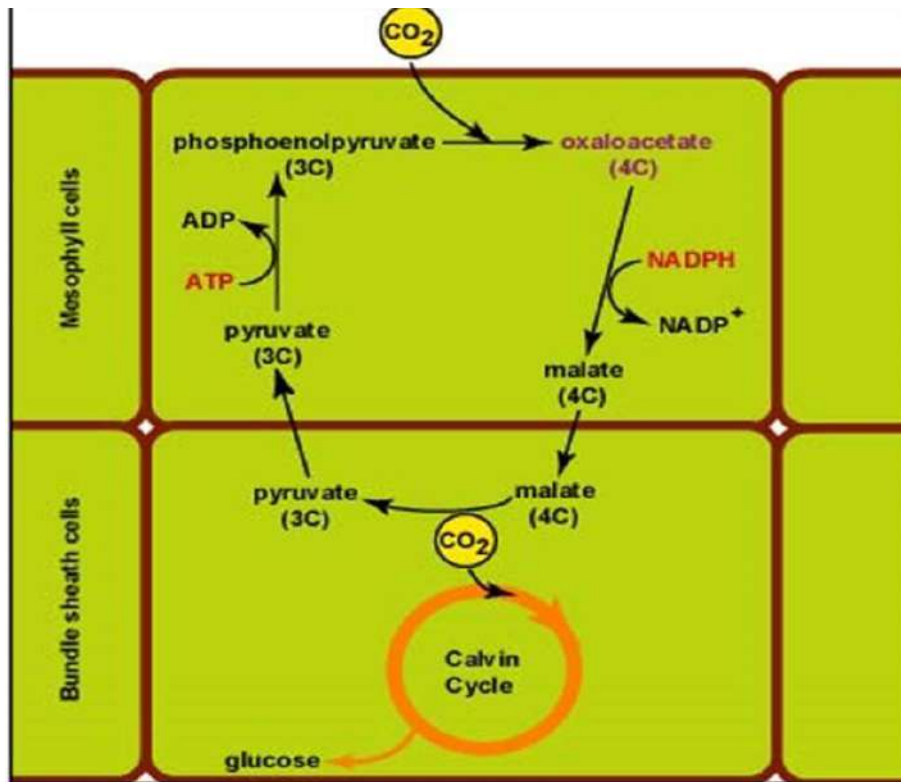
1. C₃ plants

The plants with 3C compound as a primary initial CO₂ fixation product such as wheat, tomato and date palm. The compound is PGA.

2. C₄ plants

The plants with 4C compound as a primary initial CO₂ fixation product such as corn and sugar cane. The compound is **oxaloacetate**.

* CO₂ gas is fixed first in the phosphoenol pyruvate (PEP) which is present in the **chloroplasts of the mesophyll cells** forming a 4C compound named oxaloacetate. The oxaloacetate converts into malate which moves to enter the **chloroplast of the bundle sheath cells**, then converts to pyruvate and CO₂. The CO₂ is re-fixed again in Calvin cycle while the pyruvate enters the chloroplast of the mesophyll cells to convert into PEP .



CO₂ fixation in C₄ plants.

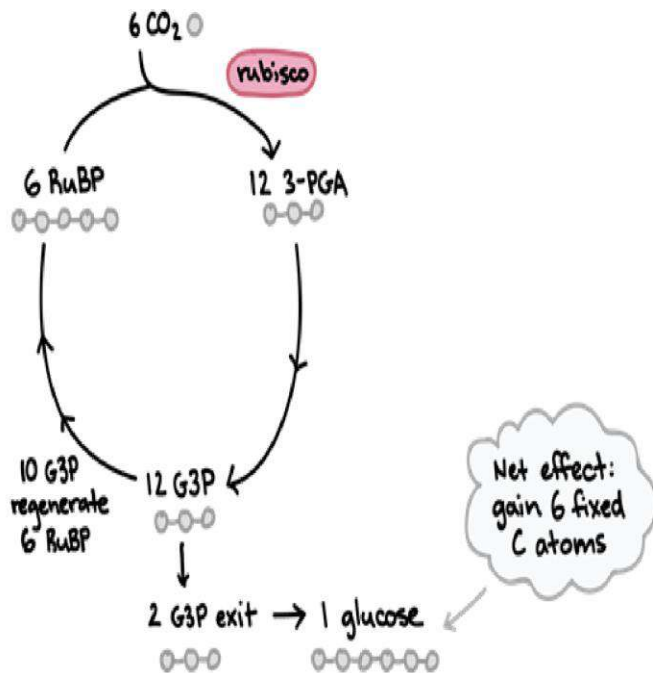
3. CAM plants (mean plants with crassulacean acid metabolism)

- * They called CAM since it was first investigated in plants of Crassulaceae family which are commonly produced crassulacean acid. Plants of this group open their stomata at night to fix CO₂.
- * **During night**, starch breaks down by reaction of glycolysis to form phosphoenolpyruvate (PEP). CO₂ is fixed into oxaloacetate by the enzyme PEP carboxylase, then this acid is converted to malate by dehydrogenase enzyme. Malate is stored in the vacuole.
- * **During day-light**, malate come out of the vacuoles and dehydrogenated to form oxaloacetate again. Oxaloacetate is decarboxylated to form CO₂ and PEP. The CO₂ is re-fixed into Calvin cycle while PEP converts to starch by reverse glycolysis. Some of starch produced by reverse glycolysis and Calvin cycle can be utilized again during night to fix CO₂ again.

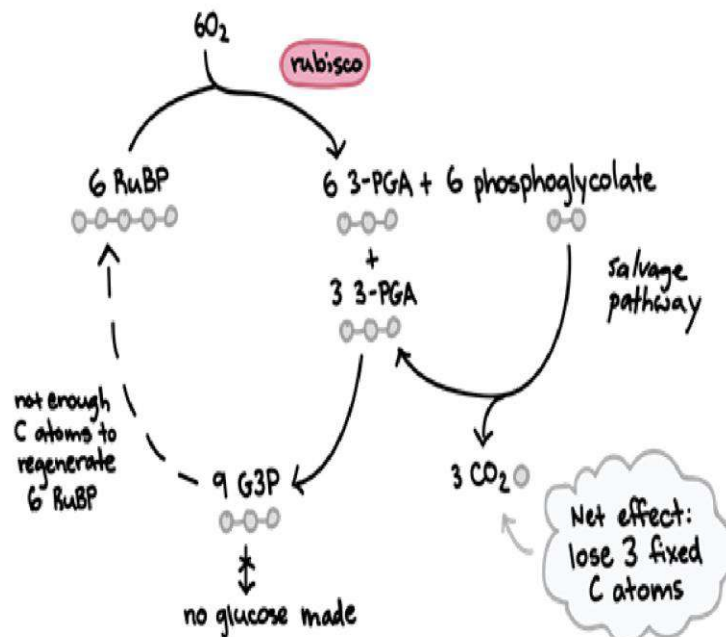
Photorespiration:

Photorespiration presents in all plants and takes place in the presence of light. The reactions of photorespiration are completed in 3 cellular structures namely *chloroplast*, *peroxisome* and *mitochondria*. The process is important for biosynthesis of the amino acids (glycine and serine) which are essential for protein biosynthesis. The Photorespiration occurs as in follow

NORMAL CALVIN CYCLE



PHOTORESPIRATION



The comparative between photorespiration and calvin cycle.

conditions :

1. Occurs on hot, dry, bright days
2. Stomates close
3. Fixation of O_2 instead of CO_2
4. Produces 2-C molecules instead of 3-C sugar molecules
5. Produces no sugar molecules or no ATP

Because of photorespiration, plants have special adaptations to limit the effect of photorespiration in C_4 plants and CAM plants.

The major differences between C_3 and C_4 plants

No	C_3 plants	C_4 plants
1	PGA is the compound produced after CO_2 fixation	OAA is the compound produced after CO_2 fixation

2	The enzyme involved in CO ₂ fixation is Ribulose diphosphate carboxylase	The enzyme involved in CO ₂ fixation is Phosphoenolpyruvate carboxylase
3	low efficiency of CO ₂ fixation under higher light intensity compared to C ₄ plants	high efficiency of CO ₂ fixation under higher light intensity
4	low efficiency of CO ₂ fixation under higher temperature compared to C ₄ plants	high efficiency of CO ₂ fixation under higher temperature
5	CO ₂ fixation conducts one time	CO ₂ fixation conducts two times
6	low efficiency of CO ₂ fixation under low moisture compared	high efficiency of CO ₂ fixation under low moisture
	to C ₄ plants	
7	low efficiency of CO ₂ fixation under low concentration of CO ₂ compared to C ₄ plants	high efficiency of CO ₂ fixation under low concentration of CO ₂
8	Low productivity compared to C ₄ plants	High productivity
9	high photorespiration	low photorespiration
10	Commonly occurred in temperate regions	Commonly occurred in tropical regions

Ribulose diphosphate carboxylase:

It is considered the most important enzyme for the life. It consists of 16 protein subunits with molecular weight equal to 550 000. It forms about 16% of the chloroplast protein. The enzyme is known to work on two substrates CO₂ and O₂, therefore it is called allosteric enzyme.

Factors affecting the rate of photosynthesis:

1. CO₂ concentration:

Both concentration and diffusion affect photosynthesis rate. It has been shown that the diffusion of CO₂ inside the internal leave tissues is controlled by diameter aperture. Photosynthesis is increased with the increased CO₂ concentration up to a definite limit after which the concentration becomes toxic to plant.

2. O₂ concentration:

The increased in O_2 concentration significantly decreased the rate of photosynthesis.

3. Water

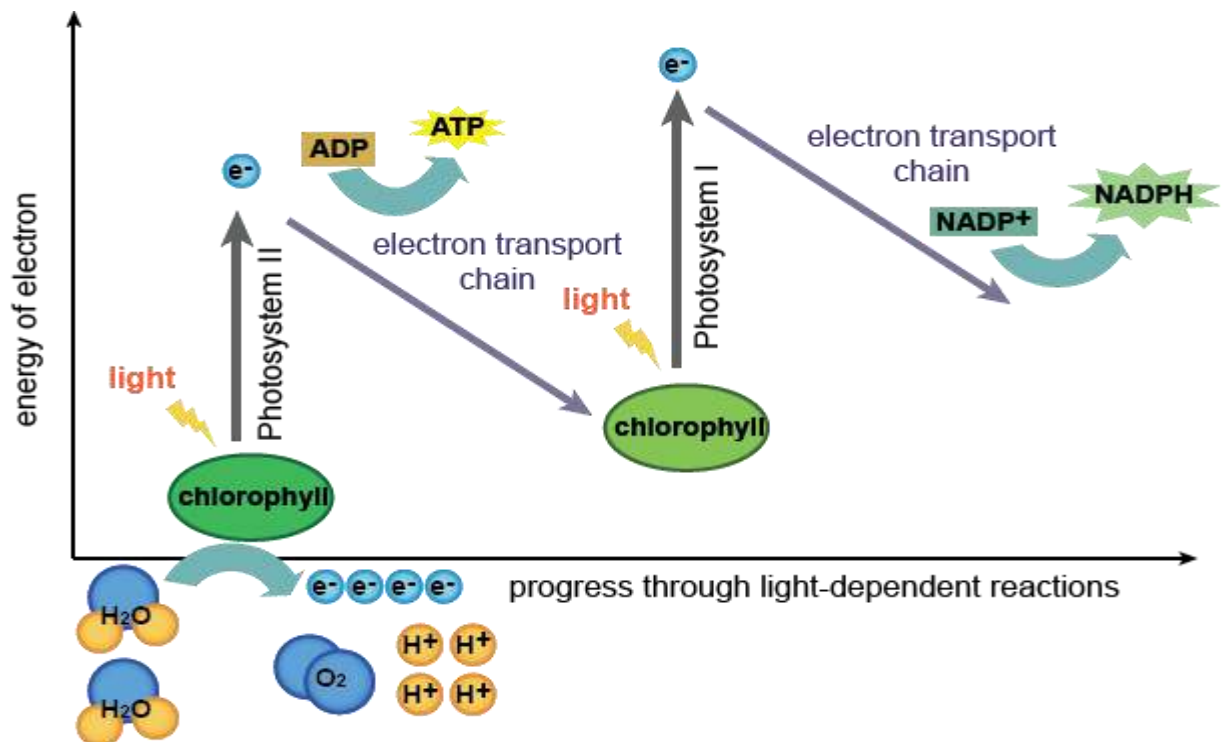
Water is very important factor that affects the rate of photosynthesis. Under drought condition, stomata close, diffusion of CO_2 and O_2 inside the leave inhibited, and these lead to inhibit the rate of photosynthesis. Flooding of plants prevents CO_2 and O_2 and thereby reducing photosynthesis rate.

4. Temperature

Extreme temperature is significantly inhibited photosynthesis rate. The low temperature freezes the water inside and outside the cells and thus preventing the movement of CO_2 and O_2 into the cells. It also causes protein denaturation. The high temperature inhibits or destroys enzymes including enzymes of Calvin cycle.

5. Light intensity

It has been shown that rate of photosynthesis increases with increasing light intensity to a limit after which, the increase in light intensity slightly increases the rate of photosynthesis. However, at very light intensity, the rate of photosynthesis inhibited due to photooxidation of chlorophylls.



Respiration

Introduction:

It is known that a large amount of the photosynthetic products (carbohydrates) is utilized in respiration to produce energy and organic compounds which utilize in growth and development of plant. During respiration, some of the energy produced liberated as a heat while most of the energy is utilized in synthesis of high energy compounds such (as ATP, NADH+H) to be used to accelerate of chemical reactions within the cells to synthesize the important biological molecules such as proteins, lipids, carbohydrates , hormones and flower and fruit formation f. Some of the energy is involved in in minerals uptake from soil.

High energy compounds:

It is already known that there are two types of reactions:

1. Excergonic reaction: it is a reaction that produced energy after completing (proceed with a release of free energy).
2. Endergonic reaction: it is a reaction that required energy for completing.

The energy produced from the excergonic reactions is stored in

chemical compounds called high energy compounds which on hydrolysis yield free energy equal to or greater than that of ATP. Most of them contain phosphate group (except thiol ester). They can be classified as follow:

1. Pyro**phosphate** compounds:

- A. Adenosine triphosphate (ATP) which is the most important high energy compounds in the living cells.
- B. Uridine triphosphate (UTP)
- C. Guanosine triphosphate (GTP)
- D. Thymidine triphosphate (TTP)

2. Acyl **phosphate** compounds such as 1, 3 biphosphoglyceric acid

3. Enol **phosphate** such as phosphoenol pyruvate (PEP)

4. Guanidine **phosphate** such as phosphocreatine

5. Thiol **esters** such as Acetyl CoA and succinyl CoA

Respiration:

Respiration can be defined as oxidation process of carbohydrates resulting energy and chemical compounds utilized in biosynthesis of important biological molecules such as proteins, lipids, hormones and chlorophylls. The respiration process includes a series of chemical reactions. The storage carbohydrates (mainly starch) should be hydrolyzed to glucose which is the first compound in respiration process. The oxygen is contributed only in the final step to oxidize the hydrogen atoms to form H₂O.

There are two main types of respiration:

(i) Aerobic and (ii) Anaerobic.

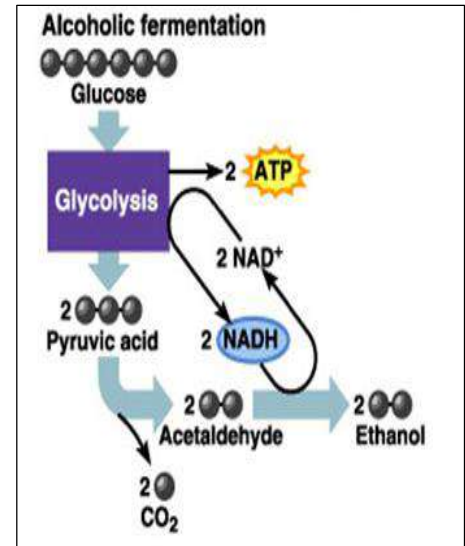
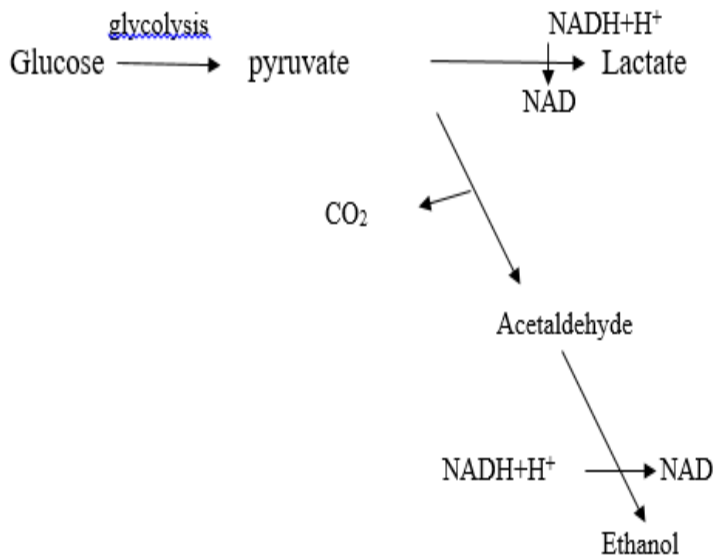
The process of aerobic respiration is divided in three stages:

1. The first step which conducted in the absence of O_2 and called **Glycolysis**.
2. The second stage which required O_2 to complete and called **Krebs cycle**.
3. Electron Transport System (ETS) and oxidative phosphorylation.

We will discuss these three processes in details.

1. Glycolysis:

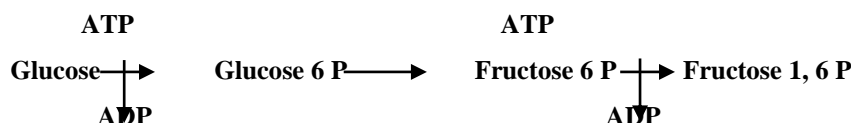
It a series of metabolic reaction started with glucose and ended with the lactate compounds in anaerobic respiration. When the process ends with ethyl alcohol and CO_2 it is called alcoholic fermentation. The production of lactate or alcohol is restricted to microorganisms, while in **higher plants** the process ends with production of **pyruvate**. Figure 1 shows the outline of the alcoholic and lactic acid fermentation.



The glycolysis process is also called Embden - Meyerhof - Parnas Pathway (EMP) to appreciate the scientific efforts of these scientists in discovering the metabolic pathways and their enzymes in this process. The process is located in cytoplasm and does not require O₂.

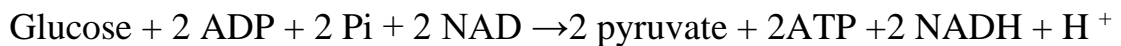
The process consists of two stages:

1. Stage 1 which includes transfer of glucose to fructose 1, 6 biphosphate. The process includes 3 chemical reactions and required 2 ATP as follows:



2. Splitting of 1, 6 fructose biphosphate to 3C compounds namely **dihydroxy acetone phosphate** and **glyceraldehyde - 3 phosphate** (the two compounds are in equilibrium state and each one can convert to another as needed by the cell). The compound glyceraldehyde - 3 phosphate converts to 1, 3 biphosphoglycerate and the cell gains $\text{NADH} + \text{H}^+$ (equal to 3 ATP). The 1, 3 biphosphoglycerate converts to 3 Phosphpglycerate and the cell is gained ATP. The 3 Phosphpglycerate coverts to 2 Phosphpglycerate which ultimately converts to phosphoenol pyruvate (PEP). The phosphoenol pyruvate converts to pyruvate and the cell gains another ATP. Figures 1 shows the pathway of glycolysis.

The process of glycolysis can be summarized as follow:



Therefore, each molecule of glucose yields 8 ATP (2NADH2+2 ATP)

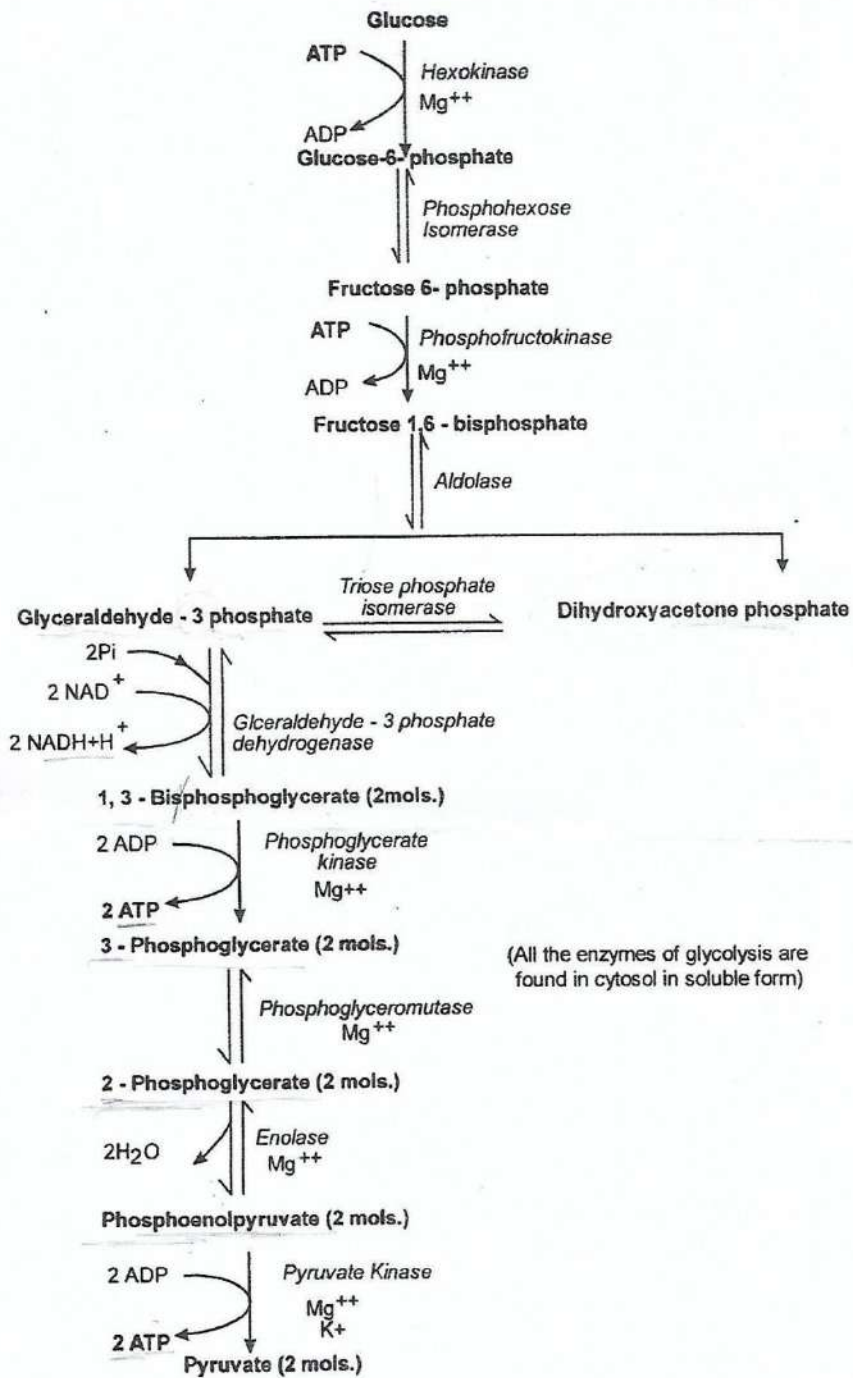
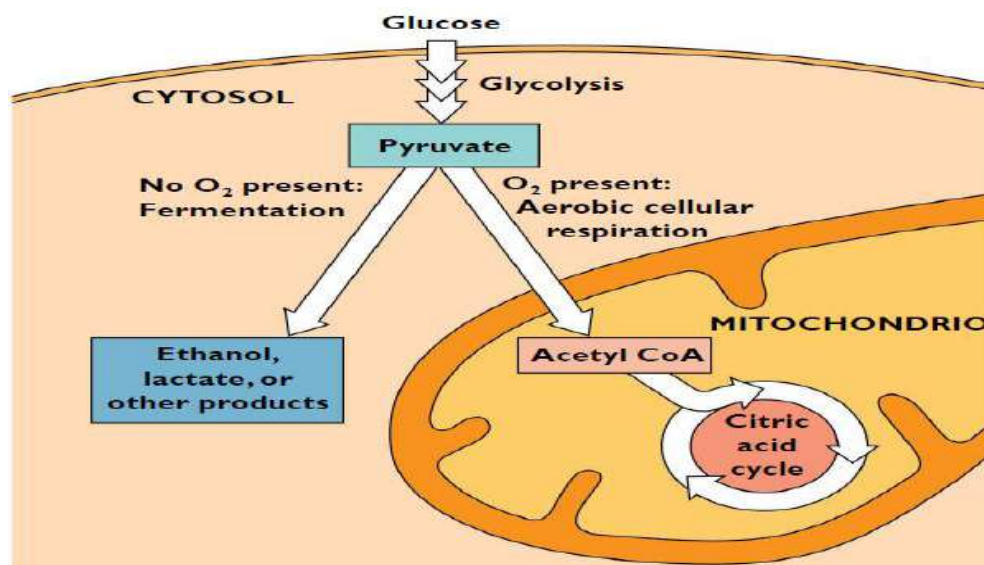


Fig. 16.1 Glycolysis (EMP-Pathway)

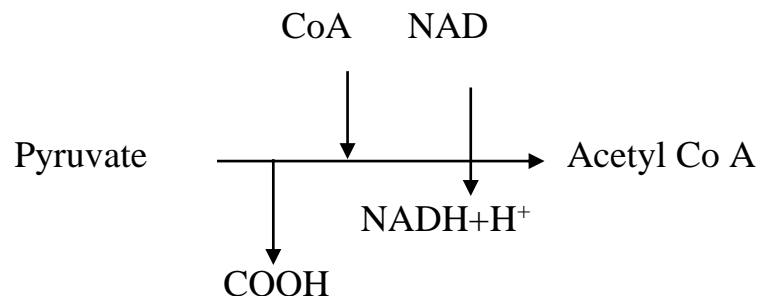
Importance of glycolysis:

1. It is necessary step for oxidation step of carbohydrates.
2. Produce the reduced compound $\text{NADH} + \text{H}^+$
3. Produce ATP
4. Source of several chemical compounds involved in the synthesis of amino acids, proteins, cell wall, lipids and other biological compounds.



Biosynthesis of Acetyl CoA:

When oxygen is sufficient, oxidation decarboxylation of pyruvate takes place forming acetyl CoA and $\text{NADH} + \text{H}^+$ as in the following equation:



The reaction required the following:

1. Thymine pyrophosphate TPP
2. Mg^{++}
3. CoA
4. Lipoic acid
5. NAD

2- Krebs cycle:

It is also called citric acid cycle or tricarboxylic acid cycle TCA. It is a cycle since the first compound of the cycle (oxaloacetate OAA) is re synthesized again in the cycle (Figures 3-1, 3-2).

The cycle is started by the reaction of **Acetyl CoA** with **OAA** to form citrate. The citrate converts to **cisaconitate** by dehydration reaction. The cisaconitate is unstable compounds and converts immediately to **isocitrate** by hydration reaction. Oxidation of isocitrate takes place to form **α k-glutarate**. In this reaction the reduced compound $NADH+H^+$ and CO_2 are produced. In the presence of NAD and α k- glutarate dehydrogenase, α k- glutarate converts to **Succinyl CoA** and $NADH+H^+$ and CO_2 are produced. Succinyl CoA is oxidized in the presence of GDP and inorganic phosphate to form **succinate** and GTP energy compound. The succinate produced is oxidized to form **fumarate** and produce $FADH_2$ in the presence of the oxidized form FAD. The fumarate is hydrated to form **malate**. In the fourth oxidation step malate is converted to OAA forming $NADH+H^+$ in the presence of malate dehydrogenase and NAD. Thus the regeneration of OAA completes the cycle.

Questions:

1. How many CO_2 molecules released during one cycle?
2. How many $\text{NADH}+\text{H}^+$ molecules formed during one cycle?
3. How many FADH_2 molecules formed during one cycle?
4. How many GTP molecules formed during one cycle?
5. How many ATP molecules formed during one cycle?
6. How many oxidation steps take place during one cycle?

Significance of Krebs cycle:

1. Source of carbon skeleton of amino acids
2. Production of the reduced forms ($\text{NADH}+\text{H}^+$ and FADH_2)
3. Production of the energy compound GTP
4. The compounds of Krebs cycle are involved in the synthesis of important biological molecules such as cytochromes, phytochromes, proteins, vitamins, hormones and nucleic acids.

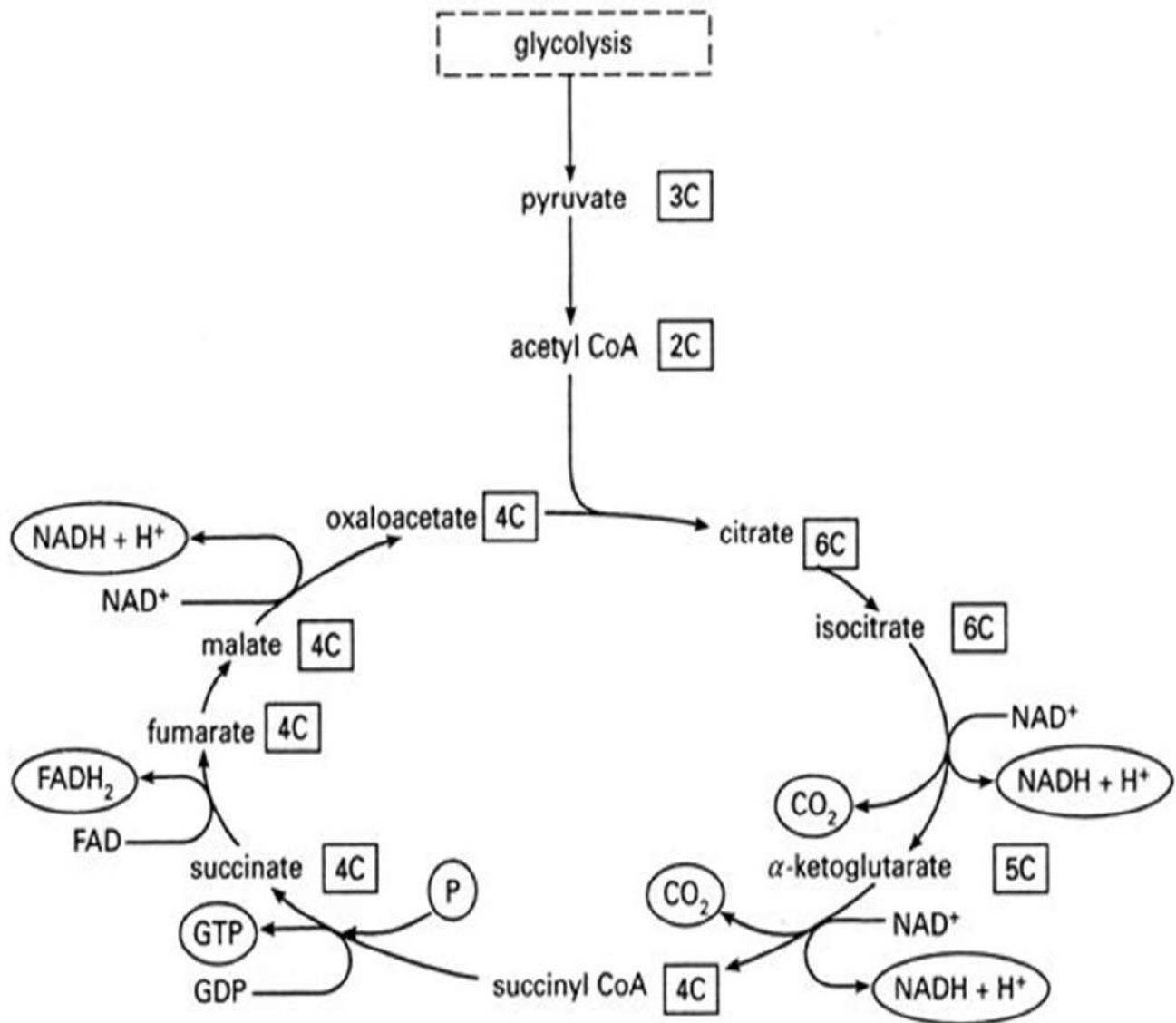


Figure 2. Metabolic pathway of Krebs cycle.

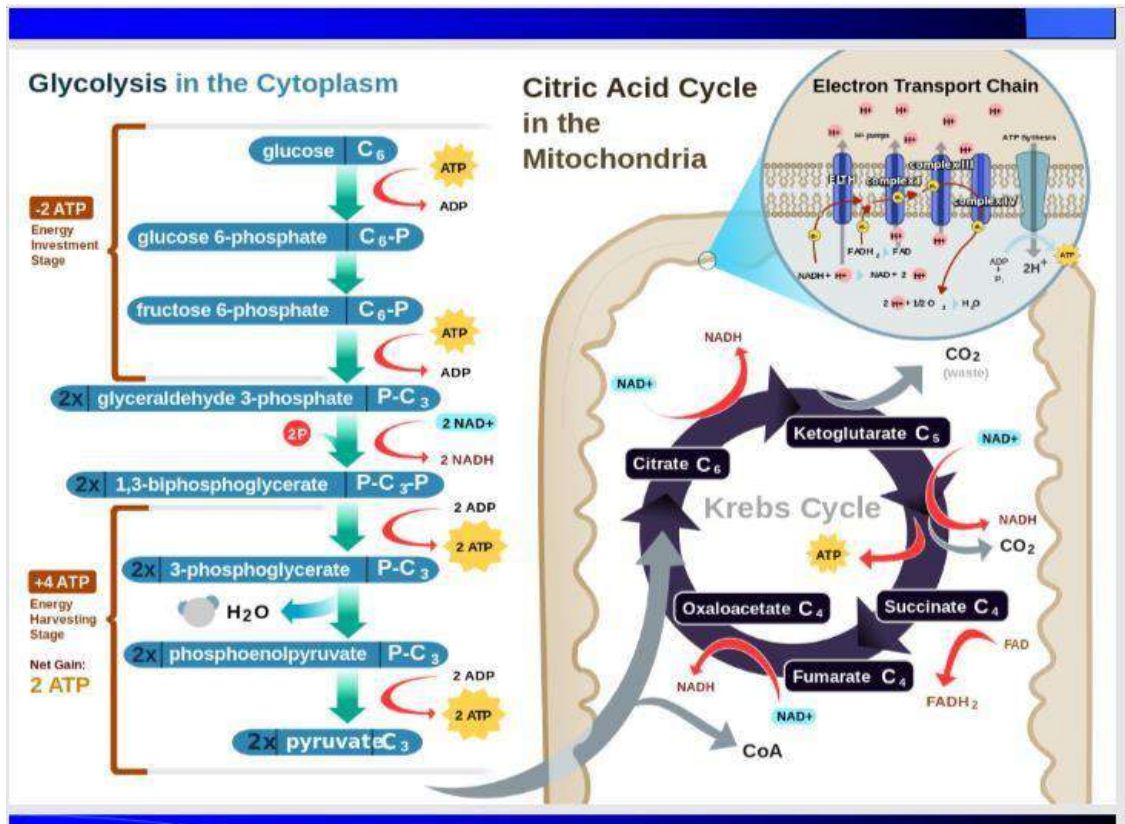
Electron Transport System (ETS) and Phosphorylation

It is the third step of aerobic cellular respiration. **Cellular respiration** is the term for how the cells produce energy from the food consumed. The electron transport chain (ETC) is where most of the energy cells are created. This "chain" is actually a series of protein complexes and electron carrier molecules inside the inner membrane of the mitochondrial cell, also known as the powerhouse or energy house of the cell.

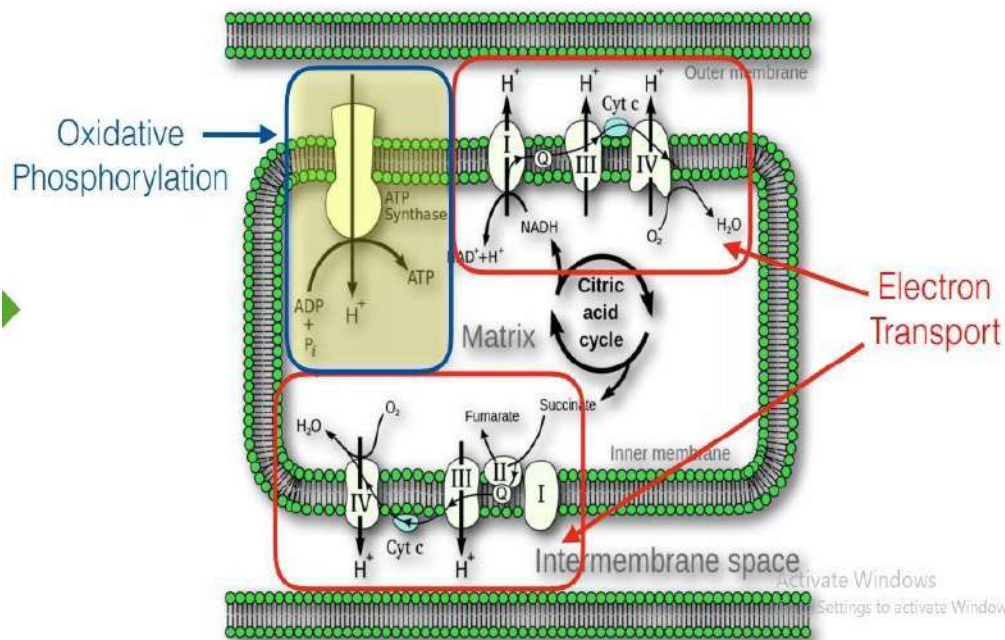
Electron transport chain (ETC):

A chain of hydrogen and electron carriers consisting of NAD⁺, FAD⁺, CoQ and cytochromes (cytochrome b, cytochrome c, cytochrome a and cytochrome a₃). The glucose molecule is completely oxidized by the end of the citric acid cycle.

The energy is not released, unless NADH₂ and FADH₂ are oxidized through electron transport system. Transfer of electrons and protons from NADH₂ and FADH₂ to oxygen through a series of components like flavoprotein, cytochrome is called **electron transport chain**. This process leads to coupling of electrons to form high-energy phosphate bonds in the form of ATP from ADP is called **oxidative phosphorylation**.



The ETC is a series of four protein complexes bound to the inner mitochondrial membrane and organic molecules, which electrons pass through in a series of redox reactions, creating an electrochemical gradient that leads to the creation of ATP in a complete system named oxidative phosphorylation. The energy released forms a proton gradient, which is used in chemiosmosis to make a large amount of ATP by the protein ATP-synthase. As shown in the figure below



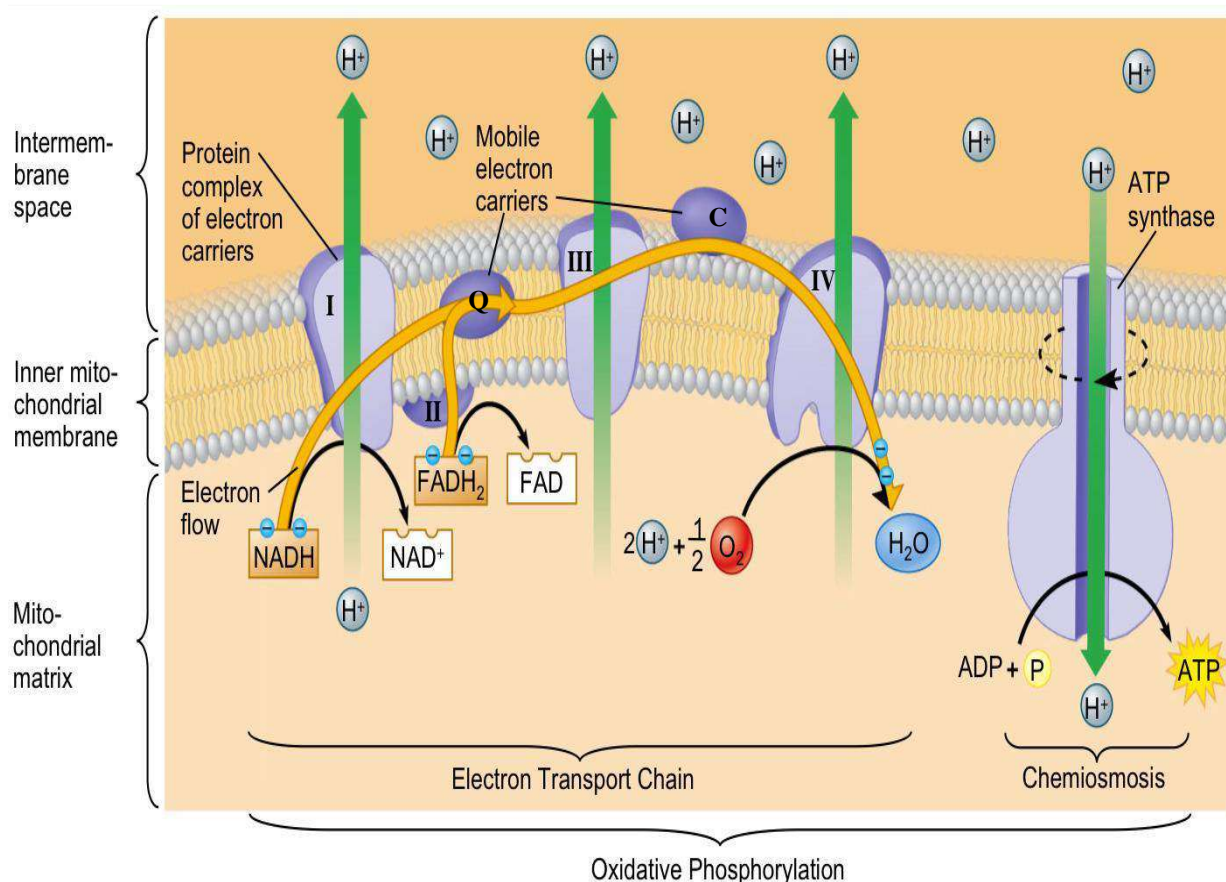
The Mechanism of ETC:

1- NADH₂ arrives directly at the ETC from the TCA cycle and immediately oxidizes to NAD⁺, with its protons (hydrogen ions) remaining in the matrix, and its electrons (e⁻) going to **complex I** which immediately goes through a series of redox (reduction and oxidation) reactions that create a proton pump within complex I, pumping (or translocating) 4 protons (H⁺) from the matrix through the protein into the intermembrane space. The electrons (e⁻) now transfer to **mobile carrier Q** and NAD⁺ returns to its original source to pick up more hydrogen ions. **2- FADH₂** arrives to the ETC from the TCA cycle. FADH₂ then oxidizes to FAD, with its electrons and protons going to complex II. FAD then returns to the TCA to pick up more electrons and protons. **Complex II** goes through redox but it does NOT create a proton pump. **3- Mobile carrier Q** also picks up all of the electrons on complex II, and shuttles the electrons it collects to cytochrome complex III, which also

immediately goes through redox (reduction and oxidation) reactions. This again creates a proton pump, pumping 4 H^+ from the matrix through complex III, directly into the intermembrane space of the mitochondrion.

4- The electrons are now shuttled from complex III to complex IV by mobile carrier C. As the electrons transfer onto cytochrome complex IV, it immediately goes through another redox reaction. This creates a final proton pump, pumping 2 protons from the matrix through cytochrome complex IV, directly into the intermembrane space of the mitochondrion.

5- Complex IV is the last step in the ETC, and the electrons that have been driving these reactions now need another place to go. To solve this problem, an oxygen atom, which has a very strong attraction for electrons, picks up two electrons from complex IV, along with two some free protons from the mitochondrial matrix, to simply form water (H_2O). In essence, the **FINAL acceptor of the electrons at complex IV is oxygen** (which forms water with the protons and electrons it accepts into its structure). This is referred to as '**metabolic water**' (because it is made in metabolism). As shown in figure below.



Oxidative Phosphorylation

The many protons that this process pumps in the intermembrane space create a high in H^+ ion concentration (charge) against a low H^+ ion concentration in the matrix that the cell does not like. All cells prefer homeostasis, so to help relieve this imbalance, **ATP synthase** (a special protein embedded next to the ETC) pumps several protons at a time back into the matrix.

That is called the **chemiosmotic hypothesis** that proposed by Peter Mitchell. This hypothesis stated that a **proton-motive force** was responsible for driving the synthesis of ATP. In this hypothesis, protons would be pumped across the

inner mitochondrial membrane as electrons went through the **electron transfer chain (ETC)**. This would result in a proton gradient with a lower pH in the intermembrane space and an elevated pH in the matrix of the mitochondria. As these protons move into the matrix by **ATP synthase**, enough energy is liberated to phosphorylate (or add another Pi to) ADP, thus synthesizing ATP. The ATP will then leave the mitochondrion and go where energy it is needed in the cells for life processes.

Oxidative phosphorylation has two parts: the electron transport chain (ETC) and chemiosmosis.

Pentose phosphate pathway (PPP):

It is also called Hexose Monophosphate Shunt. In this pathway **glucose-6-phosphate** is directly oxidized without entering glycolysis starting from 6 molecules of it. It is located in the cytoplasm and requires oxygen for oxidation of glucose.



The most important characteristics of this pathway are:

1. Only one CO₂ molecule is released during one cycle.
2. Two molecules of NADPH₂ are produced in each cycle. The NADPH₂ can convert to NADH₂ to yield 3ATP.

According to the above information, the hexose phosphate required 6 cycles to complete its oxidation. And the products of this oxidation are 6 CO₂ and 12 NADPH₂ (36 ATP).

3. It is considered as a source of erythrose sugar which is used in cell wall biosynthesis.
4. It is considered as a source of ribose and deoxy ribose sugars which are used in biosynthesis of nucleic acids (DNA and RNA). Figure 4 shows the metabolic pathways of PPP.

5. Producing Glycolytic Intermediate: In PPP Pathway, few molecules of Glycolytic intermediates are produced these are directly involved in Glycolysis. The molecules are Glyceraldehyde-3-Phosphate (GAP) and Fructose-6-Phosphate.

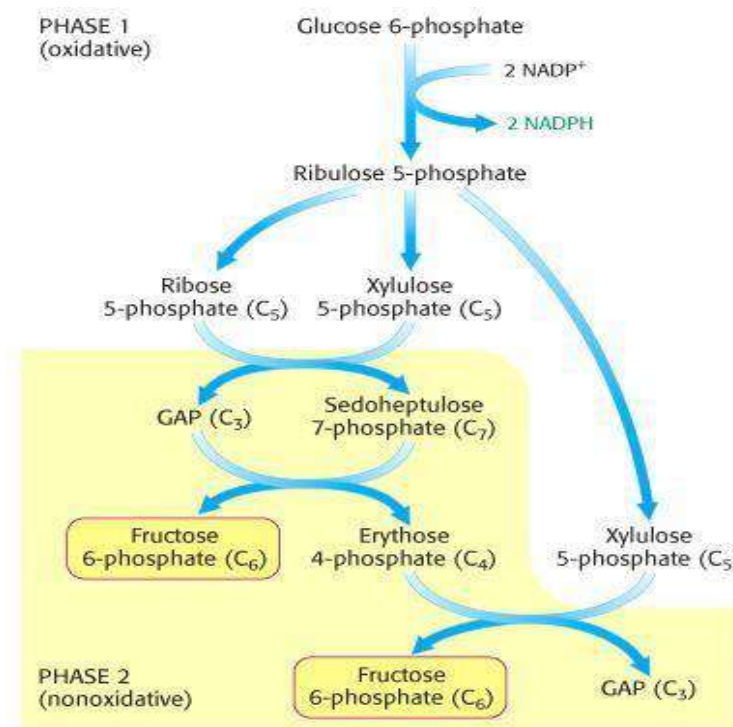


Figure 4: the metabolic pathways of PPP

Environmental factors affecting respiration:

1. Temperature:

The temperature has significant effect on enzyme activity of all enzymes including respiration enzymes. Maximum respiration rate occurred between 35-40 C. the effects of temperature on respiration rate increased with increasing temperature.

2. O₂ concentration:

In complete absence of oxygen anaerobic respiration takes place while aerobic respiration stops. Under low O₂ concentration, more CO₂ liberated from the plant due to increase of glycolysis rate. But when O₂ increased, less CO₂ liberated due to increase of aerobic respiration. **The ratio of CO₂ liberated to O₂ consumed is called respiratory coefficient (RC).** For carbohydrates, the RC=1 (amount of O₂ consumed = the amount of CO₂

liberated) while for proteins and lipids $RC < 1$ because the amount of O_2 consumed $>$ the amount of CO_2 liberated.

3. CO_2 concentration:

Increased CO_2 concentration decrease the O_2 uptake by the stomata and this leads to decrease the respiration rate.

4. Inorganic salts:

Increase the salt concentration causes increase in respiration rate. In order to provide more ATP required for uptake of salt ions.

5. Wounding:

Wounds takes place on plant body stimulate the wounding regions to produce meristematic cells for formation of callus which are characterized by high respiratory activity. The high respiratory activity may be due to the presence of respiration substrate (sugar) in the wounding region.

6. Photosynthesis:

Increased the photosynthesis causes increase the respiration rate due to increase in the amount of respiration substrate (sugar).

7. Moisture of plant tissues:

Increased the moisture content of plant stimulate growth and development and this needs more respiratory metabolites and ATP produced by respiration.

1. What are energy compounds? or numerous the energy compound in respiration process?
2. What are the different between Exergonic reaction and Endergonic reaction?
3. What are the stages in aerobic respiration process?
4. Drew the Diagram of the Glycolysis process?
5. What are the Glycolysis equations?
6. What is the importance of glycolysis?
7. How many CO_2 molecules released during one Krebs cycle?
8. How many $\text{NADH}+\text{H}^+$ molecules formed during one Krebs cycle?
9. How many FADH_2 molecules formed during one Krebs cycle?
10. How many GTP molecules formed during one Krebs cycle?
11. How many ATP molecules formed during one Krebs cycle?
12. How many oxidation steps take place during one Krebs cycle?
13. What are the significances of Krebs cycle?
14. Calculate the respiration energy budget of ATP?
15. What are the Environmental factors affecting on respiration?
16. What are the characteristics of this PPP pathway?

Plant Hormones and Growth Regulators

Most of the physiological activities and growth in plants are regulated by the action and interaction of some chemical substances in them called as **hormones** which is defined as **"organic substances produced naturally in the higher plants, controlling growth and other physiological function at a site far from its place of production and active in extremely low amounts"**. Growth regulators control cell activities by sending chemical signals or messages to cells to do or not do something, including activating the genes that code for specific enzymes or blocking gene transcription. Plant hormones inhibit as well as promote cellular activities. To distinguish them from the animal hormones they are termed as **phytohormones**.

Plant hormones are not nutrients, but chemicals that in small amounts promote and influence the growth, development and differentiation of cells and tissues. The biosynthesis of plant hormones within plant tissues is often diffuse and not always localized. They are often produced very locally within plant body; some plant cells even produce hormones that have an effect within themselves.

Overview of Hormone Action

To understand the function of a hormone, we need to know:

- *how it is synthesized and accumulates,
- *how it is transported,
- *how it is perceived, and how its perception is transduced into a response.

Hormones are transported within the plant by utilizing four types of movements:

- (1) **Localized** movement,
- (2) **Cytoplasmic streaming** within cells and slow diffusion of ions and molecules between cells are utilized.

(3) **Vascular tissues** are used to move hormones from one part of the plant to another; these include: (I) **sieve tubes** that move sugars from the leaves to the roots and flowers.

(II) In **xylem** that moves water and mineral solutes from the roots to the foliage.

The effect of plant hormones

* The functions of plant hormones are diverse, but all have profound effects on growth and development.

* Hormones affect all phases of the plant lifecycle from seed to seed, and their responses to environmental stresses, both biotic (from a living organism) and abiotic (from the physical environment).

* Not all plant **cells** respond to hormones, but cells that do so, are programmed to respond at specific points in their growth cycle; with the greatest effects occurring at specific stages during the cells life, with diminished effects occurring before or after this period.

* Generally, **the effect of plant hormones** on the plant depends on the location and the concentration of the hormone relative to other hormones in the specific tissues. Plant hormones often work in conjunction with each other, and have overlapping effects. They also work with environmental stimuli.

* The production of hormones occurs often at sites of active growth within the meristems, before cells have fully differentiated out. After production they are sometimes moved to other parts of the plant where they cause an immediate effect or they can be stored in cells to be released later.

* The concentration of hormones required for plant responses are very low (10^{-5} to 10^{-6} mol/L).

So the Plant hormones are:

1. Naturally occurrence organic substance.
2. Operates in very low concentrations.
3. Integrate many different plant parts.
4. Affects plant growth and development.
5. Actions may involve sites far removed from hormone origin.
6. Hormones elicit a wide range of responses from growth to dormancy.
7. Play key roles in signal transduction. (Signal transduction is the cascade of events that allow a signal, usually from outside the cell, to be interpreted by

the cell), so plant hormones are the major developmental and physiological signaling molecules in the plant.

Plant growth regulators

Plant synthetic growth regulators are promoter plant growth.

The difference between plant growth regulators and plant hormones are:
The plant hormone are **natural produces** in side plant body, while plant growth regulator are **synthesized** by human and because of hydrolysis enzymes effect, plant growth regulators are more stability than hormones.

How hormone is perceived, and how its perception is transduced into a response?

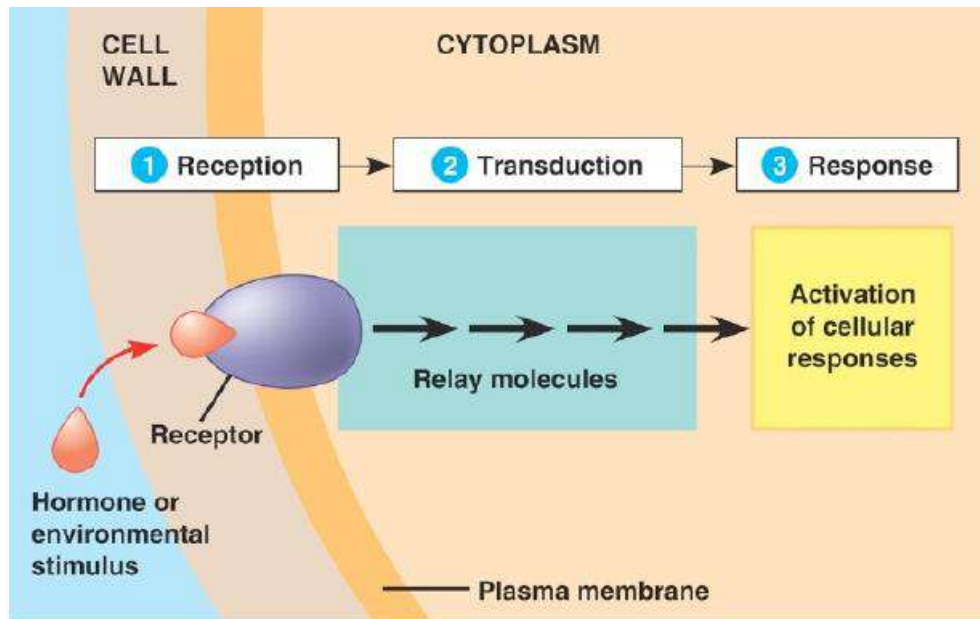
Hormones act on target tissues to activate a receptor.

1. The general mechanism is: hormone → target tissue/cell → receptor → signal amplification → response
2. Thus, for a response to occur:
 - The hormone must be present in sufficient quantity;
 - The target tissue must be sensitive (sensitized) to the hormone;
 - The target tissue recognizes the hormone (i.e., there must be a receptor to which the hormone can bind);
 - The binding of the hormone/receptor should initiate a change in the receptor (amplification).
 - The activated receptor initiates a physiological response.

Signal Transduction Pathways in Plants

Hormones can function as signal molecules that trigger signal transduction pathways in cells. Such pathways often result in the synthesis of transcription

factors that in turn promote synthesis of enzymes which facilitate chemical reactions within the cell (the response). In a similar fashion, a signal molecule may function to repress transcription. Signal transduction pathways are equally important for chemical messaging in plants as in animals and both environmental cues and hormones serve as signal stimuli.



Methods for regulating endogenous levels of hormones:

The internal levels of plant hormones must be tightly controlled so that the response occurs only at the appropriate time. Regulation of hormonal action is achieved by:

- (1) controlling the rate of hormone synthesis.
- (2) forming conjugates, which are inactive storage forms where the hormone is covalently bonded to a sugar, amino acid or other molecule.
- (3) enzyme degradation.
- (4) transporting the hormone away/toward the site.
- (5) compartmentalizing the substance in an organelle such as the chloroplast.

Classes of plant hormones

It is generally accepted that there are six major classes of plant hormones, some of which are made up of many different chemicals that can vary in structure from one plant to the next. The chemicals are each grouped together into one of these classes based on *their structural similarities and *on their effects on plant physiology. Other plant growth regulators that are not easily grouped into these classes exist naturally, including chemicals that inhibit plant growth or interrupt the physiological processes within plants. Each class has positive as well as inhibitory functions, and they most often work in tandem with each other, with varying ratios of one or more interplaying to affect growth regulation.

There are **six major groups** of hormones: auxins, gibberellins, ethylene, cytokinins, abscisic acid and brassinosteroids. Another hormones are:

- Salicylic acid- signal in defense responses to plant pathogens.
- Jasmonates- plant growth regulation and defense.
- Polyamines- growth and development; mitosis and meiosis.
- Systemin- long-distance signal that activates chemical defenses against herbivores.
- Nitric oxide- signal in hormonal and defense responses.

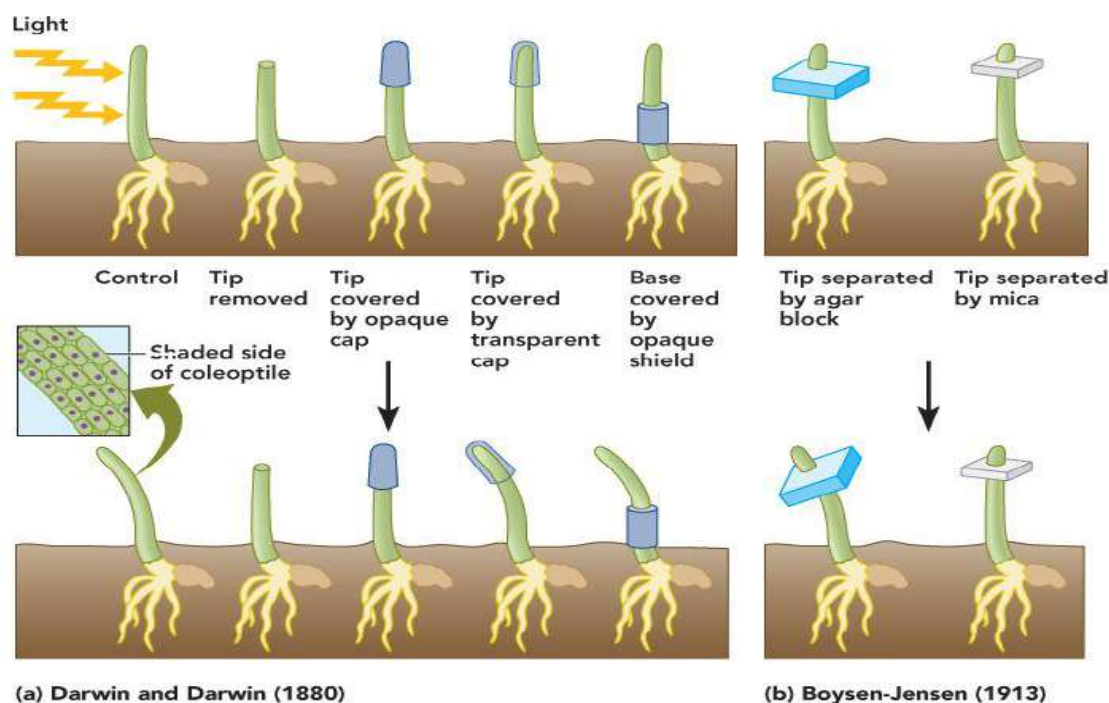
Auxins

History of Auxins

The concept of chemical messengers in plants was proposed in 1880 by Charles Darwin and his son Francis who spent time looking at the phenomenon of phototropism in grass seedlings. Plant shoots are positively phototropic. When a seedling is illuminated from the side, the shoot will bend towards the light. This directional growth makes sense, since plants need light for photosynthesis. The Darwin father and son did research on grass seedlings to try and determine what

controlled this differential growth. Interestingly, in their research, if the coleoptile of the wheat seedling was removed, the plant no longer curved towards light. They did a number of experiments and determined that a chemical located in the coleoptile traveled to the region of elongation and effected a differential elongation of cells furthest from the light sources.

- In 1913 Peter Boysen-Jensen affirmed that the signal for bending was mobile. When he placed a mica slice between the coleoptile tip and the rest of the shoot, no bending occurred. The mobile chemical was subsequently studied and named auxin by Frits Went in 1926.



Chemistry/Structure

A. Naturally Occurring Auxins

The most important auxin found in plants is indole-3-acetic acid (IAA). IAA is comprised of an **indole ring** linked to **acetic acid**. Other auxins that have been isolated from plants include:

indole ethanol, indole acetaldehyde, indole acetonitrile, phenylacetic acid (PAA), and 4-chloro-indoleacetic acid. These are probably converted to IAA *in vivo*.

B. Synthetics with Auxin Activity

There are a variety of substances that are not known to occur in plants have auxin activity. These include:

indolebutyric acid (IBA);

naphthalene acetic acid (NAA);

2,4- dichloro-phenoxyacetic acid (2,4-D),

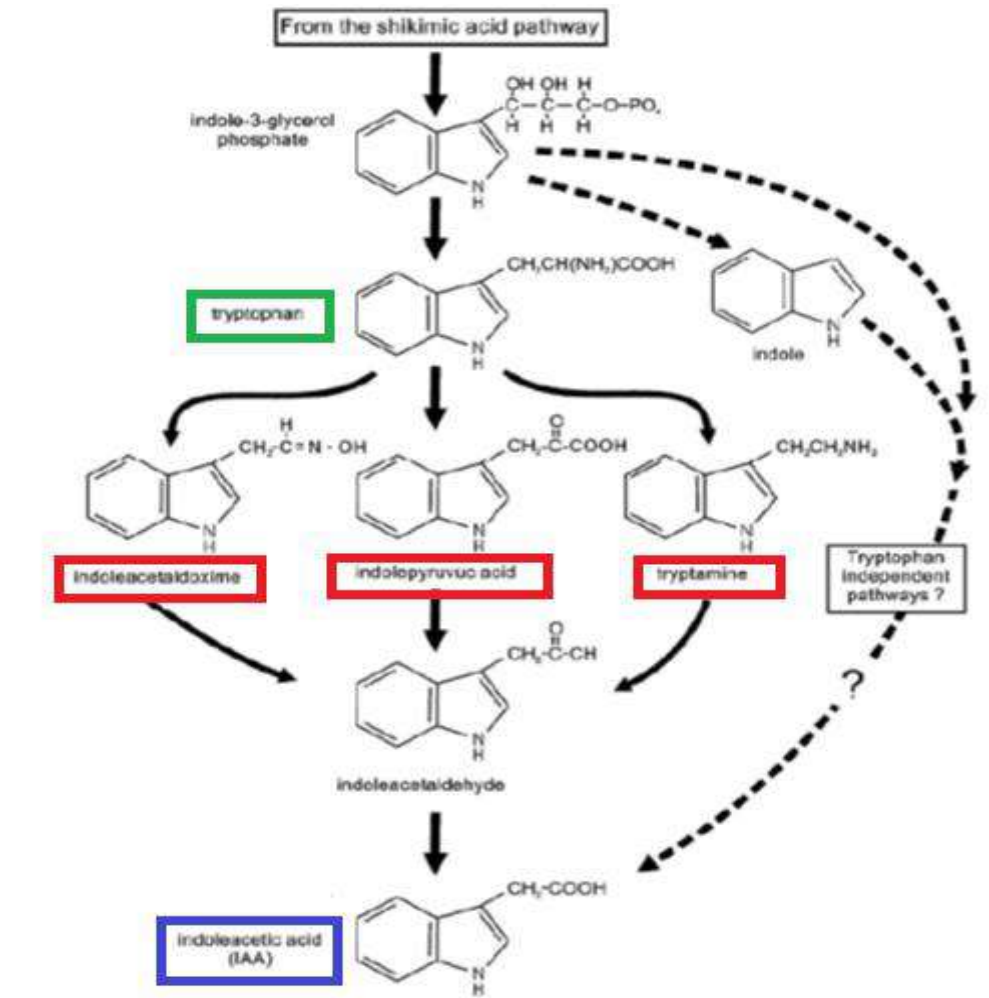
and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).

Auxin Biosynthesis

Traditionally, the indole amino acid, **tryptophan (Trp)**, which is formed as well as the other aromatic compounds by the shikimic acid pathway, has been thought to be a precursor of the most important native auxin in plants, IAA. There are several possibilities as to how plants may convert Trp into IAA:

- 1- **indole-3- pyruvic acid** (the so called “indolepyruvate pathway”).
- 2- **indole- 3-acetaldoxime** (the “indole-3-acetaldoxime pathway”) .
- 3- **tryptamine** (the “tryptamine pathway”).

The most common IAA-biosynthetic pathway in plants appears to be the indole pyruvate one.



Pathways of auxine biosynthesis

Auxins transports

Auxins are synthesized in **shoot apical meristems, young leaves, seeds and fruits**.

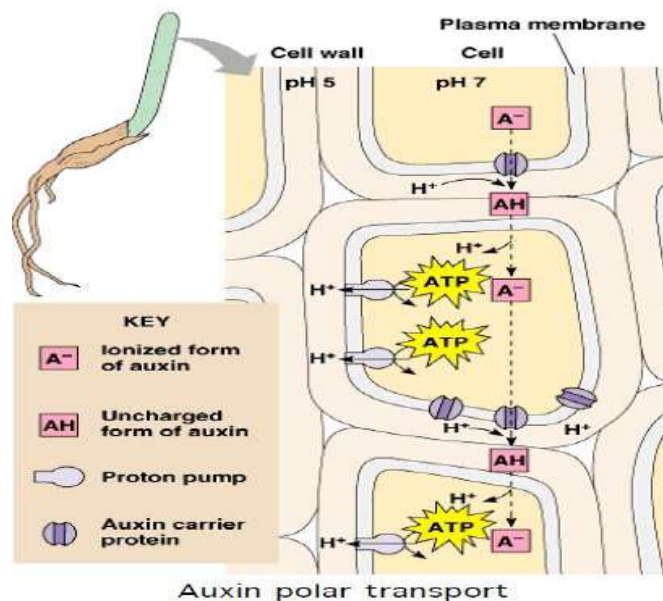
IAA is **only one** plant hormone transported **unidirectional from apical end to basal end**.

Auxin appears to be transported directly through parenchyma tissue, from one cell to the next. It travels down the stem (from the shoot apex) at about 10 mm/hr, too fast to be by diffusion but slower than translocation in the phloem. Auxin always moves down the stem parenchyma cells towards roots by polar (charged) transport. Auxin becomes negatively charged, using proton pumps, an ATP requiring process. For auxin transport to be successful, the following conditions are must be met:

- Diffusion of a polar molecule across the plasma membrane

- Carrier molecules for auxin located only at the basal (bottom) end of the cell
- Proton pumps must be present to remove H^+ from the cell, altering the pH gradient
- Weak acid ionization (Indole Acetic Acid, IAA, aka auxin (A), is a weak acid: $A^- + H^+ = AH$

Auxin enters cells as IAAH passively, or as IAA^- via active cotransport. IAAH dissociates within the cytosol and special auxin transport proteins in the basal end of the cell are needed to carry auxin through the plasma membrane to the top of the adjacent cell. Auxin destined for root tissue, however, moves through phloem sieve tubes.

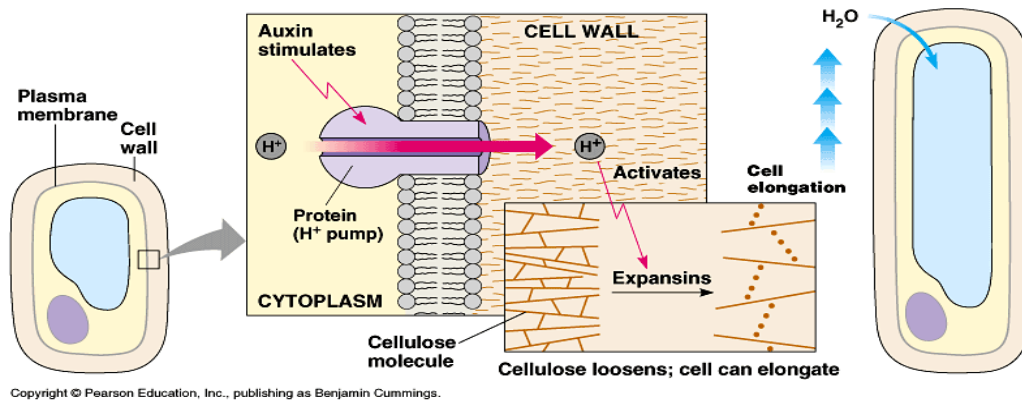


Physiological effects of auxins:

1. Cell elongation of leaves, roots and stems:

The auxins cause Cell elongation of leaves, roots and stems as following:

- increases the cell wall plasticity.
- increases the osmotic potential of the cell due to high accumulation of solutes.
- decreases the viscosity of protoplasm.
- increases synthesis of proteins and RNA. All these factors lead to absorb higher amount of water and thereby increase the size of the cells.



2-Tropism:

Tropism is the movement of plant organ towards the environmental stimulus such as light, gravity, water, chemicals and mechanical stimulus. There are several types of tropism:

- A. Phototropism:** it is bending of plant towards the light source due to translocation of auxin to the shaded side. The increased concentration in the shaded side causes increase in cell enlargement and growth of the cells in this region and cause bending.
- B. Geotropism:** movement of roots towards the earth center due to earth gravity. This happens due to accumulation of auxin in the lower region. Roots are different from stems in their response to auxin. High concentration of auxin in roots causes inhibition instead of stimulation. Root is positive geotropism while stem is negative geotropism.
- C. Chemotropism:** Bending or moving of plant organs to chemical stimulance such as elongation of pollen tube down wards to the ovule due to the effect of auxins produced by ovary and ovule walls.
- D. Thigmotropism:** Movement of plant due to the effect of mechanical factors.
- E. Hydrotropism:** movement of roots towards the moisture region in soil.



3-Apical dominance:

It is a phenomenon in which the apical bud dominates over the lateral bud and does not allow it to grow. This happens when the auxin in the apical bud is translocated to lateral buds and increases their auxin concentration. The high concentration of auxin inhibits growth of lateral buds. Cytokinins (another group of plant hormones moving upward from roots) counter the apical dominance effect of auxins and promote lateral bud development.

4-Callus formation:

Auxin was found to stimulate cell division as well as cell enlargement in the cell division in the wounding tissues forming a callus layer on wound to protect the plant organ from the pathogen.

5-Roots initiation:

The auxin stimulates cell divisions in pericycle region in which the roots are initiated forming a high number of lateral roots.

6-Fruit development:

Fruit formation is generally followed by pollination and fertilization processes. These processes induce auxin formation which is utilized in stimulation of cell divisions of ovary wall and receptacle leading to growth and development of fruit.

It has been found that auxin can stimulate formation of fruit without pollination. The fruits formed in this way are called **parthenocarpic fruits**.

7-Leaves and fruit drop:

Before leaves dropping, the plant forms abscission zone which is located at the base of the leaf petiole and consists of thin cell wall cells without superin and lignin. It was found that dropping of leaves depends on steep gradient of concentration of auxin across the abscission zone. Proximal application of IAA (end of the petiole close to the stem) is high abscission is accelerated. When the concentration of IAA is high at the distal region (Close to the leaf) abscission does not occurred.

8- Vascular differentiation:

Auxin affects secondary growth by increasing cambial activity and influencing the differentiation of cambial initials.

9-Increase rate of respiration:

The auxin increases the respiration rate. The reason is that the auxin stimulates several metabolic processes which require energy and this would increase respiration to provide the required energy.

Cytokinins

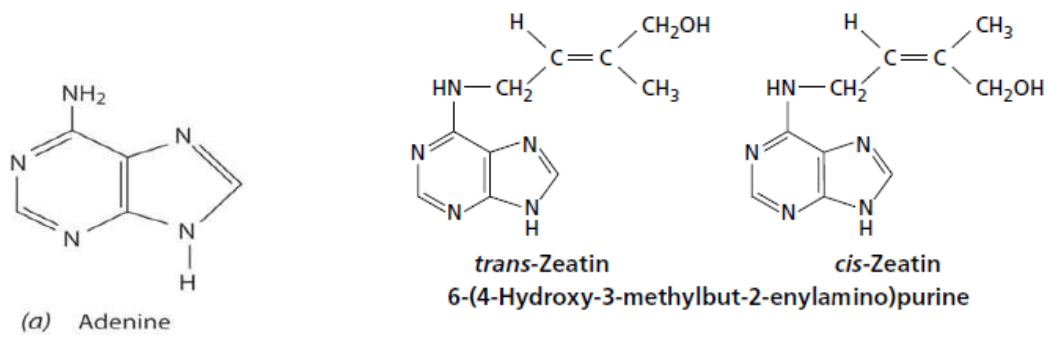
Cytokinins are plant hormones consist of purine ring. These hormones were discovered when scientists found that some compounds containing adenine have the ability to induce cytokinesis cell division, (adenine is a nitrogen base found in the structure of DNA and RNA). At that time scientists discovered the first compound in Cytokinins group and named kinin. However animal physiologists used the term kinin to include hormones related to polypeptides group, therefor they used the term phytokinin and later replaced by cytokinin.

Thirteen out of eighteen cytokinins have been extracted and purified from higher plants. Some of these compounds bound with 5 carbon sugar and some time with phosphate to form nucleotides and nucleosides. Cytokinins are 0.01 to 1.0 mM in plants

Chemistry:

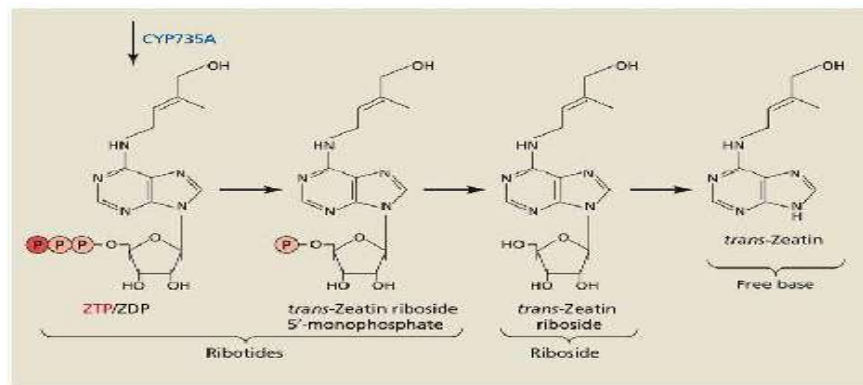
Generally

- **All cytokinins have basic Adenine ring structure.** CKs are adenine derivatives (amino purines)
- approximately 40 different structures known.
- **Zeatin (Z)**, which was the first, isolated from maize (*Zea mays*). It is one of the most common cytokinins in plants.
- **Other naturally occurring** cytokinins include: dihydrozeatin (**DHZ**) and isopentenyladenosine (**IPA**).



Biosynthesis:

- Cytokinins occur in free form (free base) or in tRNA
- The major site of biosynthesis of free cytokinins is root tip in the meristematic region of the root and move upward in xylem and pass into the leaves and fruits where they are required for normal growth and cell differentiation– also produced in developing buds, developing seeds.
- CK biosynthesis is synthesized through the biochemical modification of adenine as shown in the figure below.



Physiological Effects:

- 1- **Cell division** - applications of CKs induce cell division in tissue culture in the presence of auxin. The presence of CKs in tissues with actively dividing cells indicates that CKs may naturally perform this function in the plant. **It was found that IAA is necessary for nucleus division while cytokinin is required for cytoplasm division.**
- 2- **Cell elongation.** cytokinins stimulate cell enlargement as IAA and GAs.
- 3- **Morphogenesis** - in tissue culture, CKs promote shoot initiation. It was found that the parenchyma cells of callus don't differentiate into organs or tissues when there is **a balance** between IAA and cytokinin, but when the **ratio of cytokinin/IAA increased** differentiation of the parenchyma cells started to form buds and shoots.
- 4- **Growth of lateral buds** - CK applications can cause the release of lateral buds from apical dominance.
- 5- **Prevent the aging and senescence:** Cytokinin are found to be inhibit aging and senescence due to:
 - A- preventing the formation of hydrolytic enzymes such as nucleases, proteases which cause senescence and aging.
 - B- Withdraw of nutrient from the surrounding tissues to the region in which the cytokinin accumulates.
 - C- CKs delay leaf senescence. Chloroplast development - the application of CK leads to an accumulation of chlorophyll and increasing the rate of synthesis of photosynthetic enzymes then promotes the conversion of leucoplasts into chloroplasts.

Gibberellins

The nature of gibberellins

Unlike the classification of auxins which are classified on the basis of function, gibberellins are classified on the basis of structure as well as function. Gibberellic acid, which was the first gibberellin to be structurally characterized, is GA₃. There are currently more than 125 GAs have been isolated and chemically characterized. The discovery of gibberellins was related to their effects on shoot elongation as assessed in the “foolish seedling” disease of rice. This disease, caused by the fungus *Giberrella fujikuroi*, is characterized by accelerated rates of stem elongation resulting in the production of tall, turned, very thin pale seedlings and yield reductions compared with non-diseased fields. Later work at the University of

Tokyo showed that a substance produced by this fungus triggered the symptoms of foolish seedling disease and they named this substance "**gibberellin**".

Gibberellins Biosynthesis:

Gibberellins Are Biosynthesized in Apical Tissues

*The highest levels of gibberellins are found in immature seeds and developing fruits. However, because the gibberellins level normally decreases to zero in mature seeds. *Gibberellins are believed to be synthesized in **young tissues of the shoot**. It is uncertain whether young root tissues also produce gibberellins.

*Gibberellins are a large family of tetracyclic diterpenes that synthesized from **acetyl CoA via the mevalonic acid pathway**.

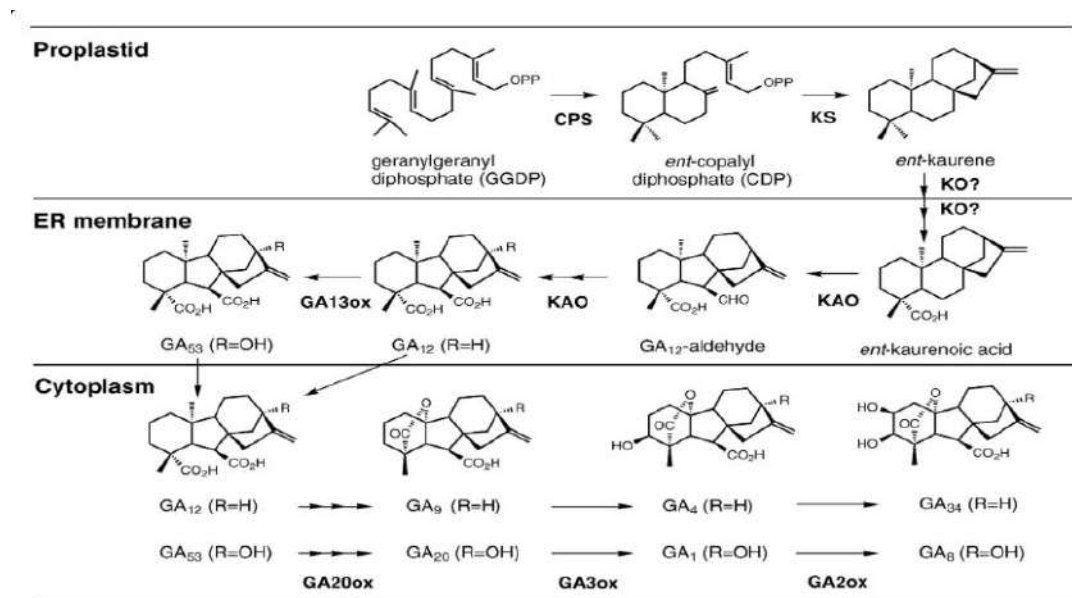
*GAs are synthesized in chloroplasts and then released to cytoplasm and then they are transported along sieve tubes.

The three stages of gibberellin biosynthesis are:

In stage 1, geranylgeranyldiphosphate (GGDP) is converted to ent-kaurene in plastids.

In stage 2, which takes place on the endoplasmic reticulum, ent-kaurene is converted to GA₁₂ or GA₅₃.

In stage 3 in the cytosol, GA₁₂ or GA₅₃ are converted other GAs.



Functions of Gibberellins

Active gibberellins show many physiological effects, each depending on the type of gibberellins present as well as the species of plant. Some of the physiological processes stimulated by gibberellins are outlined below.

1. Control genetic dwarfism:

Genetic dwarfism is caused by gene mutation in some crop cultivars such as corn, pea and broad bean. It was found that application of GA_3 on the dwarf plants returned the plant to normal shape and size.

2- Stimulate stem elongation by stimulating cell division and elongation.

3-Stimulate of flowering and growth of flower branches:

A-In some plants, growth of leaves become very dense and the branch of flower are stunted (Rosette). This happens in long day plants which does not receive enough light.

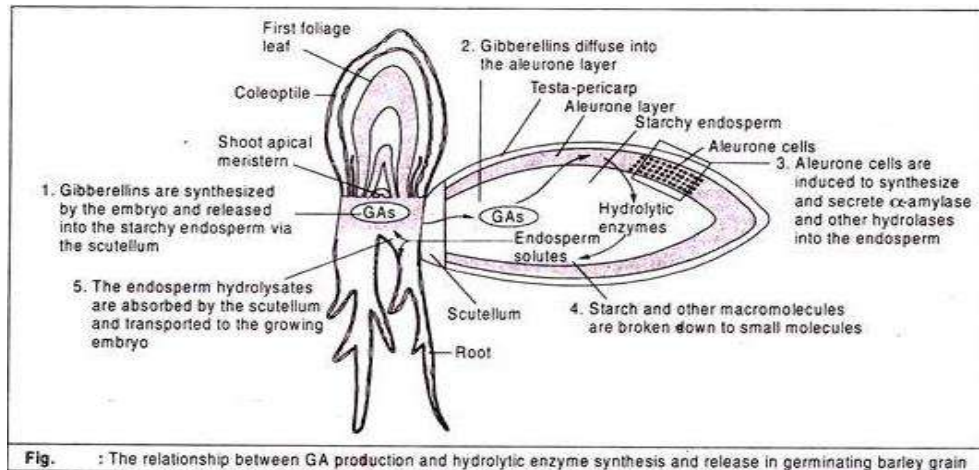
B-In other plants the flowering branch is elongated abnormally because the plant does not received enough cold temperature.

It was found that GA_3 can substitute the cold treatment and the long day in plants. The plant becomes normal after treatment with GA_3 .

4-Break seed dormancy in some plants which require stratification or light to induce germination.

Seed dormancy can be defined as the state or a condition in which seeds are prevented from germinating even under the favorable environmental conditions for germination. There are certain major causes for the seed dormancy. Light, Temperature, Hard Seed Coat, Germination inhibitors, Immaturity of the seed embryo, Impermeability of seed coat to water, Impermeability of seed coat to oxygen, and Presence of high concentrate solutes.

5-Induction of Seed Germination and stimulates enzyme production (α -amylase) during germination of cereal grains. The biochemistry of GA -induced α -amylase synthesis in the aleurone of cereal grains has been studied extensively. Gibberellins from the embryo of germinating grains are necessary for the synthesis of α -amylase by the cells of the aleurone layer, which, in turn is necessary for the hydrolysis of starch within the endosperm. Then endosperm hydrolysates are transported to the growing embryo.



6- Formation of parthenocarpic fruits:

GA₃ is similar to Auxin in that it can stimulate parthenocarpic fruits.

However GA₃ is more suitable to induce parthenocarp in apple, peach, pear and grapes than auxins.

Abscisic acid or Abscisin II

*This hormone is isolated in 1965 from fruits of cotton crop and named Abscisin II. Later was named abscisic acid (abbreviated as ABA). *It is considered as growth inhibitor hormone.

*ABA is synthesized in **mature leaves** and translocated to shoot apex through leaf petiole.

Biosynthesis of ABA in Plants:

Abscisic acid is a sesquiterpenoid containing 15 carbon atoms with molecular Formula: C₁₅H₂₀O₄.

The **initial steps** of ABA biosynthesis take place **in chloroplasts** or other plastids while **final steps** occur **in cytosol**. ABA is synthesized in higher plants **indirectly through carotenoid pathway** as breakdown products of 40-C xanthophylls such as **violaxanthin** or **neoxanthin**. Finally it is formed in cytosol.

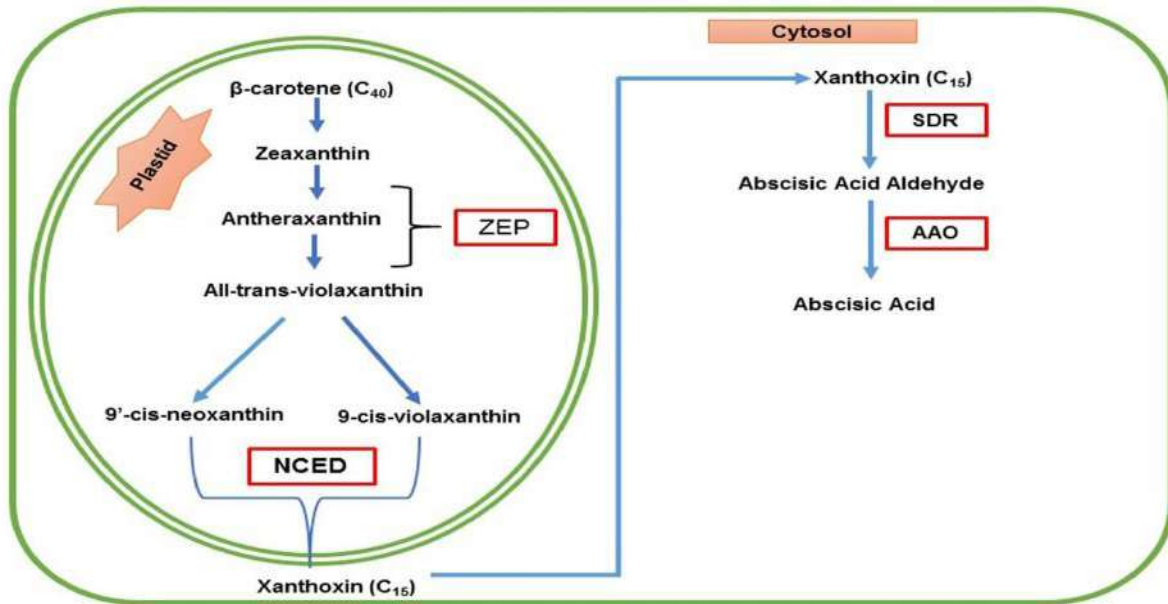
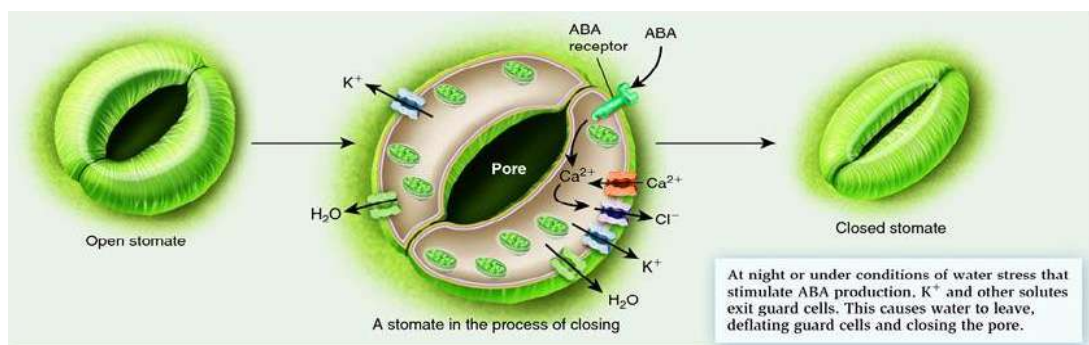


Fig: Biosynthesis of ABA in higher plants

Physiological Effects of Absciscic Acid (ABA):

(1) Stomatal Closing in plants undergoing water-stress:

During drought, leaves synthesize large amounts of ABA which causes stomata to close. Biosynthesis of ABA continues in mesophyll chloroplasts during period of water-stress. ABA produces its effect via regulating guard cell ion fluxes **by binding to proteins on the outer surface of the plasmalemma of guard cells**. This describes the plasmalemma more positively-charged, thereby **stimulating transport of ions (especially K⁺) from guard cells to epidermal cells**. The loss of these ions causes water to leave guard cells (via osmosis) which then deflates, thus closing the stomatal aperture. When water potential of the plant is restored (*i.e.*, increased), the movement of ABA into the guard cells stops. ABA disappears from the guard cells a little later.



(2) Other Effects:

ABA has also been shown to play some role in:

(i) inducing bud dormancy: Most of plant species form buds which grow later to leaves or flowers. Buds of most plant species may enter dormancy under unfavorable conditions such as drought, high and low temperature and salinity. The dormancy is regulated by endogenous hormonal levels. It was found that high concentration of ABA stimulates dormancy while High level of GA3 breaks the dormancy.

(ii) **inducing dormancy of seeds.**

(iii) **Abscission and senescence of leaves and fruits:** It was observed that higher concentration of ABA stimulates juvenile leave and fruit dropping. Concentration of ABA in mature leaves (Ready to drop) was 200% more than that in juvenile leaves. Senescence is also accelerated due to the inhibition of GA3 by ABA.

Ethylene

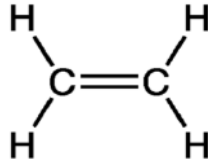
During the 1800, the city streets of Germany were illuminated by lamps that burned

“illuminating gas”. Soon after these lamps were installed, it was observed that plants growing near the lamps had short thick stems and leaves falling from most of them. In 1901, Dimitry Neljubow identified ethylene as the minor product of “illuminating gas” that was responsible for defoliation and stunted growth of plants growing near the lamps. Later at the half of the 20th Century, with the advent of gas chromatography, ethylene is involved as an endogenous regulator of plant growth and development.

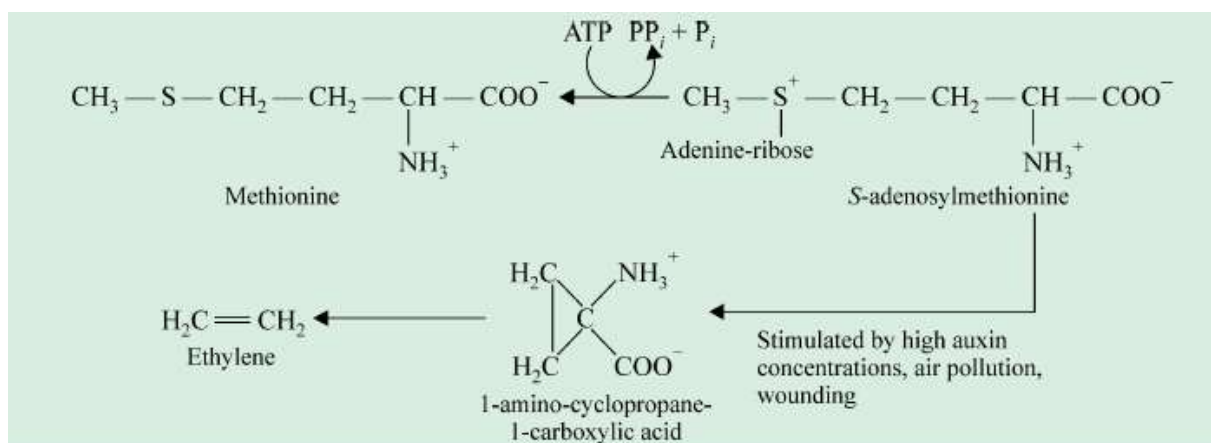
Ethylene is growth retardant hormone, **gas** under natural condition, simple chemical structure compared to other hormones, found in a very low concentration in plants, and it affects essential physiological process alone or in combination with auxin.

Biosynthesis of ethylene in plant

- Ethylene (C₂H₄) is flammable and highly volatile substance with a molecule weight 28, has the following formula,



- Ethylene is produced **from** essentially **all parts of higher plants**, including leaves, stems, roots, flowers, fruits, tubers, and seeds.
- Ethylene production is regulated by a variety of developmental and environmental factors. During the life of the plant, ethylene production is induced during certain stages of growth such as **germination**, **ripening** of fruits, **abscission** of leaves, and **senescence** of flowers. Ethylene production can also be induced by a variety of external aspects such as mechanical wounding, environmental stresses, and certain chemicals including auxin and other regulators. Also, the synthesis of ethylene is inhibited by CO₂. When plants are placed in pure CO₂ (or O₂-free air), ethylene synthesis decreases markedly.
- Ethylene is synthesized in plant tissues **from the amino acid methionine** in **three steps**: methionine to S-adenosyl-methionine (SAM). SAM is then converted to **1-aminocyclopropane-1-carboxylic acid** (ACC), Final step is formation of the ethylene. ACC synthesis increases with high levels of auxins, especially indole acetic acid (IAA) and cytokinins.



Biosynthesis of ethylene from methionine

Physiological effects of ethylene

1) **Stimulates fruit ripening:** One of the most distinct effects of ethylene is ripening of fruits therefore it is also known as **fruit ripening hormone**.

The stimulation of fruit ripening by ethylene is a consequence of many ongoing processes such as:

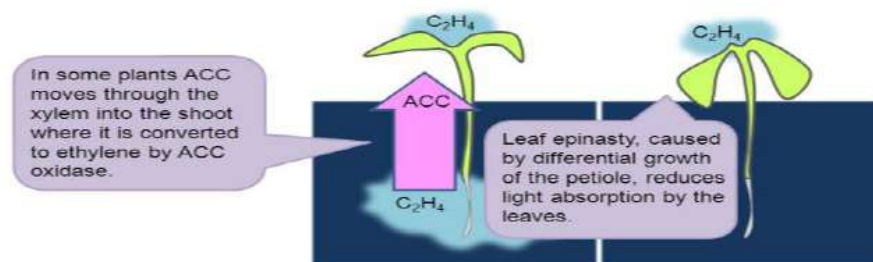
- (a) the breakdown of chlorophyll and synthesis of other pigments; for example, apples changing from green to red during ripening,
- (b) fruit softening due to breakdown of cell walls by cellulase and pectinase, and
- (c) conversion of starches and acids to sugars.

Fruits such as apples, bananas, kiwi, avocado, and tomatoes can be stored under ethylene-free, low oxygen conditions for months without ripening. When they are needed for market, about 1 ppm ethylene is added to the air.

2) **Inhibition of root growth:** ethylene inhibits linear growth of roots of dicot plants

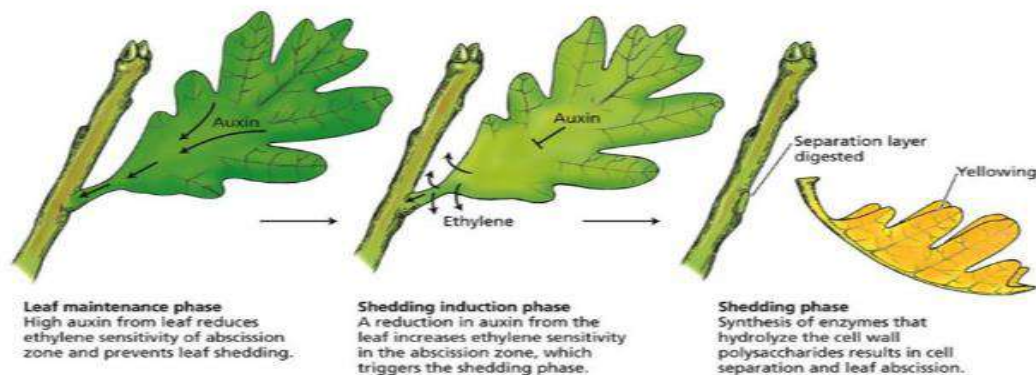
3) **Induces leaf epinasty:** When upper side of the petiole of the leaf grows faster than the lower side, the **leaf curves downward**. This is called **epinasty**. Ethylene causes leaf epinasty in tomato and other dicot plants such as potato, pea, and sunflower. However, **monocots do not exhibit this response**. Higher concentration of auxin, stress conditions such as salt stress, water-logging and pathogen infection also induce leaf epinasty **indirectly** through increased ethylene formation. In tomato and other plants, water-logging creates **anaerobic condition** around the roots resulting in **accumulation of ACC** (the immediate precursor of ethylene formation) **in roots**. ACC is then translocated to shoots along with transpiration stream where it is converted into ethylene in presence of oxygen and induces leaf epinasty.

ACC moving from root to shoot induces ethylene formation and epinasty



- 4) Inhibits flowering: Although ethylene **inhibits flowering in most species**, but induces it in a few plants including mangoes, pineapples and some ornamentals.
- 5) Leaf abscission: Ethylene promotes abscission of leaves in plants. Older leaves are more sensitive than the younger ones. The relative conc. of auxin on two sides of the abscission layer has regulatory effect on the production of ethylene that stimulates leaf abscission.

At the time of abscission, *conc. of **auxin in laminar region decrease** with simultaneous **increase in ethylene production**. *This also increases sensitivity of cells of abscission zone to ethylene which **synthesize cell wall degrading enzymes** (e.g. cellulases and pectinases). Activity of these enzymes results in cell wall loosening and cells separation **leading to leaf abscission**.



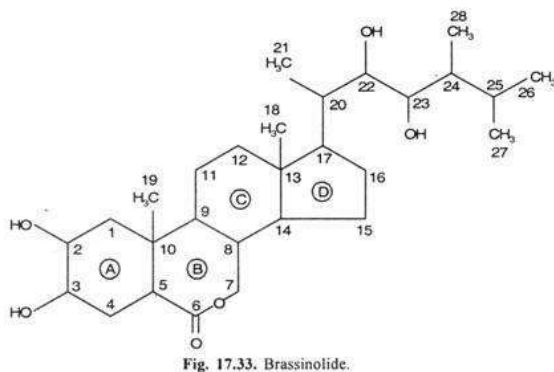
Brassinosteroids

Brassinosteroids or brassins have been recognized as a **sixth** class of plant hormones. They are a recently discovered group of steroids that have distinct growth promoting activity in some plants especially in stems. These compounds were first isolated in 1979 from bee collected pollen grains of rape (*Brassica napus*), of the Mustard Family (Brassicaceae), for which this class of chemicals are named,

although they are present in all plants and some algal species as well. In chemical structure, brassinosteroids resemble steroid hormones of animals.

More than 60 brassinosteroids have so far been identified from different parts of plants such as pollens, seeds, leaves, stems, roots and flowers. They cause marked biological effects on plant growth at very low concentrations (micro-molar concentrations).

One of the very common, well known and biologically active brassinosteroids in plants is brassinolide whose structure is given in the following Figure:



Biosynthesis of Brassinosteroids in plant

Brassinosteroids are biosynthesized from a C_{28} plant sterol called **campesterol** by a reductive step followed by several oxidative steps. Campesterol is in turn derived from a triterpene called squalene.

Effects of Brassinosteroids:

1. BRs promote both cell elongation and cell division in shoot. In soybean hypocotyls, brassinosteroids were found to affect cell elongation and gene expression quite independently of auxins. BR-induced genes include genes that loosen cell walls to permit cell expansion.
2. BRs both promote and inhibit root growth:

BRs are required for normal root elongation, exogenous applied BRs may have positive or negative on root growth, depending on the concentration. When applied exogenously, BRs promote root growth at low concentration and inhibit root growth at high concentration. BRs promote gravitropism responses.

3. BRs promote xylem differentiation during vascular development.
4. BRs are required for the growth of pollen tubes.
5. BRs promote seed germination.
6. BRs delay leaf abscission.
7. Brassinosteroids also have important agricultural implications in **increasing productivity of many crop plants**