Lecture 1: Introduction and importance of Crop Physiology in Agriculture – An over view of Plant cell

Introduction

Crop physiology is important to appreciate the structural economy or the efficiency of biological structures. The chloroplast and mitochondria are sub microscopic organelles, which bring about energy conversions so efficiently that man, made solar panels; hydroelectric and atomic power plants are far less efficient in terms of ratio of energy generated per unit space. Thus, Crop physiology offers an opportunity to view this intricate and finely interwoven fabric of interplay of functions- the gossamer of life. Crop physiology has remained very fascinating on account of its extra and sometimes sole emphasis on understanding the basic principles like structure-function relationship, energy-function relationship, and physico-chemical explanations of functional manifestations and molecular basis of cellular regulation. It has always enriched the treasure of knowledge and elevated human minds with the joy of enlightenment. Hence, Crop physiology is the investigation of the plant processes driving growth, development, and economic production by crop plants.

Plant physiology is the branch of plant sciences studying plants structure, processes, and responses of economically important and their interactions to the environmental variables at sub-cellular, cellular, tissue, organ and whole plant level during their growth and development. The notion of physiology originated from Greek by joining the words *physis*, which means "function" and logos is "science". The physiology of plants can be an amalgam of three fundamental events in their life cycle. 1. The structure of a plant is the initial task that provides the pattern of plant body to its environment, 2. Processes in plants enlightened the vital functions of various structures in plants were triggered by its genetic and environment, 3. Responses are nothing but the outcome discloses the changes in plant growth and development.

In overall view, Plant physiology includes, transport of water, nutrient absorption, transpiration, photosynthesis, translocation, assimilation, energy production, respiration and development are the key physiological processes occur in entire cycle of plant growth and development in response to the environment. The manipulation of these process and its responses were play significant role in Agricultural crops in order achieve higher production through higher biomass and yield.

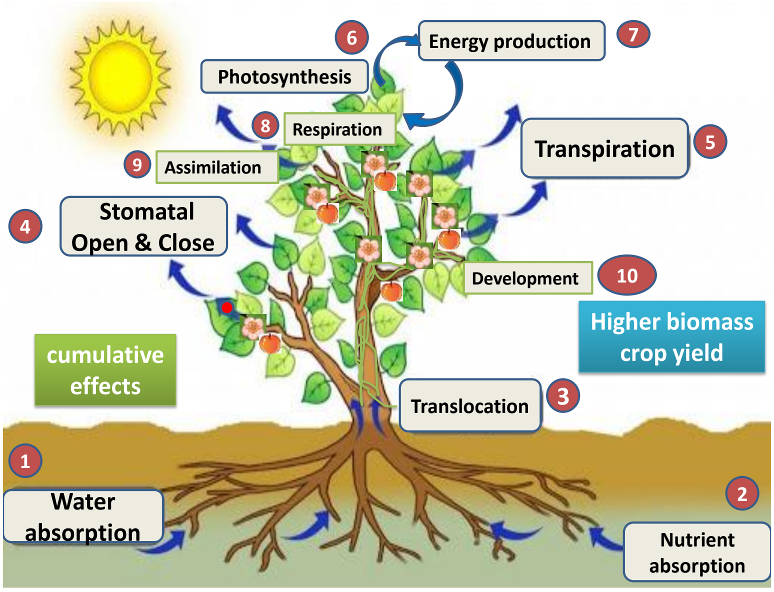


Figure 1. Over view of key physiological process in plants

Over view of Plant cell

Cell is the fundamental and organizational unit of all living organisms including plants. All plant cells have basic eukaryotic organization contain Cell wall, Cytoplasm and Nucleus.

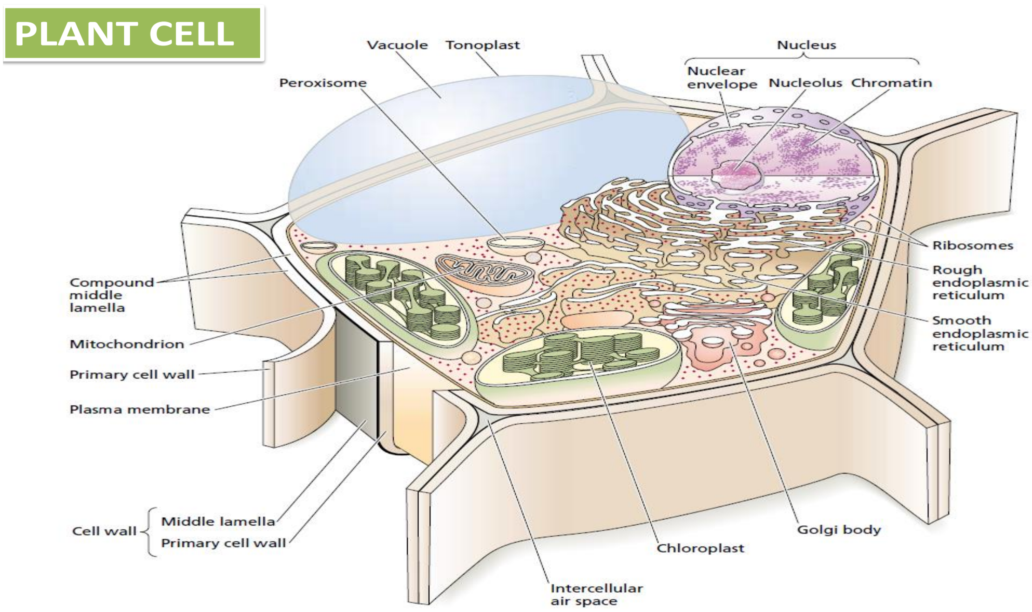
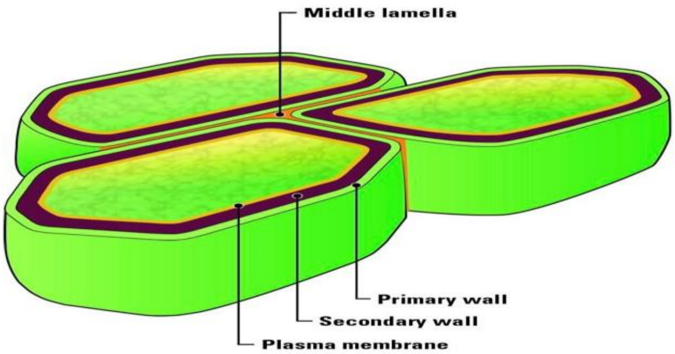


Figure 2. The over view of plant cell (Courtesy: Plant physiology; Taiz and Zeiger)

Cell wall

Cells are enclosed by a membrane that defines their boundaries called **cell wall**. It is stiff, rigid and nonliving provides a support and protection. Cell wall is permeable to water, oxygen and CO_2. Cell wall consists of three parts namely, Primary wall, Secondary wall, and Middle lamellae with Plasmodesmata.

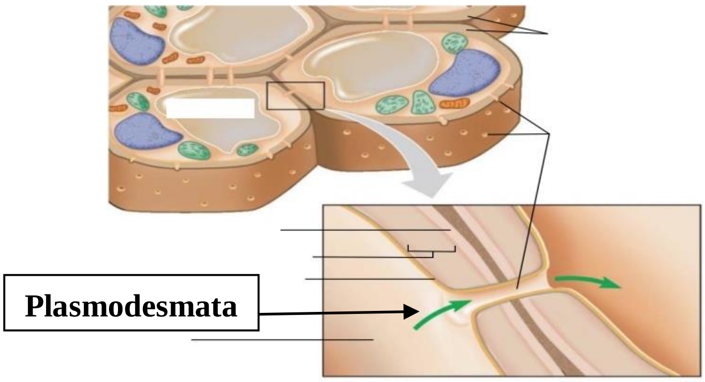


Components of cell wall

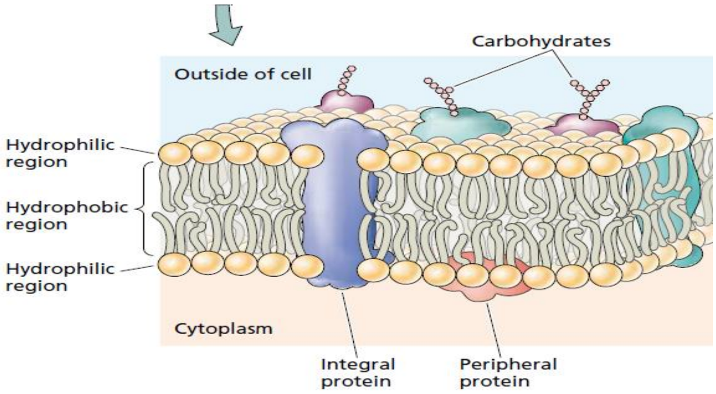
Primary cell wall chiefly contain cellulose which is long chain polysaccharides joined together by (1:4) glycosidic linkages. They are characteristic of young and growing cells. Secondary cell wall chiefly contain lignin, thicker and stronger than primary walls.

Middle lamella contains pectic acid in the form of Ca and Mg. It is a long chain polygalactouronic acid with (1:4) glycosidic linkage.

Plasmodesmata are a tubular extension of the plasma membrane which connects the cytoplasm of adjacent cells.

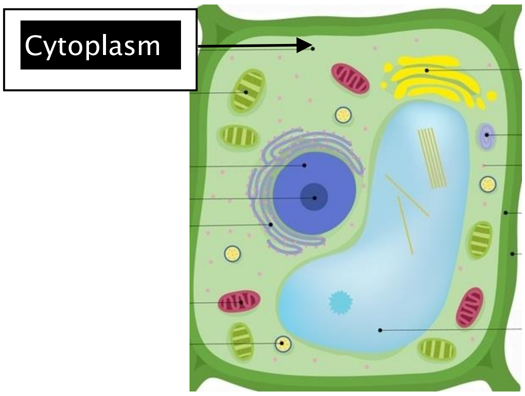


Cell Membrane in other words called Plasma Membrane, Plasma lemma and Cytoplasmic membrane. It forms the outer most boundary of cytoplasm made up of lipids and proteins. It is selectively permeable or differentially permeable to solutes in to the cytoplasam. It also protects and supports the cell Organelles in its environment.



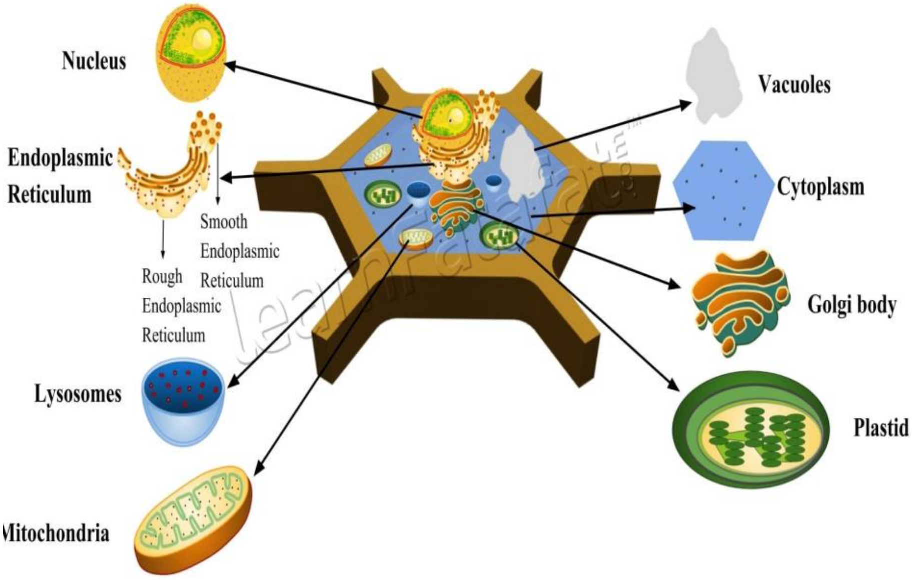
Cytoplasm

The cell substance between the cell membrane and the nucleus, containing the cytosol, organelles, cytoskeleton, and various particles is called cytoplasam.



Cell Organelles

Cell Organelles are cellular components found inside the plasma membrane. They coordinate with their functions efficiently for the normal functioning of the cell. They are are classified into three categories based on the presence and absence of membrane.



2.1. Organelles with or without membrane

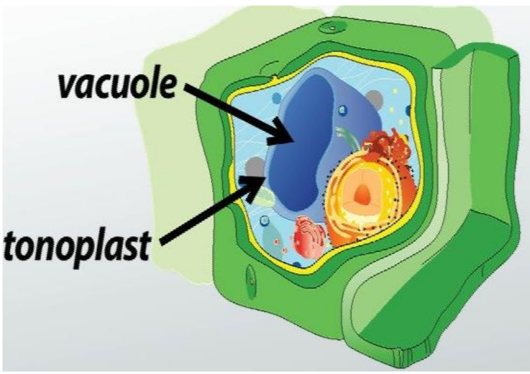
The cell wall, ribosome, and cytoskeleton are membrane-bound cell organelles. They present both in prokaryotic cell and the eukaryotic cell.

Single membrane-bound organelles: Vacuole, lysosome, golgi apparatus, endoplasmic reticulum are single membrane-bound organelles present only in a eukaryotic cell.

Double membrane-bound organelles: Mitochondria and chloroplast are double membrane-bound organelles present only in a eukaryotic cell.

2.2 Vacuoles

Vacuoles are storage bubbles found in cells. It is a membrane-bound organelle. The membrane is called as tonoplast found in both animal and plant cells. Vacuoles stored food and maintain the cell pH by buffering the salts. They can also serve to store waste products and protect the cell from contamination.



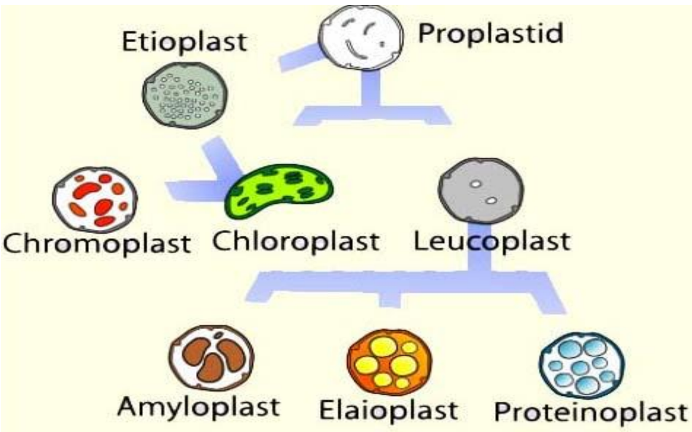
2.2. Plastids

Plastids are large membrane-bound organelles which contain pigments. Plastids are classified into three types based on the pigments: Chloroplast, Chromoplasts and Leucoplast

Chloroplasts serve as the site of photosynthesis. Chloroplasts contain a green pigment known as chlorophyll. It captures the light energy and utilizes the water and carbon dioxide into glucose for food.

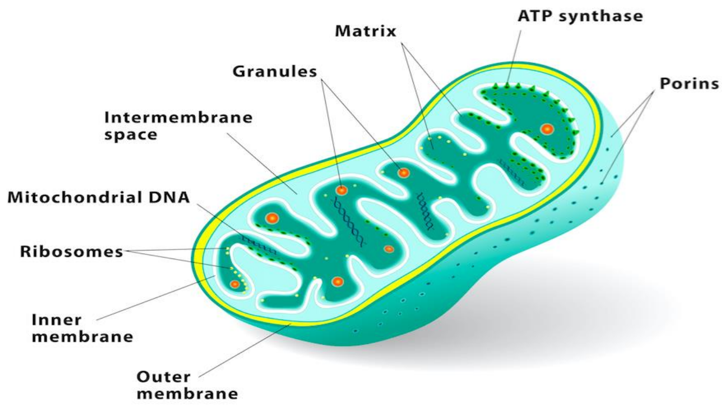
Chromoplasts are pigments primarily involved in the production and storage of (carotenoid pigments). These are accessory pigments assisting in absorption of light energy as well as protecting the chlorophyll from high light stress.

Leucoplasts are unpigmented organelles and specifically involved in storage of starch (amyloplasts), lipids (elaioplasts) and proteins (proteinoplasts).



2.3. Mitochondria

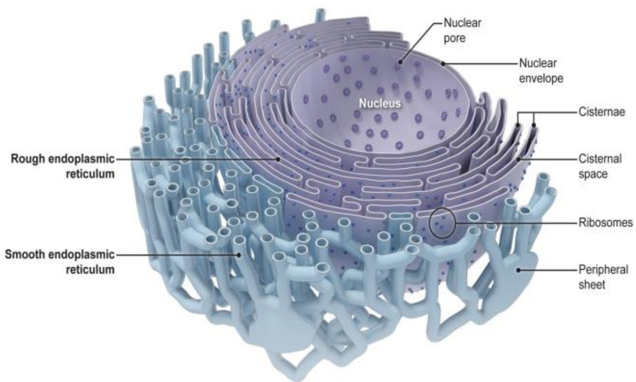
They are semi autonomous double-membrane-bound organelle found in most eukaryotic organisms. It generates most of the chemical energy needed to power the cell's biochemical reactions called "Powerhouse" of the cell. It is the site of aerobic respiration in the cell and produced energy- rich molecules in the form of ATP which helps in the transformation of the molecules. It also acts as cell's 'digestive system'.



2.4. Endoplasmic Reticulum

It is a network of membranous canals filled with fluid. It is a transport system of the cell involved in transporting of materials throughout the cell. There are two different types of Endoplasmic Reticulum:

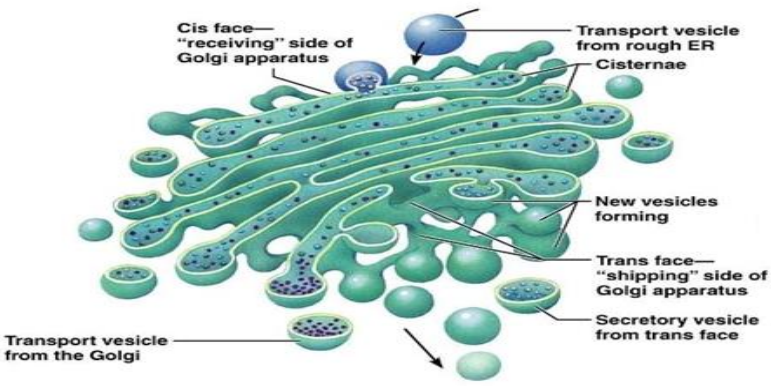
A net work of membranous tubules within the cytoplasm of a eukaryotic cell having continuous attach with the nuclear membrane.



It usually has ribosomes attached and is involved in protein and lipid synthesis. The endoplasmic reticulum is a type of organelle made up of two subunits – rough endoplasmic reticulum, and smooth endoplasmic reticulum.

2.4.1. Rough Endoplasmic Reticulum is composed of cisternae, tubules, and vesicles which are present throughout the cell and assisting in protein synthesis. Ribosomes are located in the rough ER.

2.4.2. Smooth Endoplasmic Reticulum are the storage organelle, associated with the production of lipids, steroids, and also responsible for detoxifying the cell.

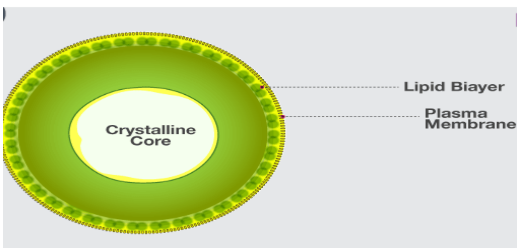


2.5. Golgi apparatus

Golgi complex is a complex of vesicles and folded membranes within the cytoplasm of most eukaryotic cells, involved in secretion and intracellular transport responsible for further modification of the proteins transported from the rough ER. The processed proteins are then stored in the Golgi or packed in vesicles to be shipped elsewhere in the cell.

2.6. Microbodies

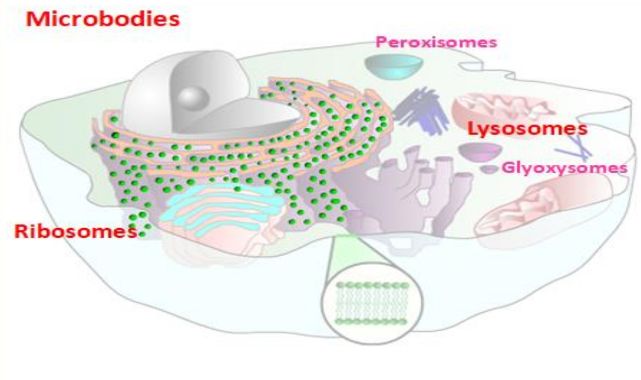
A microbody is a type of organelle that is found in both plant and animal cells. The organelles in the microbody family include peroxisomes, glyoxysomes, glycosomes, and hydrogenosomes. In vertebrates, microbodies are especially prevalent in the liver and kidney organs. A microbody is usually a vesicle with a spherical shape, ranging from 0.2-1.5 micrometres in diameter. The microbodies are found in the cytoplasm of a cell, but they are only visible with the help of an electron microscope.



They are surrounded by a single phospholipid bilayer membrane and they contain a matrix of intracellular material including enzymes and other proteins, but they do not seem to contain any genetic material to allow them to self-replicate.

2.6.1. Peroxisomes are surrounded by single layer membrane. It contains key enzymes for glycolate cycle called Peroxisomal enzyme ie., catalase. It is a site of photorespiration.

2.6.2. Ribosomes or microsomes are membrane less organelle present in ER, Cytoplasm, Chloroplast and Mitochondria. It is a site of protein synthesis



2.6.3. Lysosome is commonly referred to as suicidal sacs. They are membranous organelles that contain acidic enzymes (hydrolase enzymes) that serve to digest various macromolecules (e.g. lipids and nucleic acids) in the cell.

2.6.4. Spherosome are half unit membrane bound. They also called as oleosomes or Oil-bodies. It stored mainly fats and contain lipase enzyme.

2.6.5. Glyoxysomes found in fat rich seeds (Oil storing seeds) contains key enzymes for Glyoxylate cycle conversion of fatty acids in to sugars.

3.Nucleus

Nucleus is the largest organelle that stores the cell's DNA (deoxyribonucleic acid). The nucleus controls all of the cell's activities using the DNA's genetic information. Within the nucleus is a smaller structure called the nucleolus, which houses the RNA (ribonucleic acid). RNA helps to convey the DNA's orders to the rest of the cell and serves as a template for protein synthesis.

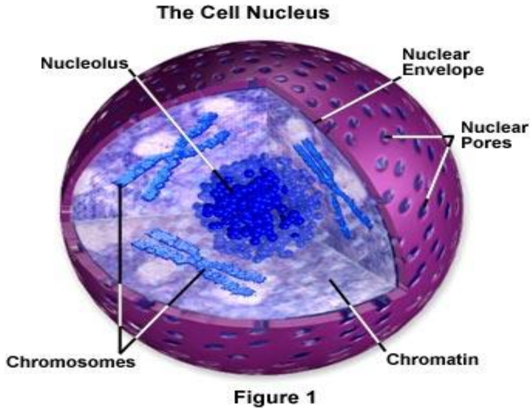


Figure 1

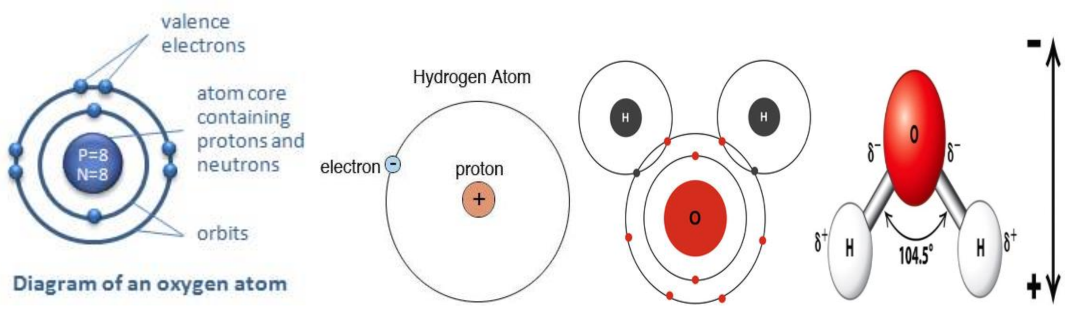
Lecture 2: Structure and role of water –water potential and its components – Diffusion – Osmosis – imbibition– Plasmolysis - Field Capacity and Permanent Wilting Point

I. Plant water relations

Water is the liquid gold or elixir of life. Without water, life as we know it could not exist. Water is the most abundant constituent of most organisms. The actual water content will vary according to tissue and cell type and it is dependent to some extent on environmental and physiological conditions, but water typically accounts for more than 80 to 90 percent by weight of non-woody plant parts. Water is dynamic in plant and the water content of plants is in a continual state of flux, depending on the level of metabolic activity, the water status of the surrounding air and soil, and a host of other factors.

1.1. Structure of Water

The water molecule consists of an oxygen atom covalently bonded with two hydrogen atoms. The chemical formula of water is H_2O. It is a tiny V-shaped (Tetrahedral) molecule attached as H-O-H bond in an angle of 104.5°. The oxygen atom is more electronegativethan hydrogen and tends to attract the electrons of the covalent bond.



This attraction results in a partial negative charge (δ−) at the oxygen end and a partial positive charge (δ+) at each hydrogen molecules making water as a polar molecule.These partial charges are equal, so that the water molecule carries no net charge. The resulting electrostatic attraction between the oxygen atom of one water molecule and the hydrogen atom of another water molecule constitutes a hydrogen bond.

1.2.Role of water

The Polarity and hydrogen bonding of water molecules are the two major factors generate many physical properties of water which play crucial role in plant system.

1.2.1. Physical Properties of water

1. **Excellent Solvent**
   The excellent solvent properties of water are due to the smaller size molecules, highly polar character of and hydrogen bonding the water molecule
2. **High specific heat**
   The specific heat is defined as the heat energy required to raise the temperature of a substance by a set amount (1 Cal (4.184 J) / g H_2O / °C)
3. **High latent heat of vaporization**
   The energy needed to separate water molecules from the liquid phase and move them into the gas phase at constant temperature during transpiration (44 KJ / mol at 25°C)
4. **High latent heat of Fusion**
   The quantity of heat necessary to change one gram of a solid to a liquid with no temperature change (80 cal /1 g ice / 0°C)
5. **Surface tension**
   The energy required to increase the surface area is known as surface tension
6. **Cohesion**
   The mutual attraction between water molecules due to extensive hydrogen bonding in water
7. **Adhesion**
   The attraction of water to a solid phase such as a cell wall called asAdhesion
8. **High Tensile Strength**
   Cohesion induced a maximum force per unit area that form a continuous column of water can withstand before breaking
9. **Capillarity**
   Cohesion, adhesion, and surface tension give rise to a phenomenon known as capillarity
10. **Density**
    It is the weight of the water per its unit volume, which depends on the temperature of the water.The density of water is around approximately about 1 gram/ cubic centimetre (1 g/cm^3)

1.2.2. Functions of water in plant system

The various functions of water in plants include: maintaining cell turgidity for structure and growth; transporting nutrients and organic compounds throughout the plant; comprising much of the living protoplasm in the cells up to 90-95 per cent of its total weight; participates directly in many metabolic processes and inter-conversion of organic compounds upon hydrolysis and condensation reactions: comprising serving as a raw material for various chemical processes, including photosynthesis;

1.5. Water Potential and its components

The activation physical properties require energy or potential is called as water potential. It is nothing but the measure of free energy of water molecules capable of doing work under system in constant temperature and pressure per unit volume (J m^{-3}).

In general, water potential of solution is determined by the four major factors. They are

1. Solute potential or osmotic potential (ψ_S)
2. Pressure potential or turgor potential (ψ_p)
3. Matric potential (ψ_m)
4. Gravity (ψ_g)

$$\psi_w = \psi_s + \psi_p + \psi_m + \psi_g \text{ -------------------------- } 1$$

Matric potential or gravitational components are generally omitted in considerations of water transport at the cell level due to meager difference as compared to osmotic potential and pressure potential. Thus, the water potential in plant system is quantified by using the following components.

$$\psi_{w} = \psi_{s} + \psi_{p} \quad \text{---------------------------------} \; 2$$

1.6. Role of water potential in plant

The movement of water molecules took place in plants depends on the water potential maintained in and out of the plant cells. There are four physical processes usually involved in plant cells based on the water potential level. They are diffusion, osmosis, plasmolysis and imbibitions.

Diffusion

The movement of particles or molecules from a region of higher (water or gas) concentrations to a region of lower concentration is called as diffusion. The rate of diffusion of gases is faster than liquids or solutes. The diffusion particles develop a certain pressure called as the diffusion pressure (D.P).

Osmosis

The movement of water molecules from a region of higher water potential to a region of lower water potential through a selectively permeable membrane is called as osmosis. In short, osmosis is nothing but the diffusion of solvent (water) molecules into the solution through a semi permeable membrane. It is also called as Osmotic diffusion. A pressure developed during osmosis called as osmotic pressure (O.P).

Significance/ benefits/uses of osmosis in plants

1. Absorption of water by roots in to xylem is due to osmosis
2. Osmosis is a selective process which protects cells
3. Opening and closing of stomata of guard cells by osmotic diffusion
4. Turgidity of the cells maintained through osmosis

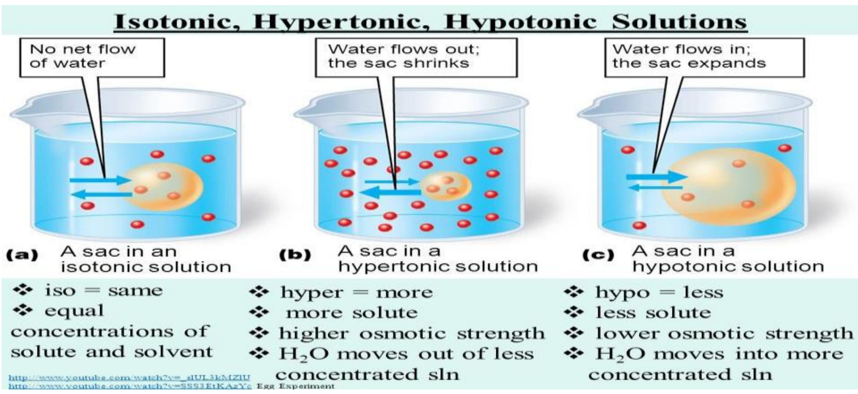
1.7. Cell solution and osmotic pressure

The Plant cell itself is an osmotic system due to the presence plasma membrane (semi-permeable) experiences the different forms of cell solution according to its osmotic pressure. The cell sap usually contained an aqueous solution in their vacuoles hold comparatively high osmotic pressure. The contact of cell with Hypotonic, Hypertonic and Isotonic solutions cause the different osmotic movement of cell solutes.

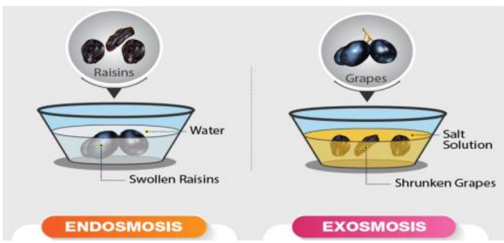
Hypotonic: Hypotonic solution is in lesser in concentration and its osmotic pressure is low. ie., ion concentration inside the cell is higher than outside.

Hypertonic:Hypertonic solution is in higher concentration and its osmotic pressure is higher. ie., ion concentration inside the cell is lower than theoutside.

Isotonic: Isotonic solution is in equal concentration and its osmotic pressure is equal ie., ion concentration inside the cell is equal to the concentration outside the cell.



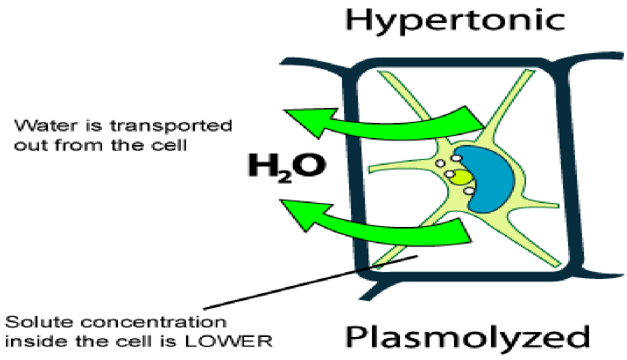
Living plant cells in contact with Hypotonic and Hypertonic solutions generate two types of osmosis: **(i) Endosmosis (ii) Exosmosis**



End-osmosis: The cell under hypotonic condition pass the water in to the cell called End-osmosis. As a result cell becomes turgid. The entry of water inside the cell developed certain pressure called as turgor pressure.

Ex-osmosis: The plant cell under hypertonic condition exudes the water from a cell called Ex-osmosis. The cell becomes flaccid.

Plasmolysis: The outward movements of water from the cell during exosmosis cause the shrinkage of protoplasm from cell wall in plant cell called as Plasmolysis.



Plasmolysis is determined by the degree of protoplasm hydration and by the microstructural features of the plasma membrane. It occurred in two stages namely (i) incipient plasmolysis (initial contraction of protoplast from the cell wall) (ii) evident plasmolysis to reach flaccid condition. Reentry of water into the cell becomes turgid and the protoplasm reassumes the normal position is called deplasmolysis.

Advantages of plasmolysis

1. It indicates the semi permeable nature of the plasma membrane.
2. It is used to determine the osmotic pressure of the cell sap.
3. Plasmolysis is used in salting of meat and fishes.
4. Addition of concentrated sugar solution to jam and jellies check the growth of fungi and bacteria which become plasmolysed in concentrated solution.

Imbibition: Substances which absorbs water or liquid and swell up. These substances are called as imbibants and the phenomenon is imbibition. A pressure developed during imbibition is called as imbibition pressure or matric potential (ψ_m). Absorption of water by the seeds is an imbibition process.

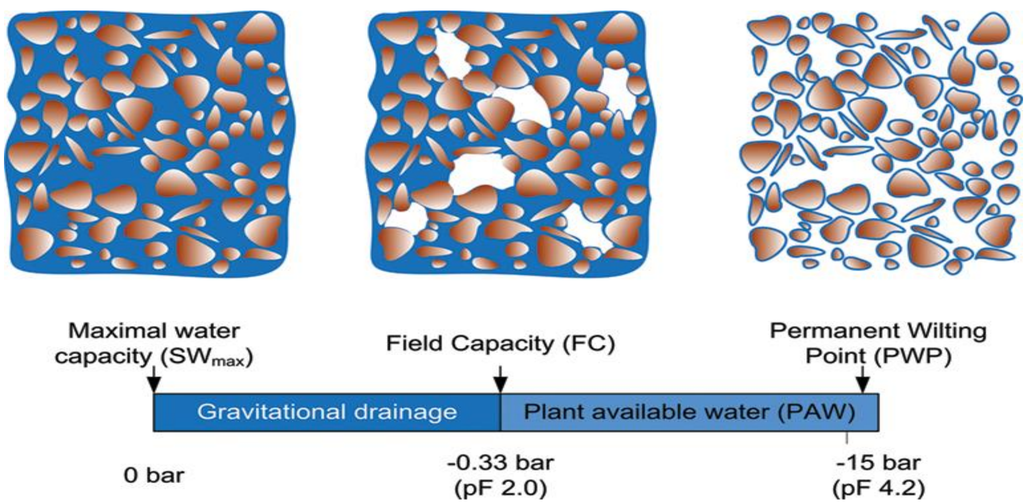
1.8. Field capacity, Available soil water and permanent wilting point

Field capacity or water holding capacity of the soil

Field Capacity is the amount of soil moisture or water content held in the soil after excess water has drained away and the rate of downward movement has decreased. This amount of water retained by the soil is called as field capacity or water holding capacity of the soil. At field capacity, the soil water potential is -0.1 to -0.3 bars.

Available soil water or Plant available water is nothing but the soil's ability to retain water and make it sufficiently available for plant use. Available water capacity is held between its field capacity and permanent wilting point. The available soilwater potential is -0.1 bars to -15 bars.

Permanent wilting point is soil water left after the plants growing in that soil has permanently wilted is called as permanent wiling point. The soil water potential at permanent wilting is -15 to -18 bars.

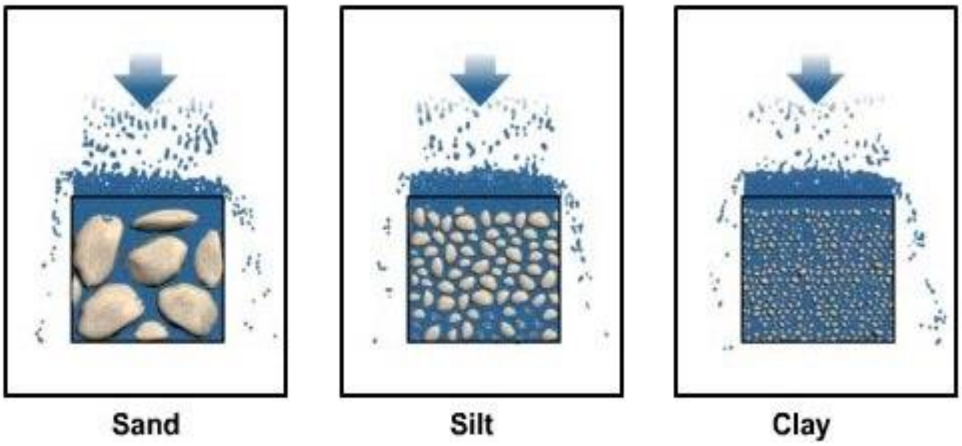


Lecture 3: Mechanisms of water absorption – Pathways of water movement – Apoplast and symplast

Mechanisms of water absorption

1. Water in the soil

The water content and rate of water movement in soils are the prerequisite for water absorption by the plants. However, it solely depends on the soil type and soil structure. At one extreme is sand, in which the soil particle size may be 1mm or more in diameter. Sandy soils have low surface area and it formed only lesser water channels between soil particles. The other extreme is clay and the soil particle size less than 2 µm in diameter. Clay soils usually have greater surface area formed more water channels between soil particles.

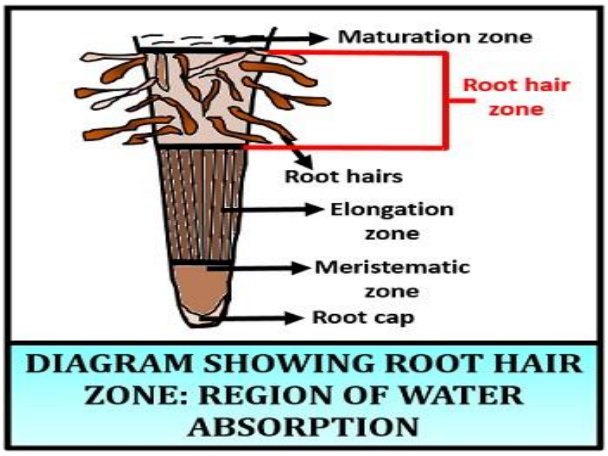


2. Water Movement in Soil

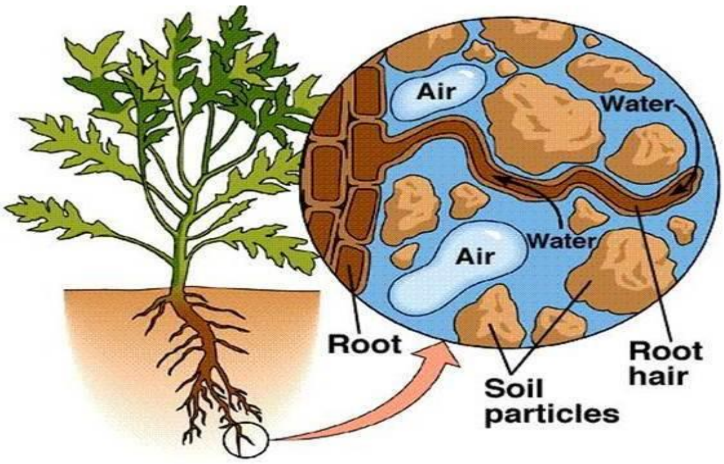
Water moves through soils predominantly by bulk flow driven by a pressure gradient. In addition, diffusion of water vapor accounts for some water movement. As plants absorb water from the soil, they deplete the soil of water near the surface of the roots. This depletion reduces ψ_p in the water near the root surface and establishes a pressure gradient with respect to neighboring regions of soil that have higher ψ_p. Because of the water-filled pore spaces in the soil are interconnected, water moves to the root surface repeatedly by bulk flow through these channels down the pressure gradient. The rate of water flow in soils depends on two factors ie., the size of the pressure gradient through the soil, and the hydraulic conductivity of the soil.

3. Absorption of soil water by the roots

Intimate contact between the surface of the root and the soil is essential for effective water absorption by the root. This contact provides the surface area needed for water uptake and is maximized by the growth of the root and of root hairs into the soil.



Root hairs are outer most microscopic extensions of root epidermal cells that greatly increase the surface area of the roots, thus providing greater capacity for absorption of ions and water from the soil. Root hairs are delicate cells that occur in a small zone at the rear the root's growing tip. They are very tiny in appearance and alive for short time.



4. Mechanism of water absorption;

There are two types: 1. Active Absorption 2. Passive Absorption.

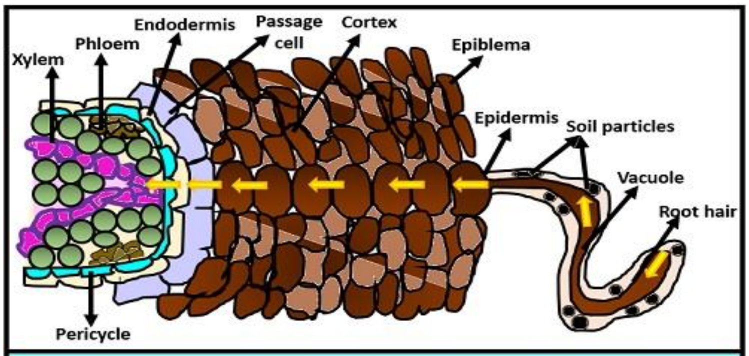
Active Absorption

A. Osmotic absorption

1. Water absorption takes place according to the osmotic gradient.
2. Root cells play active role in water absorption by spending metabolic energy released during respiration.
3. The hydrophilic cell walls of root hairs imbibes water from the soil is the first process in active absorption.
4. The high osmotic pressure (low water potential) in the root cells generates a high diffusion pressure deficit (DPD) create the soil water (high water potential) to enter into the root cells by osmotic diffusion.

5. Root cells become turgid and turgor pressure increased. The osmotic pressure and diffusion pressure deficit are decreased.
6. The cortical cells had drawn the water from the turgid root cells by osmotic diffusion.
7. In the same way, the water enters to the inner most cortical cells and reaches the endodermis.
8. The passage cells of the endodermis pass the water into pericycle by osmotic diffusion.
9. The cells of pericycle become turgid and finally, the xylem had drawn the water from pericycle cells.
10. The drawn water in the xylem of root required certain pressure to raise the water in the xylem is called root pressure.

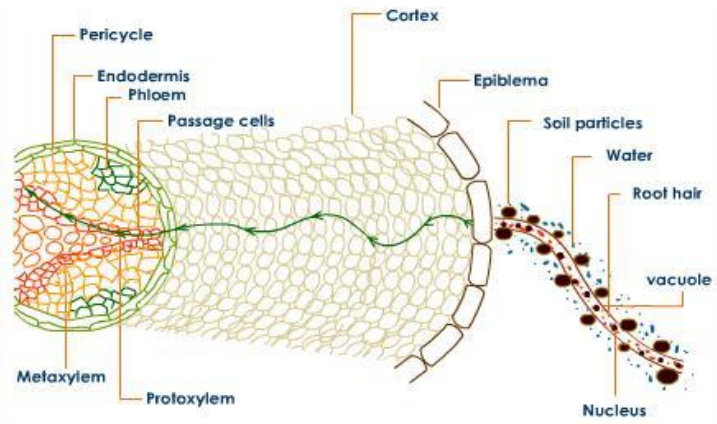
B. Non-osmotic absorption: Water absorption takes place against the osmotic gradient by spending metabolic energy. Absorption of water takes place even when the water potential of soil water is lower than the water potential of cell sap. This process is comparatively insignificant and this process regulated when plants are under water stress.



MECHANISM OF WATER ABSORPTION IN PLANTS

C. Passive Absorption

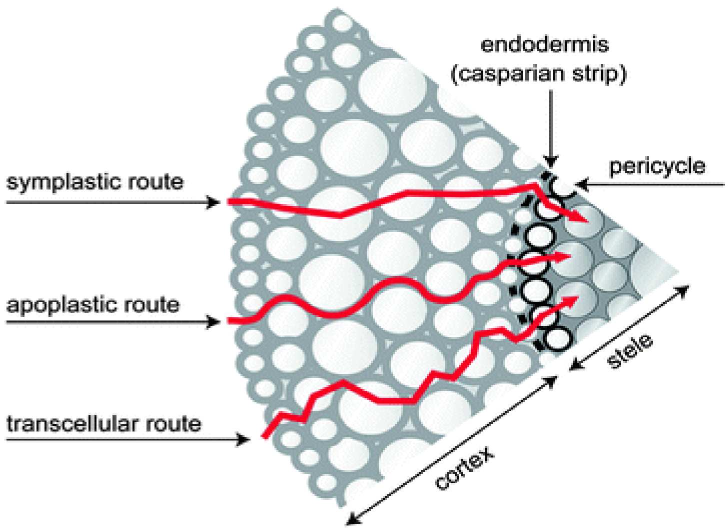
1. Water absorption occurs due to high rate of transpiration.
2. A tension created in the leaves during rapid transpiration is transmitted to the base of root's xylem.
3. The water in the root xylem is raised upward to reach the transpiring surfaces of leaves.
4. The consequence of this process, soil water enters into the root hairs through cortical cells to the xylem of roots.
5. The force of water entry is developed by the transpiring leaves. Root cells remain passive in this process.



5. Water Moves in the Root use pathways

The bulk flow of water from the soil is made available between soil particles for the roots absorption. When the water comes in contact with the root surface, the nature of water transport from the root hairs to the xylem of roots takes place through three pathways namely, apolpast, symplast and transmemberane.

1. Apoplast: Water moves exclusively through the cell wall without crossing any membranes. The apoplast path way is the continuous system of cell walls and intercellular air spaces in plant tissues.
2. Symplast: Water travels from one cell to next cell using plasmodesmata. The symplast consists of the entire network of cell cytoplasm interconnected by plasmodesmata.
3. Transmembrane: In this pathway, water crosses through the plasma membrane on entering and in the same way on exiting in its path. Water transport across the tonoplast may also be involved.



6. External factors affecting absorption of water

1. Soil water

The availability of soil water and its forms determined the absorption ability of plant roots. Presence of high amount of capillary water makes easy water absorption by the roots. High amount of soil water limits the water absorption due to poor root respiration.

2. Ions Concentration

Presence of more salts in soil solution (poor drained soils) results in higher osmotic pressure which hinders the water absorption of root cells due to higher osmotic pressure in the cell sap under.

3. Soil air

Water logged soils are poor in aeration which develop oxygen deficiency (O_2) and accumulated of carbon-dioxide (CO_2) in soil. This condition retards the metabolic activities of roots by limiting the root respiration and affects the water absorption. It also inhibits the growth and elongation of the root hairs.

4. Soil temperature

Optimum soil temperature (<30°C) facilitate water absorption. Higher temperature (>30°C) or low temperature (0°C) decreased the water absorption.

The differences between active and passive water absorption

Sl. No.	Active absorption	Passive absorption
1.	Active role by the root cells	Active role by the transpiring leaves
2.	Metabolic energy is required	Energy is not required
3.	Osmotic diffusion of water from root cells to xylem	Mass flow of water through transpiration
4.	Water path way movement is symplast	Water path way movement is Apoplast
5.	Root pressure raises the water in xylem roots. Eg: Guttation	Transpiration ratio in leaves raise the water in xylem roots

Leture 4: Translocation of water – Ascent of sap – Mechanisms of xylem transport

1. Translocation of water

The conducting cells of xylem have specialized anatomy that enables water transport with greater efficiency to all the parts of the plants. Xylem contains two types of tracheary elements: tracheids and vessel elements. In the intact plants, water is brought to the leaves via the xylem (tracheary elements) and distributed to the leaf vascular bundle.

Ascent of sap

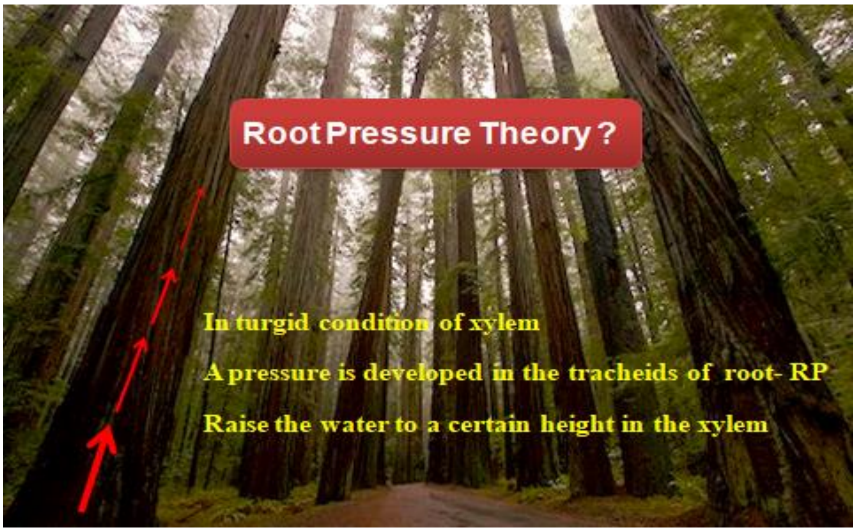
The entry water through root hair travels via apoplast or symplast or transmembrane pathways reach the xylem. Subsequently, the xylem water rose either by using roots pressure or transpiration. The raised water in the xylem is move upward in order to reach the topmost part of the plant. The unidirectional upward movement of xylem water in the plant is called "Ascent of sap".

2. Mechanism of xylem transport

Two theories have explained the translocation of water in the xylem

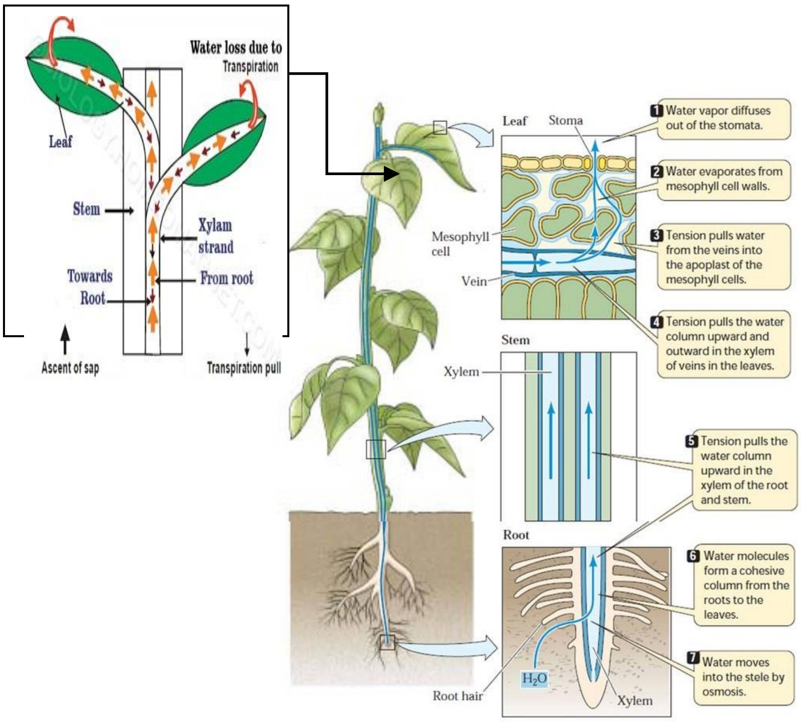
1. **Root Pressure Theory**

A pressure developed in the xylem of roots raises the water in the xylem to a certain height. However, the root pressure in tall trees alone not enough to supports the water column in xylem to rise and reach the top without break. This can be justified that the range of root pressure usually occurs between 2 atm to 6 atm in plants, whereas the actual requirement of plants is up to 30 atm.

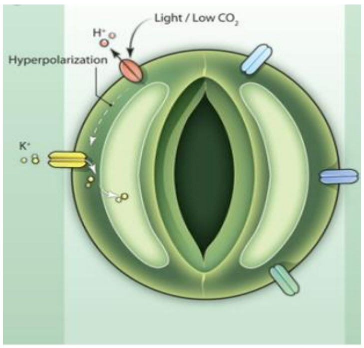


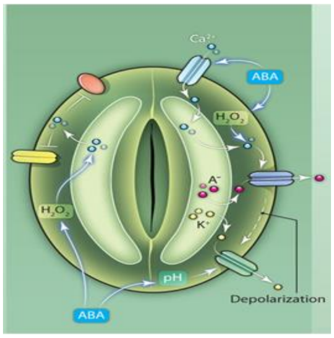
2. Transpiration Pull Theory - The Cohesion–Tension Theory (Dixon & Jolly,1924)

A continuous loss of water from the leaves created a large tension in the root xylem during the transport of water. A tension developed in the xylem vessel is called Transpiration Pull and this tension pulls the water through the xylem. The upward movement of water in the xylem requires cohesive and adhesive forces to sustain the large tension continuously in the xylem without break of water column. In short, transpiration pull is responsible for the ascent of sap in plants. It was justified that the cohesive force of water molecule might be extended up to 350 atm.



The total force required to raise water column to height of 350 feet's is only12 atm, however, the inclusion of force required for the water transport by cross-walls (due to cavity) and other resistances in xylem may reach up to 30 atm. Hence, this theory is very convincing and has now been widely supported by many workers.

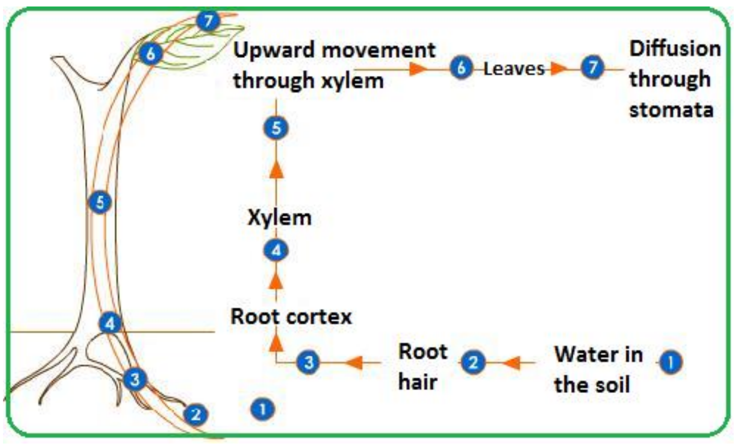




Lecture 5: Transpiration – significance – Stomatal physiology: structure of stomata with mechanisms of stomatal opening and closing – guttation - antitranspirants

1. Mechanism of Transpiration

Water movement from the leaf to the atmosphere is takes place during day time. The water in the form of water vapour is lost through the stomata from the aerial parts of plant to the atmosphere is called transpiration. This can be studied in three steps.



1.1. Osmotic diffusion of water from xylem to the intercellular spaces of mesophyll cells

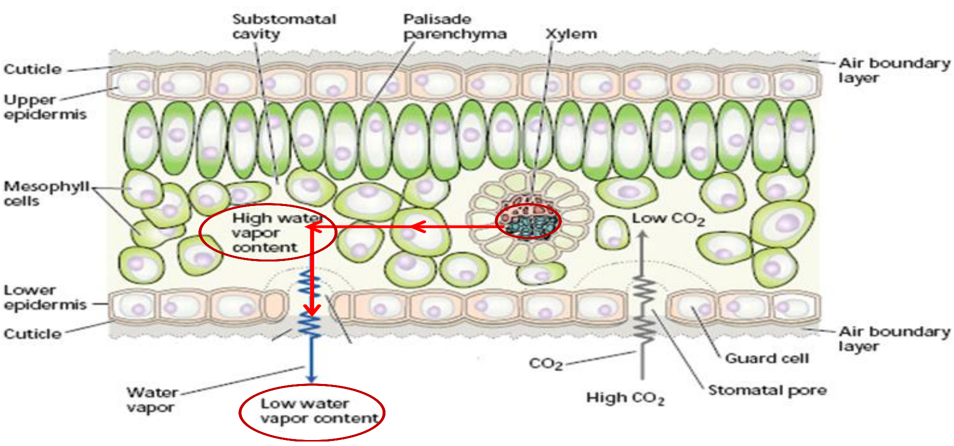
Water is pulled from the xylem into the cell walls of the mesophyll. Water moves along this pathway predominantly by diffusion. Mesophyll cells are drawn water from xylem due to high diffusion pressure deficit (DPD) and osmotic pressure (OP) and become turgid. Water is released from the mesophyll cells, and it evaporates into the air spaces of the leaf in the form of water vapour close to stomata. The process of water drawn from the xylem to mesophyll cells is continued by osmotic diffusion.

1.2.Opening of stomata

Consequently, the osmotic entry of water into the guard cells occurs from the surrounding turgid epidermal cells and mesophyll cells. The turgor pressure (TP) of the guard cells increased and they become turgid (accumulation of osmotically active substances). The guard cells swell and stomata is opened. The water vapor then exits from the leaf through the stomatal pore by osmotic diffusion.

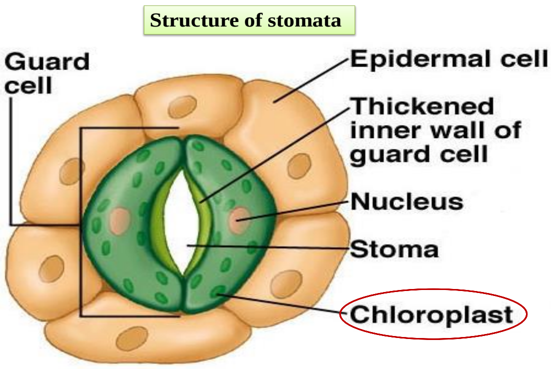
1.3.Closing of stomata

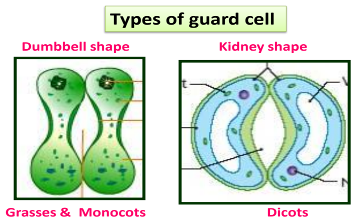
In the absence of transpiration, the osmotic pressure (OP) and diffusion pressure deficit (DPD) of the guard cells is decreased due to the depletion of osmotically active substances. Consequently, water is released back to the surrounding epidermal and mesophyll cells and the guard cells become flaccid. The thickened surfaces of the guard cells come close to each other, thereby closing the stomatal pore and stomata.



1.4. Structure of stomata

A typical leaves of all vascular plants contain a specialized cells called guard cells. The guard cells, epidermal cells or subsidiary cells, and tiny pore are collectively called as stomatal complex or stomatal apparatus.Stomata are usually most abundant on the lower surface of the leaf. Morphological diversity of guard cells has been distinguished by two types. One is typical of grasses and the other is found in all dicots. In dicot plants, guard cells are kidney-shaped have an elliptical contour with the pore at its center. In grasses, guard cells are dumbbells shaped and flanked by a pair of differentiated epidermal cells called subsidiary cells, which help the guard cells to control the stomatal pores.





1.5. Mechanism of opening and closing of stomata:

A distinctive feature of the guard cells is the specialized structure of their walls. Portions of these walls are substantially thickened and have a differential thickening pattern results in very thick inner and outer (lateral) walls, a thin dorsal wall (the wall in contact with epidermal cells), and thickened ventral (pore) wall. The alignment of cellulose microfibrils, are also an important determinant of cellshape. The presence of chloroplast in guard cells is involved in photosynthesis which plays an essential role in the opening and closing of the stomatal pore. Mainly, three theories are explained the mechanism of opening and closing of stomata.

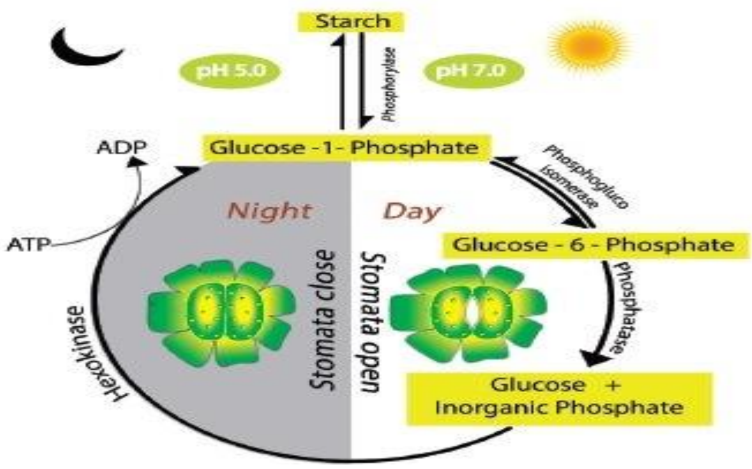
1. Theory of Photosynthesis
2. Starch – Sugar Inter-conversion Theory
3. Theory of Proton Transport

1. Theory of Photosynthesis

The presence of guard cell in stomata contains chloroplast which is not in epidermal cell. In the presence of light in day time, photosynthesis takes place in the guard cell and produced sugars. The increased solutes decreases the water potential of guard cell creates water to enter from epidermal cell. The entry of water makes the guard cell become turgidto open the stomata.At night, the sugar is converted into starch and osmotic potential of the guard cell is reduced and stomata are closed.

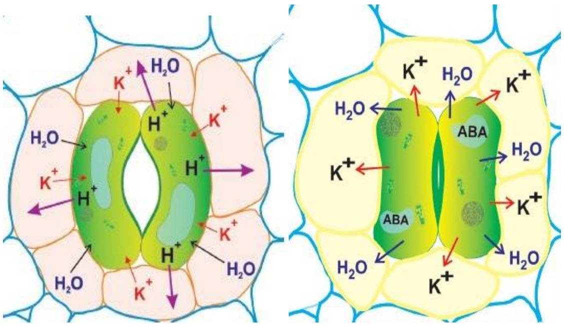
2. Starch – Sugar Inter-conversion Theory – Steward Theory

Classical theorybased on effect of pH on starch and the activity of phosphorylase enzyme. In guard cells, the insoluble starch undergo phosphorylation in the presence of light with high pH which covert the insoluble starch intosoluble form of glucose-1-PO_4. The soluble form decreases the water potential in guard cells make thewater to enter in guard cell from epidermal cell. Guard cell becomes turgid cause the opening of stomata. Theinsoluble form of starch during dark with low pH hold higher water potential cause the water to comes out from the guard cell. Guard cell becomes flaccid cause the closer of stomata.



3. Theory of Proton Transport

The synthesis of Malic acid ($C_3H_5O_3COOH$) during photosynthesis in day time dissociates and produce malate $C_3H_5O_3COO^-$ and Proton (H^+) in guard cell. The Proton (H^+) is released out from the guard cell and cause Potassium (K^+) and Cl^- to enter in the guard cell. The K+ and Cl^- were act as osmolytes and decrease the water potential in guard cell. The process lead to entry of water into the guard cell makes turgid to open stomata. During Night, the process is reversed due to nil photosynthesis which causes the release of K+ and Cl^- and increases the water potential in guard cell. The water comes out from the guard cells become flaccid and closes the stomata.



4. Factors affecting transpiration

1. Light: Light stimulates stomata and speed-up the transpiration by warming the leaf. Hence, the opening of stomata is rapid in the light than in dark.

2. Temperature: Plants transpire at faster rate in higher temperatures due to increased water evaporation. Transpiration is always directly proportional to the temperature.

3. Humidity: The rate of diffusion of any substance increases as the difference in concentration of the substances in the two regions increases. When the surrounding air is dry, diffusion of water out of the leaf goes on more rapidly. Hence lower humidity leads to higher transpiration.

4. Wind: When there is no breeze, the air surrounding a leaf becomes increasingly humid thus reducing the rate of transpiration. When a breeze is present, the humid air is carried away and replaced by drier air.

5. Soil water: A plant cannot continue to transpire rapidly if its water loss is not made up by replacement from the soil. When absorption of water by the roots fails to keep up with the rate of transpiration, loss of turgor occurs, and the stomata close. This immediately reduces the rate of transpiration (as well as of photosynthesis). If the loss of turgor extends to the rest of the leaf and stem, the plant wilts. The volume of water lost in transpiration can be very high. It has been estimated that over the growing season, one acre of corn plants may transpire 400,000 gallons of water. As liquid water, this would cover the field with a lake 15 inches deep. An acre of forest probably does even better.

6. Advantages of transpiration

1. Role of movement of water

Transpiration has important role in upward movement of water *i.e.*Ascent of sap in plants and absorption and translocation of mineral salts. Absorption of water and mineral salts are entirely independent process. Therefore transpiration has nothing to do with the absorption of mineral salts. However, once mineral salts have been absorbed by the plants, their further translocation and distribution may be facilitated by transpiration through translocation of water in the xylem elements.

2. Role of regulation of temperature

Some light energy absorbed by the leaves is utilized in photosynthesis; rest is converted into heat energy which raises temperature of plants. Transpiration plays an important role in regulating the temperature of the plants. Rapid evaporation of water from the aerial parts of the plant through transpiration brings down their temperature and thus prevents them from excessive heating.

7. Transpiration as a necessary evil

1. When the rate of transpiration is high and soil is deficient in water, an internal water deficit is created in the plants which may affect metabolic processes

2. Many xerophytes have to develop structural modification and adaptation to check transpiration.

3. Deciduous trees have to shed their leaves during autumn to check loss of water.

In spite of the various disadvantages, the plants cannot avoid transpiration due to their peculiar internal structure particularly those of leaves. Therefore, many workers like Curtis (1926) have called transpiration as necessary evil.

Antitranspirants

A substance sprayed on plant leaves to reduce the rate of transpiration and conserve moisture.

Types of antitranspirants

a.Stomatal closing type. By spraying certain chemicals, stomata closes and hence transpirations stops.

Eg. Phenyl mercuric acetate, ABA

b.Thin film forming type: These substances form a thin film on the leaf surface

Eg. Wax, rice gruel

C.Reflective Type: The principle of using this type of chemicals is to increase the light reflection by the leaved and thus reducing leaf temperature and hence transpiration.

Eg. Kaolin, lime water spray

Guttation

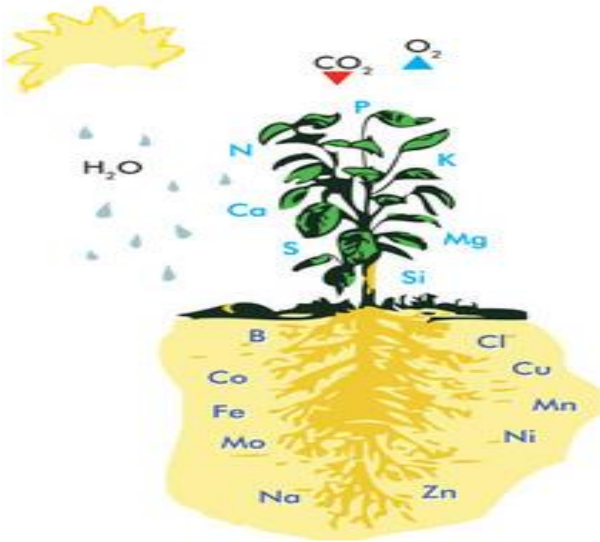
Secretion of water on to the surface of leaves through specialized pores called as hydathodes. The process occurs most frequently during conditions of high humidity when the rate of transpiration is low. Drops of water found on grass in early morning are often the result of guttation, rather than dew. Sometimes the water contains minerals in solution, such as calcium, which leaves a white crust on the leaf surface as it dries.

Lecture 6: Mineral nutrition of plants – criteria of essentiality - classification of nutrients – macro, micro, mobile and immobile – beneficial elements, mechanism of nutrient uptake

1. MINERAL NUTRITION OF PLANTS

Mineral Nutrients are elements acquired primarily in the form of inorganic ions from the soil. The chemical compounds required by an organism are termed as nutrients. The study of how plants obtain, translocate, and consume the mineral nutrients for their biological functions to synthesize organic compounds called as mineral nutrition**.**

Plant tissue analysis contains all essential and non essential elements. An essential element is one whose absence prevents a plant from completing its life cycle (Arnon and Stout 1939). Plants required seventeen (17) elements which are essential for its growth and metabolism. They are C, H, O, N, P, K, Ca, S, Mg, Fe, Mn, Zn, B, Cu, Mo, Ni and Cl.



2. Arnon's Essentiality criteria of elements

1. Plant unable to complete its life cycle in the absence of particular element

2. The functions of an element must not be replaceable by another element

3. The element must be directly involved in plant metabolism

Root takes up Nutrients - Ions

Ion is an atom or molecule which has lost or gained one or more electrons, making it positively or negatively charged

Cation:

Positively charged ion

H^+ NH_4^+ K^+ Ca^{2+}

Mg^{2+} Cu^+ Cu^{2+}

Fe^{2+} Fe^{3+} Mn^{2+}

Zn^{2+} Co^{2+} Ni^{2+} Na^+

Anion:

Negatively charged ion

NO^{2-} NO_3^-

$H_2PO_4^{3-}$ HPO_4^{2-}

SO_4^- $H_2BO_3^-$

Cl^- MoO_4^{2-}

Clay Particles

Organic Matter

3. Three classifications of essential elements

a. **Classification based on amount required or amount present in the tissue**

These essential elements are classified into two groups

1. Major elements (Macro nutrients)

2. Micro nutrients (Trace elements)

1. Major elements

The essential elements which are required by the plants in comparatively large amounts are called as major elements or macro nutrients. They are C, H, O, N, P, K, Ca, S, Mg. In this C, H, O, N, P and K are called non mineral obtained primarily from water or carbon dioxide elements and Ca, S, Mg are called as mineral elements from soils.

2. Micro elements

The essential elements which are required in very small amounts or traces by the plants are called as minor elements or micronutrients or trace elements. They are Fe, Zn, Mn, B, Cl, Cu and Mo.

b. Classification based on mobility inside the plant system

Based on the mobility, elements are also classified into three types.

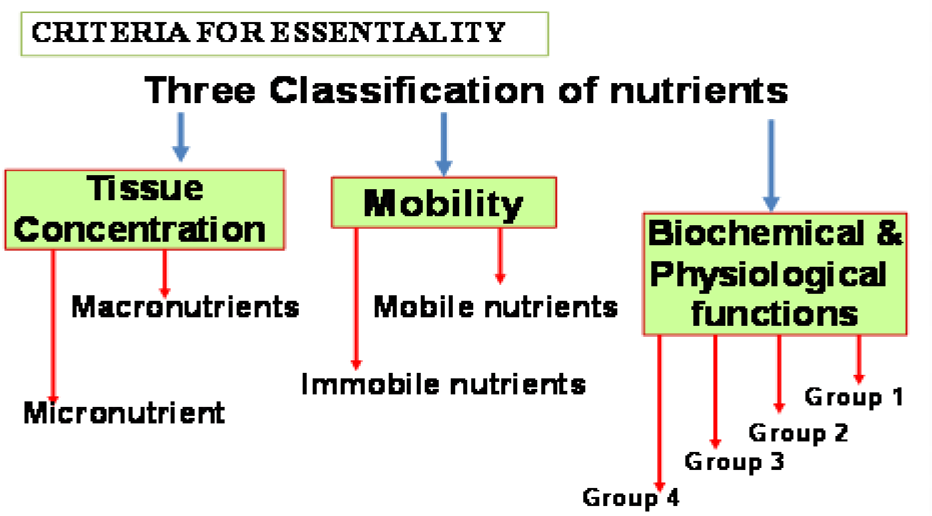
1. Mobile elements : N, P, K, S and Mg

2. Immobile elements : Ca, Fe and B

3. Intermediate in mobility : Zn, Mn, Cu, Mo

c**. Classification based on biochemical function**

1. Group 1 – major constituent of organic material - N and S
2. Group 2 – Nutrient important for energy storage or structural integrity – P and B
3. Group 3 – Nutrient that remain in ionic form – K, Ca, Mg, Cl, Mn and Na
4. Group 4 – Nutrient that are involved in redox reaction – Fe, Zn, Cu, Ni and Mo



4. Beneficial Elements

Elements which promote plant growth in particular or many plant species but are not absolutely necessary for completion of the plant life cycle, or fail to meet Arnon and Stout's criteria **Eg: Sodium, Silicon, Selenium, & Cobalt.**

5. Mechanism of nutrient uptake

It is a two step process: 1. Adsorption 2. Absorption

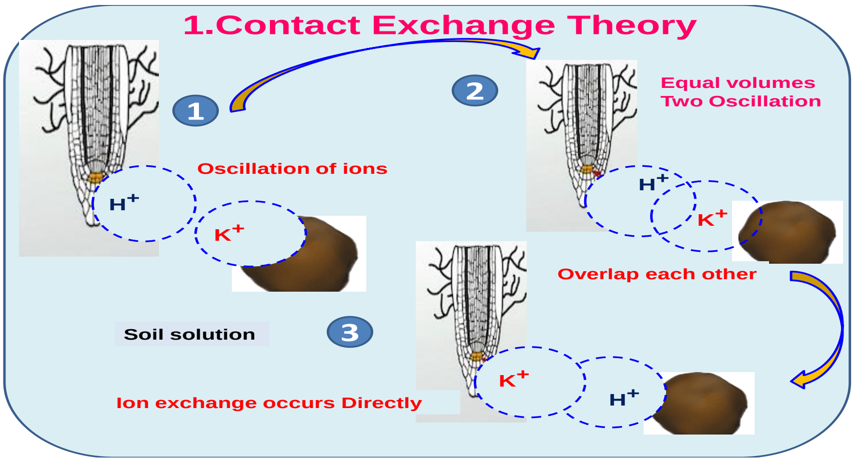
1. Mechanism of nutrient Adsorbtion:

I. **Adsorption** is a process of ion exchange between the roots surface and soil solution is known as ion exchange. The ion exchange is discussed in three theories. They are

1. Contact Exchange Theory
2. Carbonic Acid Exchange Theory
3. Donnan Equilibrium

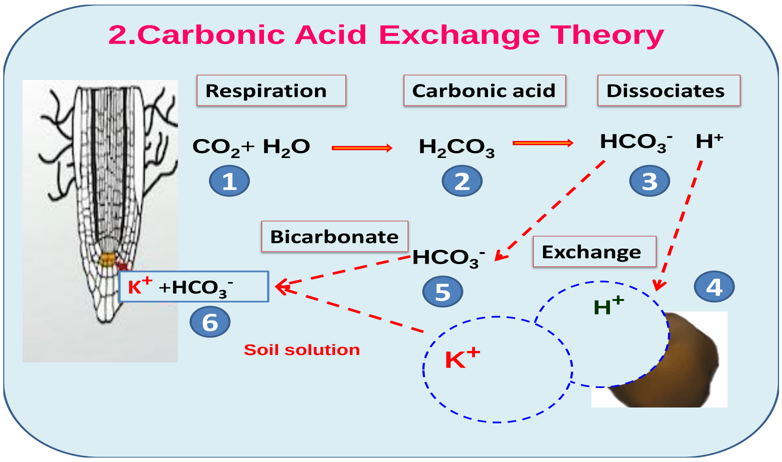
1. Contact Exchange Theory

The ions in soil solution are usually in oscillation at their respective adsorbed surface of the roots and soil particles. When the roots are in contact with soil particles, the two oscillated ions from the root surface and soil particle are overlapped each other under equal volume and ions are exchanged directly without the influence of soil solution.



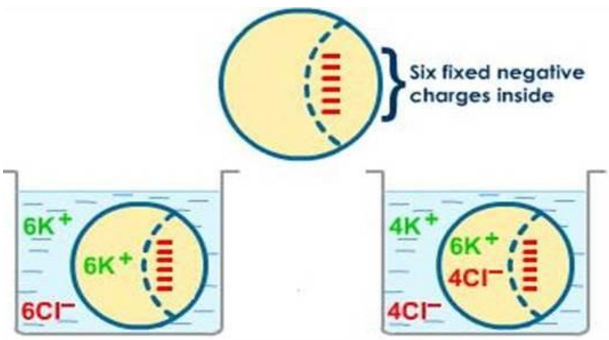
2. Carbonic Acid Exchange Theory

The carbon-dioxide (CO_2) is released during the respiration of roots under soil solution. The CO_2 under soil solution formed the carbonic acid (H_2CO_3) by reacting with the soil water. The carbonic acid then is dissociated in the soil solution as bicarbonate **(**HCO_3^-) and hydrogen ions **(**H^+) The released hydrogen ion is adsorbed by the soil particle and liberates the cation in to the soil solution. The liberated cations is taken by the bicarbonates as K^+ $+HCO_3^-$ adsorbed by the root surface.



3. Donnan's Equilibrium

Fixed or non-diffusible ions are accumulated on the inner surface of the outer membrane of the cell during the ion exchange. Due to this, additional load of cation / anion adsorbed to the inner surface of cell forced to maintain the equilibrium in the external solution. Hence, this process leads to increase the inside concentration of cation or anion and maintain the equilibrium in the external solution.



2. Mechanism of Nutrient Absorption:

1. Active Absorption

a. Carrier Concept Theory

b. Cytochrome Pump Theory

2. Passive Absorption

a. Mass Flow Theory

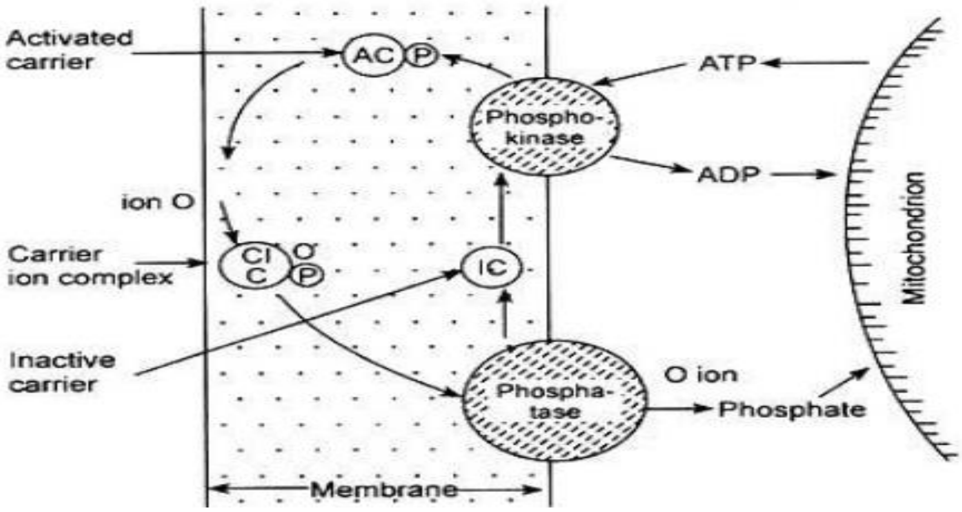
1. Active Absorption:

Absorption of mineral nutrients is against the concentration gradient by spending metabolic energy.

a. Carrier Concept Theory

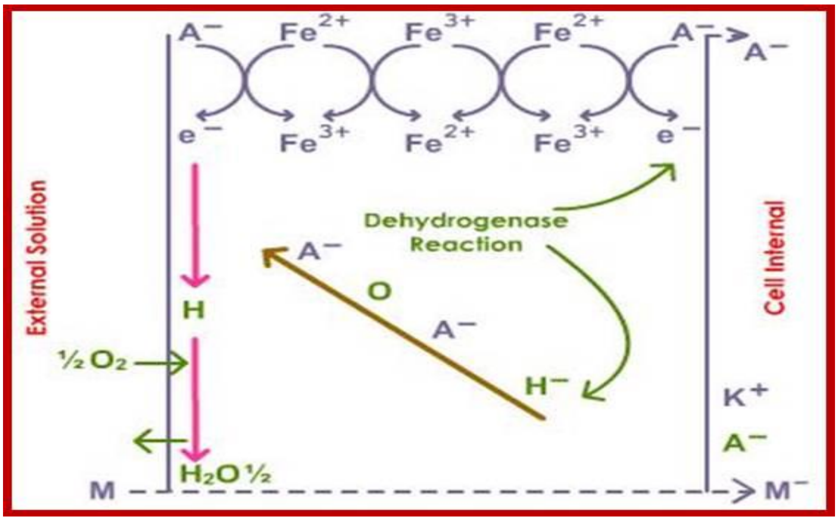
The nutrient absorption takes place by a precursor present in the plasma membrane. The nutrients placed at the outside of the plasma membrane required certain transporter to diffuse inside the plasma membrane in order to reach the cytoplasm of cell. Precursor present in the plasma membrane act as carrier ion complex which carry the ions from outer membrane to inner side. The metabolic energy is required for the precursor to perform as a carrier.

Bennet – Clark (1956) has proposed the Carrier concept theory through Protein - Lecithin structure. The carrier is a protein which associated with phosphatide and choline called as lecithin. Lecithin protein structure acted as two groups to carry the ions. Phosphatide is one gruop act as a cation binding centre and choline is another for anion binding centre. The enzymes choline acetylase, and choline esterase in the cation binding centre converts the Phosphatide into phosphatidic acid. Likewise, Lecithinase converts choline in anion binding centre. The phosphatidic acid and choline bind the cation and anions are transported from external to inside by utilizing the metabolic energy.



a) Cytochrome Pump Theory

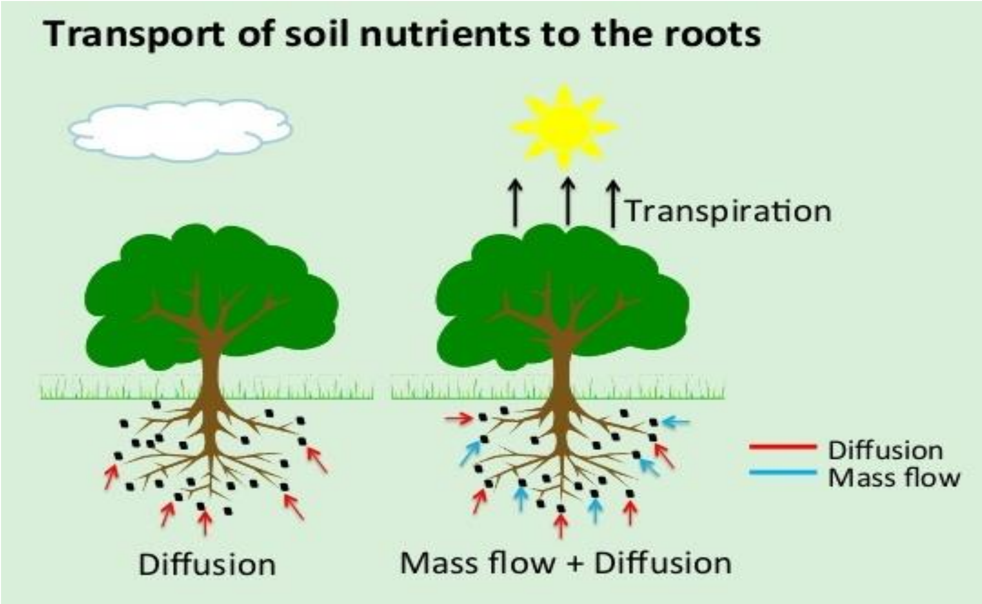
Lundegardh and Burstrom (1933) have proposed Cytochrome pump theory. Cytochrome is a heme protein act as important intermediate in electron transport system of chloroplast and mitochondria. During the oxidation of ferrous ion (Fe^{2+}) into ferric, the anions are transported across the membrane by cytochrome system.The continuous oxidation process actively absorbed the anions via cytochrome pump in to the cell by utilizing the metabolic energy supplied through dehydrogenase reaction from respiration. Cations are passively absorbed into the memberane and they are transported into the cell.



2. Passive Absorption

a) Mass Flow Theory

It is a physical absorption of nutrients based on the concentration gradient by diffusion. Rapid uptake of ions occurs when the root tissues are transferred from a medium of low concentration to high concentration. The nutrients are absorbed by the mass flow of water under the influence of transpiration. Nutrients are absorbed passively by the roots without the expenditure of metabolic energy.



Lecture 7: Physiological functions and disorders of macronutrients, Hidden hunger

Physiological roles of essential mineral nutrients - Macronutrients

1. **Nitrogen**

Important constituent of amino acids, proteins, nucleic acids, coenzymes, hormones, cytochromes, alkaloids and chlorophylls. Involved in Protein synthesis, Photosynthesis, Respiration, Growth and Reproduction

Deficiency symptoms

- Symptoms appears first at older Leaves
- Complete Yellowing of leaves (Chlorosis)
- Stunted growth
- General Starvation

2. **Sulphur**

Component of Amino acids : Cystine, Cysteine & Methionine and proteins. Constituent of Vitamins : Biotin & Thiamine.Coenzyme A & Fd, glutathione, catalyst in Protein. Sulphur helps to stabilize the protein structure and photosynthesis

Deficiency symptoms

• Deficiency causes chlorosis of the leaves

• Tips and margins of the leaf roll in ward

• Stem becomes hard due to the development of sclerenchyma.

3. **Phosphorus**

Important component of sugar phosphates, nucleic acids, nucleotides, coenzymes, phospholipids, phytic acid, coenzymes NADP, $NADPH_2$ and ATP etc., Key role in protein synthesis and ATP production. Helps in better function of membranes, energy transfer reactions of cell Metabolism. Eg. Photosynthesis, respiration and fat metabolism etc.,

Deficiency symptoms

• P deficiency may cause premature leaf fall

• Dead necrotic areas are developed on leave or fruits

• Leaves may turn to dark green to blue green colour. Sometimes turn to purplish colour looks like rusted pustules due to the synthesis and accumulation of anthocyanin pigments.

• Sickle leaf disease

4. **Potassium**

Principal cation for cell turgor and regulation of osmotic potential and cell electro neutrality. Co-factor of more than 40 enzymes. It is an activator of enzyme like DNA polymerase. It also act as a activator for many enzymes involved in carbohydrate metabolism, protein synthesis, respiration and photosynthesis. It involves in stomatal movement and regulates water balance. Important for phloem translocation

Deficiency symptoms

• Mottled chlorosis of leaves occurs

• Neurotic areas develop at the tip and margins of the leaf (marginal necrosis)

• Plants growth remains stunted with shortening of internodes.

• Deficiency causes easy lodging in cereals

• Deficiency leads to increase in pest and diseases

5. **Calcium**

It is important constituent of cell wall and It is essential in the formation of cell membranes. It helps to stabilize the structure of chromosome, involved in the hydrolysis of ATP (ATPase) and phospholipids. Acts as a second messenger in metabolic regulation and calcium protein (Calmodulin) complex regulates metabolic processes

Deficiency symptoms

• Disintegration of growing meristematic regions of root, stem and leaves

• Chlorosis occurs along the margins of the younger leaves

• Malformation of young leaves takes place

• Blossom end rot in tomato

6. **Magnesium**

Important constituent of chlorophylls. Required by many enzymes involved in phosphate transfer. It activates many enzymes in nucleic acid synthesis and carbohydrate metabolism. It plays important role in binding ribosomal particles during protein synthesis.

Deficiency symptoms

• Mottled chlorosis with veins green and leaf tissues yellow or white

(Interveinal Chlorosis) appearing first on older leaves

• Dead neurotic patches appear on the leaves

• Reddening of cotton leaves

Hidden Hunger

In some instances, a nutrient element insufficiency may be such that no symptoms of stress will visually appear with the plant seeming to be devel-oping normally. This condition has been named hidden hunger, a condition that can be uncovered using either a plant analysis and/or tissue test. A hidden hunger occurrence frequently affects the final yield and the quality of the product produced. For grain crops, the grain yield and quality may be *less than expected; for fruit crops, abnormalities, such as blossomed rot and internal abnormalities may occur, and the post-harvest characteristics of fruits and flowers will result in poor shipping quality and reduced longevity. Another example is potassium (K) insufficiency in corn, a - deficiency that is not evident until at maturity when plants easily.*

Lecture 8 - Physiological Functions and Disorders of Micronutrients

Iron:

Function:

Iron *is necessary for many enzyme functions and as a catalyst for the synthesis of chlorophyll. Iron is an important constituent of iron porphyrin – proteins like cytochromes, ferridoxin, plastocyanin. Iron is Co-factor of enzymes viz., peroxidase, catalases, dehydrogenase etc. Iron is essential for the young growing parts of plants and it lost by leaching process to held in the lower portions of the soil structure. Iron is unavailable to plants under alkaline condition even it is abundant.and acid soil causs toxic symptom. Applications of an acid nutrient formula containing iron chelates, held in soluble form, should corrects the problem.*

Deficiency:

Iron deficiency causes chlorosis of young leaves which is usually interveinal. Indicator crop is sugar cane. During initial stages chlorosis and severe deficiency causes papery white coloured leaves.

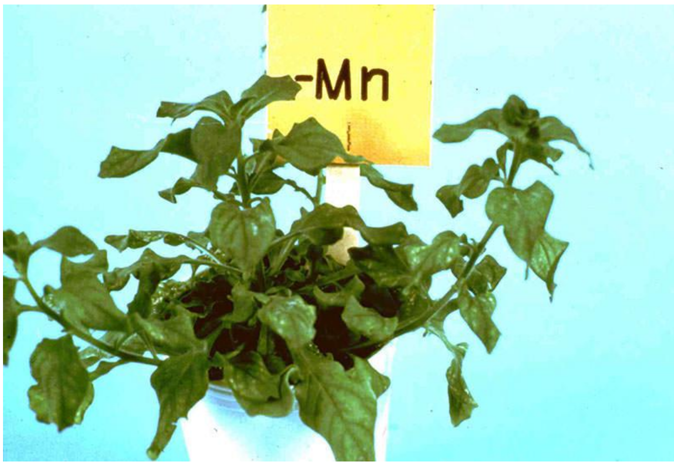
Manganese:

Function:

Manganese *is involved in enzyme activities of photosynthesis, respiration, and nitrogen metabolism processes. It is an activator of many respiratory enzymes. It is also an activator of the enzyme nitrite reductase. It is necessary for the evolution of oxygen (photolysis) during light reaction of photosynthesis. It is Co-factor of carboxylase and decarboxylase. In alkaline soils plants often show deficiency symptoms. In highly acid soils, manganese may be available to the extent that it results in toxicity.*

Deficiency:

The young leaves are affected by mottled chlorosis. Small necrotic spots developed on the leaves with yellow strips. Grain formation reduced and heads may be blind (as in sulphur). Pahala blight of sugar cane; Chlorotic spots develop as long streaks in young leaves. These spots fuse together and turn red and coalesce to form long streaks from which lamina may split. Marsh spot of pea: Brown spots or cavities develop on the internal surface of cotyledon and thus disorders appear in the seeds.Speckled yellow of sugar beet: interveinal chlorosis in the leaves and leaf margin may curl upward over the upper surface of leaf. The indicator crop is sesamum.



Boron:

Function:

Boron is necessary for cell wall formation, membrane integrity, calcium uptake and may aid in the translocation of sugars. Boron is essential for pollen germination, growth of pollen tube, fruiting, cell division, lignification, carbohydrate metabolism, RNA metabolism, respiration, indole acetic acid (IAA) metabolism, phenol metabolism, membrane integrity, water relationships and the movement of hormones. Boron has to available throughout the life of the plant. It is highly immobile and easily leached from soils.

Deficiency

Boron deficiency causes death of shoot tip, Flower formation is suppressed, Root growth is stunted. The boron deficiency causes drop of buds, flowers and developing fruits. The other disorders caused by B deficiency is heart rot of beet, Stem crack of celery, brown heart of cabbage, browning of cauliflower, water core of turnip, Internal cork formation in apple, Hen and chicken disorder in grapes, button shedding and barren nut in coconut, hard fruit in guava, top sickness in tobacco, hallow spot in peanuts. Leaves are thick, curled and brittle. Fruits, tubers and roots are discolored, cracked and marked with brown spots. In boron-deficient fleshy fruits, the quality may also be severely affected by malformation (eg. 'internal cork' in apple') or, in citrus, by a decrease in the pulp/peel ratio.

Zinc
Function:

Zinc is a component of enzymes or a functional cofactor of a large number of enzymes and plant growth hormones like auxins. It is essential for carbohydrate metabolism, protein synthesis and internodal elongation (stem growth). It act as activator of carbonic anhydrase, carboxy peptidase and alcohol dehydrogenase, CuZn – superoxide dismutase (antioxidative enzyme) etc. Zn is required for maintenance of integrity of biomembranes. Deficiency occurs on eroded soils and soil pH range of 5.5 - 7.0. Lowering the pH can render zinc more available to the point of toxicity.

Deficiency

Zinc deficiency causes chlorosis of the young leaves which starts from tips and the margins. The size of the young leaves is very much reduced. This disorder is called as 'little leaf disease' which cause rosette appearance (reduced leaf size, shortened inter node, chlorosis). Stalks will be very short. Indicator crop is brinjal and mango. Kaira disorder in paddy in which the entire old leaves show rusty brown appearance due to chlorosis and ultimately die.White bud of maize (tip), unfolded newer leaves are often pale yellow to white.

Deficient plants have mottled leaves with irregular chlorotic areas. Zinc deficiency leads to iron deficiency causing similar symptoms.

Copper
Function:

Copper is concentrated in roots of plants and plays a part in nitrogen metabolism. It is a component of several enzymes and may be part of the enzyme systems that use carbohydrates and proteins. It is the metal component of plastocyanin, which function in electron transfer and ascorbate oxidatase, phenol oxidases and cytochrome oxidase which catalyze terminal oxidation in mitochondria. The Cu atom in thr CuZnSOD is directly involved in mechanism of detoxification of of free radical (Super oxide) generated in photosynthesis. It is essential for liginification of cell wall. Copper is bound tightly in organic matter and may be deficient in highly organic soils. It is not readily lost from soil but may often be unavailable. Too much copper can cause toxicity.

Deficiency

Copper deficiency causes leaf distortion, necrosis of the tip of the young leaves, stunded growth followed by a failure of the plants to set seed. It also causes die-back (exanthema) of citrus and fruit trees. Also causes reclamation disease or white tip (bleaching of young leaves) disease of cereals and leguminous plants. The indicator crop is banana.

Molybdenum
Function

In higher plants only a few enzymes have been found to contain molybdenum as a cofactor. In these enzymes molybdenum has both structural and catalytical functions and is directly involved in redox reactions. These enzymes are nitrate reductase, nitrogenase, xanthine oxidase/dehydrogenase and presumably, sulfite reductase. The functions of molybdenum are therefore closely related to nitrogen metabolism and the molybdenum requirement strongly depends on the mode of nitrogen supply. Without it, the synthesis of proteins is blocked and plant growth ceases. Seeds may not form completely, and nitrogen deficiency may occur if plants are lacking molybdenum.

Deficiency:

Molybdenum deficiency causes whiptail disorder and inhibit flower formation in cauliflower plants, produce necrotic spots on lower surface of leaves in citrus, scald of legumes(leaf shows paling, wilting and marginal rolling).

Chlorine

Function

In higher plants more than 130 chlorinated organic compounds have been found. Water-splitting system of photosystem II (PSII) requires chlorine for O_2 evolution during light reactions of photosynthesis. The activation of Tonoplast Proton-Pumping ATPase is enhanced by chlorine. This pump is essential for pH regulation of cytosol and ion uptake in roots. Chlorine is essential for stomatal regulation. Chlorine is involved in osmosis (movement of water or solutes in cells), the ionic balance necessary for plants to take up mineral elements and in photosynthesis. Chloride, the ionic form of chlorine used by plants, is usually found in soluble forms and is lost by leaching. Some plants may show signs of toxicity if levels are too high.

Deficiency

Deficiency symptoms causes wilting of leaves, especially at leaf margins, stubby roots (subapical swelling and enhanced formation of short laterals), chlorosis (yellowing) and bronzing. In severe deficiency curling of youngest leaves followed by shriveling and necrosis.Odors in some plants may be decreased.

Lecture 9 - Foliar Nutrition - Root Feeding & Fertigation – Sand Culture, Hydroponics & Aeroponics

Foliar Nutrition

Foliar nutrition refers to the application of nutrients on the leaves. Usually 500 litres of spray solution are needed per hectare. Foliar spray can be repeated 2 or 3 times at 15-20 days intervals in order to control and correct the nutritional deficiency in crops.

Mechanism

Penetration of nutrient solution occurs through cuticle, the layer of polymerized wax which occurs on outer surface of the epidermal cells of leaves.Further penetration take place through fine, thread like semi-microscopic structure called ectodesmata. This extends through the outer epidermal cell wall, from the inner surface of the cuticle to the plasma membrane.Once the substance reaches plasma membrane of an epidermal cell, it will be observed by mechanism similar to those which operate in root cells' nutrient absorption.

Advantages

1. *Foliar nutrition may serve as a mean of applying supplemental macronutrients during critical growth periods when it is impracticable to apply fertilizers to soil. Eg. Unusual period of dry weather.*
2. *Foliar nutrition may afford remedy for the time lag between the nutrients applied in soil and absorbed by the plant.*

Necessity for Foliar spray

Foliar application of nutrients is more successful than soil application of nutrients under the following circumstances.

- *Short interval in preparation of land*
- *Sloppy land*
- *Sandy soil*
- *Thick growth of the crop which renders to the soil difficult*
- *Highly acidic and alkaline soil*
- *Under drought conditions*
- *In temperature and rainfed zones*
- *When fertilizer are costly and in short supply*
- *When soil conditions or competitions crops make nutrients from the soil unavailable to the crop.*
- *When deficiency is found in the later stage of growth*
- *When it is desired to improve quality*
- *When fertilizer is to be applied at a particular time*

Points to be followed for increasing effectiveness of foliar spray of nutrients

1. *Do not exceed the recommended solution concentration*
2. *Use 500 litresof spray solution per hectare*
3. *Whenever necessary, neutralize the solution with lime.*
4. *Use only clean filtered solutions to avoid blocking in the nozzle.*
5. *Repeat sprays as when recommended*
6. *Avoid spray during rainy condition and rain expected*
7. *Double check calculations used for preparing the final solutions*
8. *Do not store spray solutions, particularly of iron which prefer freshly made solutions*
9. *Use a wetting agent to improve effectiveness of spray @ 1 ml per litre of spray fluid ie.*

Tween 20, Sandowit, Teapol or liquid soap.

Absorption

Once the element has entered the leaf if may or may not be moved or translocated readily to other parts of the plant. The rate of entry of nutrients into the leaf varies considerably as indicated below.

Nutrient	***Time for 50% absorption***
Nitrogen	*½ - 2 hours*
Magnesium	*2-5 hours*
Potassium	*10-24 hours*
Calcium	*1-2 days*
Manganese	*1-2 days*
Zinc	*1-2 days*
Phosphorus	*5-10 days*
Iron	*10-20 days*
Molybdenum	*10-20 days*

ROOT FEEDING

Root feeding is a method of fertilizing or feeding a tree that injects beneficial fertilizer directly to the roots. Root feedingcan help in preventing against insects that like to feed on roots. Root feeding also helps to maintain good tree growth and prolongs the life of trees by offering the other nutrients not found in the soil alone.

Necessity for root feeding

The farmers are giving nutrients through organic manure or some ash, lime, but most of the times secondary and micronutrients are not given to the palms. This resulted in a button shedding, barren nuts, pencil tip, loss of vigour, sensitive to pest and disease attack and finally reduce the yield.

Advantages of Root Feeding

1. *Maintaining good healthy palms resulting with disease and pest free.*
2. *Root feeding leads to enhance crop production and crop productivity.*
3. *Root feeding technique is well suited for monocot tress*

How to do the root feeding

1. *Dig a pit of one feet depth at 3-4 feet away from the main trunk*
2. *Select the youngpencil thickness root from root zone*
3. *Cut the root tip slantingly*
4. *Insert the packet contain nutrient solution at the one end and tie the packet along with root tightly.*
5. *The packet has to be removed after the solution has been absorbed by the tree root.*

Points to be followed for increasing the effectiveness of root feeding of nutrients

1. *The irrigation to be withheld for three to four days prior to root feeding.*

2. *The appropriate (pencil thickness with pink / cream color in coconut palm) root should be selected*
3. *Undamaged root should be used for root feeding*

Fertigation

Fertigation is the application of fertilizers, soil amendments, or other water-soluble products through an irrigation system.

Practical Usage in Agriculture and Horticulture Crops

- *Fertigation is used extensively in commercial agriculture and horticulture work and it started working in general landscape applications.*
- *Fertigation is used to spoon-feed additional nutrients or correct nutrient deficiencies detected in plant tissue analysis.*
- *Usually practiced with high-value crops such as vegetables, turf, fruit trees and ornamentals*
- *Injection during middle one-third or the middle one half of the irrigation recommended for fertigation using micro propagation*
- *Water supply for fertigation kept separate from domestic water supply to avoid contamination.*

Commonly used nutrients

* *Most plant nutrients can be applied through irrigation systems.*
* *Nitrogen is most commonly used nutrient.*
* *Other nutrients include nitrate, ammonium, urea, phosphate and potassium*
* *TNAU Liquid fertilizers are most suited for this technique.*

Determining the nutrient to be used in fertigation

- *A soil fertility analysis is used to determine*
- *The more stable nutrients should be used.*

Advantagesover the traditional broadcast or drop-fertilizing methods

- *Increased nutrient absorption by plants with reduction in solid fertilizer requirement.*
- *Reduced leaching to the water table*
- *Reduction in water usage due to the plant's resulting increased root mass's ability to trap and hold water*
- *Application of nutrients can be done at the precise time and quantity they need.*

Disadvantages

- *Concentration of solution decreases as fertilizer dissolves, leading to poor nutrient placement, results in pressure loss in main irrigation line*
- *Limited capacity of fertigation.*
- *High cost of liquid fertilizers*

Methods used in fertigation

- *Drip irrigation, which reduces per water and nutrient application rates relative to sprinklers*
- *Sprinkler systems, which increase leaf and fruit quality*

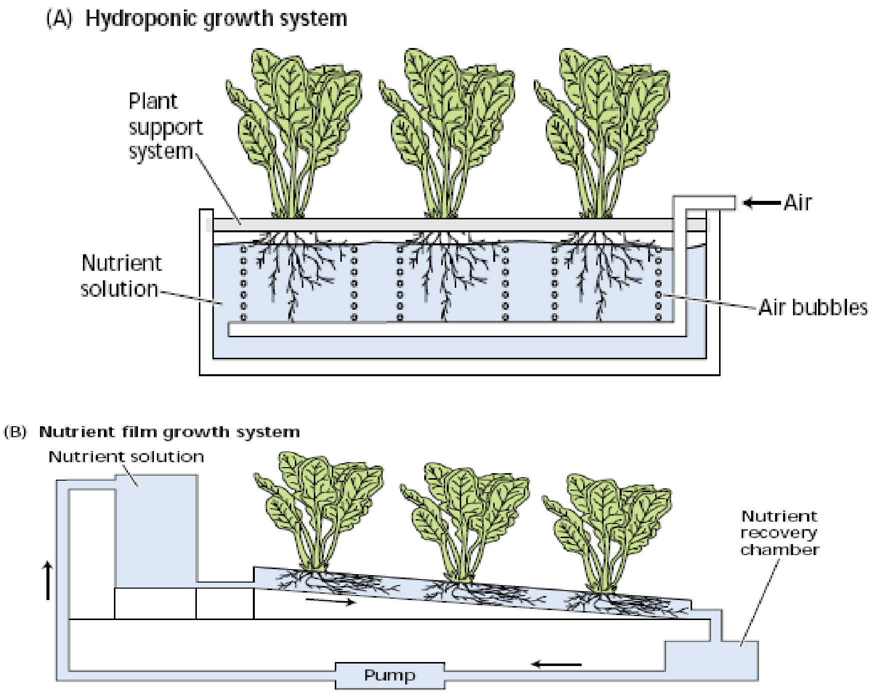
Sand culture

Sand culture is a method of growing plants hydroponically, without the use of soil. It is a variation of gravel culture where the sand, which is used primarily to anchor plants in the grow bed or tray, is a lot finer than the gravel grow medium option.Sand culture is also known to be one of the most affordable type of soilless growing methods due to the abundance of sand on the planet and the fact that it can be re-used over and over again. However, due to its high salt content, the sand must be treated before use.

Sand culture is thought to be more efficient than traditional hydroponic methods because the sand largely decreases the risk of botanical ailments such as Verticilium *and* Fusarium. *Because the plants receive fresh nutrient solutions after each watering cycle, they also tend to remain healthy, with no nutrient imbalances.*

SOIL LESS GROWTH OR HYDROPONICS

The practice of growing plants in nutrient enriched water without soil is called as soil less growth or hydroponics. However,thetermhydroponicsisnowbeingappliedtoplants rootedinsand,gravelorothersimilarmatterwhichissoakedwitharecyclingflowof nutrient – enriched water.



According to a recent limited nations report on hydroponics: In area of tropics, where the water deficiency is the limiting factor in crop production, the soil less methods hold out much promise because of the more economical use of water.

The report also indicated that in some areas, lack of fertile soil or very thin soil layers may also move soil less methods worth serious consideration.

Vegetables grown hydroponically under controlled environment are cucumbers, egg plants, peppers, lettuces, spinach.

Advantages of hydroponics farming

1. *The regulation of nutrients*
2. *Control of pests and diseases*

3. *Reduction of labour cost*

4. *Sometimes quicker yield*

***Drawbacks of hydroponics farming**.*

1. *Firstly the cost of settling up the system is very high*

2. *Secondly it requires skills and knowledge its operation*

AEROPONICS FARMING

- *Aeroponics is the process of growing plants in an air or mist environment without the use of soil or an aggregate medium (known as geoponics) .*
- *The word "aeroponic" is derived from Greek meaning of air and pones.*
- *Aeroponic culture differs from both conventional hydroponics, aquaponicsand in-vitro (plant tissue culture) growing.*
- *Unlike hydroponics which uses a liquid nutrient solution as a growing medium and essential minerals to sustain plant growth, aquaponics which uses water and fish waste , aeroponic .*

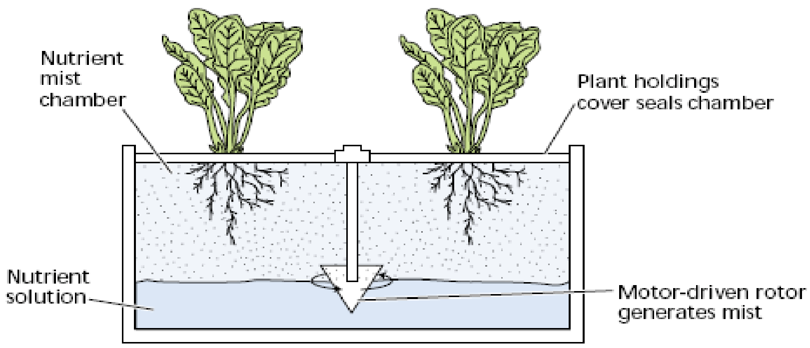
Methods

- *The basic principle of aeroponic growing is to grow plants suspended in a closed environment by spraying the plants dangling roots and lower stem with an atomized or sprayed , nutrient – rich water solution.*
- *The leaves and crown , often called canopy, extend above .*
- *The roots of the plant are separated by the plant support structure.*
- *Often, closed cell foam is compressed around the lower stem and inserted into a opening in the aeroponic chamber , which decreases labour and expense for larger plants , trellising is used to suspend the weight of vegetation and fruit .*

Benefits of aeroponics

- *It uses considerably less energy and water than traditional agriculture*
- *Since, air act as a medium to grow plants considerably less maintenance is needed.*
- *In this system, the plant roots are exposed to sufficient oxygen and they can easily absorb it.*

(C) Aeroponic growth system



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Lecture 10 - Light reaction of Photosynthesis - Photolysis of water and Photophosphorylation – Z scheme

Photosynthesis (photo- light, synthesis- building block) is a physiological process, in which carbohydrate is synthesized by aerial green parts of the plants by trapping solar energy, taking CO_2 from atmosphere and water from soil. Green plants trap solar energy by their photosynthetic pigments. Here, solar energy is converted into chemical energy and locked in high energy bonds. The conversion of unusable sunlight energy into usable chemical energy, is associated with the actions of the green pigment chlorophyll. Most of the time, the photosynthetic process uses water and releases the oxygen that we absolutely must have to stay alive. the overall reaction of this process as:

$$6H_2O + 6CO_2 \text{ ----------> } C_6H_{12}O_6 + 6H_2O + 6O_2$$

Six molecules of water plus six molecules of carbon dioxide produce one molecule of sugar plus six molecules of oxygen plus six molecules of water.

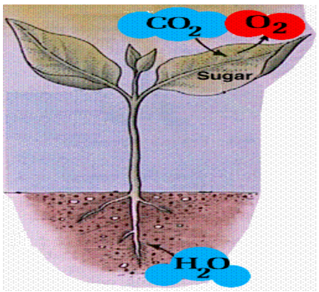


Diagram of a typical plant, showing the inputs and outputs of the photosynthetic process.

If oxygen is evolved it is called oxygenic photosynthesis (distributed in all eukaryotes and cyanobacteria) and if oxygen is not evolved, it is called anoxygenic photosynthsis (common in photosynthetic bacteria.

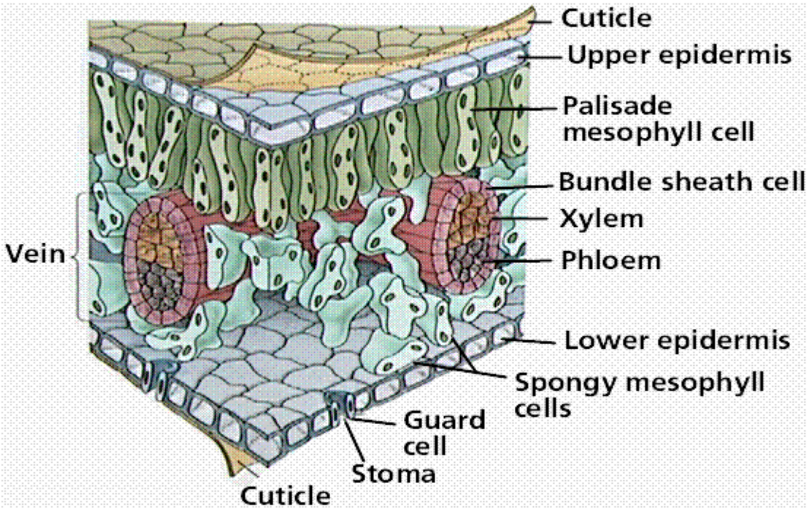
Importance of photosynthesis:

1. Photosynthesis is the monopoly of plants. They not only manufacture food for themselves but also for living world. In photosynthesis, O_2 is evolved vis-à-vis the consumption of CO_2. Thus this process purifies the air Phytoremediation as well as supplies food to entire universe (herbivores and carnivores) directly or indirectly.
2. Simple carbohydrates produced is the process are further converted into lipids, nucleic acids, proteins and other organic molecules.
3. Photosynthesis provides raw material (substrate) for respiration. Energy released by respiration is used in various metabolic processes.

Leaves and Leaf Structure

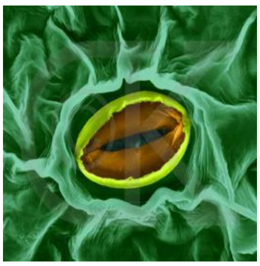
Plants are the only photosynthetic organisms to have leaves (and not all plants have leaves). A leaf may be viewed as a solar collector crammed full of photosynthetic cells.

The raw materials of photosynthesis, water and carbon dioxide, enter the cells of the leaf, and the products of photosynthesis, sugar and oxygen, leave the leaf.



Cross section of a leaf, showing the anatomical features important to the study of photosynthesis: stoma, guard cell, mesophyll cells, and vein

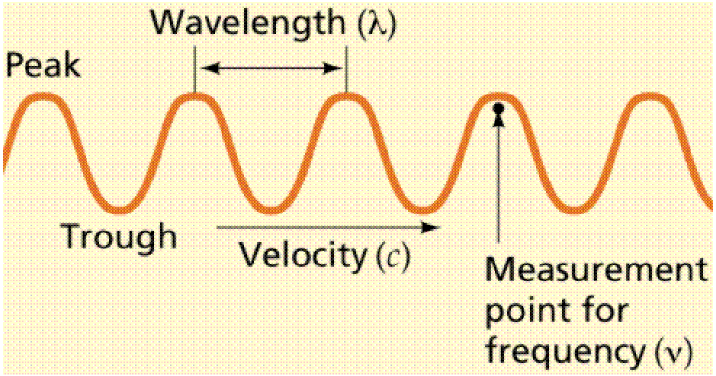
Water enters the root and is transported up to the leaves through specialized plant cells known as xylem (pronounces zigh-lem). Land plants must guard against drying out (desiccation) and so have evolved specialized structures known as stomata to allow gas to enter and leave the leaf. Carbon dioxide cannot pass through the protective waxy layer covering the leaf (cuticle), but it can enter the leaf through an opening (the stoma; plural = stomata; Greek for hole) flanked by two guard cells. Likewise, oxygen produced during photosynthesis can only pass out of the leaf through the opened stomata. Unfortunately for the plant, while these gases are moving between the inside and outside of the leaf, a great deal water is also lost. Cottonwood trees, for example, will lose 100 gallons of water per hour during hot desert days. Carbon dioxide enters single-celled and aquatic autotrophs through no specialized structures.



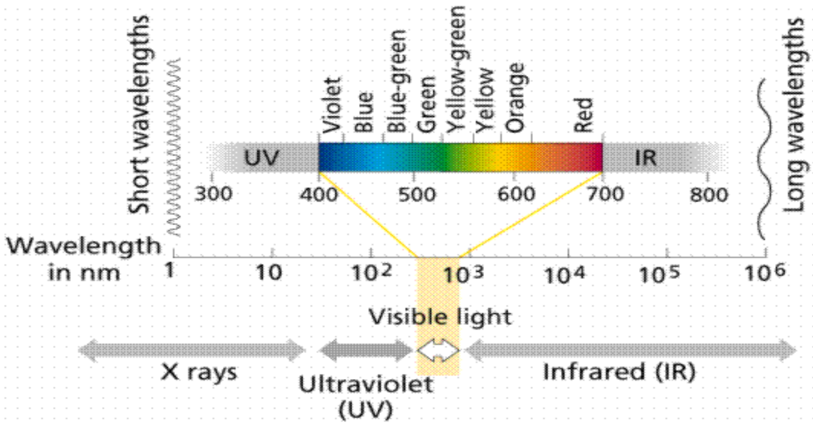
Pea Leaf Stoma, Vicea *sp.*

The Nature of Light

White light is separated into the different colors (=wavelengths) of light by passing it through a prism. Wavelength is defined as the distance from peak to peak (or trough to trough). The energy of is inversely porportional to the wavelength: longer wavelengths have less energy than do shorter ones.



The order of colors is determined by the wavelength of light. Visible light is one small part of the electromagnetic spectrum. The longer the wavelength of visible light, the more red the color. Likewise the shorter wavelengths are towards the violet side of the spectrum. Wavelengths longer than red are referred to as infrared, while those shorter than violet are ultraviolet.



Light behaves both as a wave and a particle. Wave properties of light include the bending of the wave path when passing from one material (medium) into another (i.e. the prism, rainbows, pencil in a glass-of-water, etc.). The particle properties are demonstrated by the photoelectric effect. Zinc exposed to ultraviolet light becomes positively charged because light energy forces electrons from the zinc. These electrons can create an electrical current. Sodium, potassium and selenium have critical wavelengths in the visible light range. The *critical wavelength is the maximum wavelength of light (visible or invisible) that creates a photoelectric effect.*

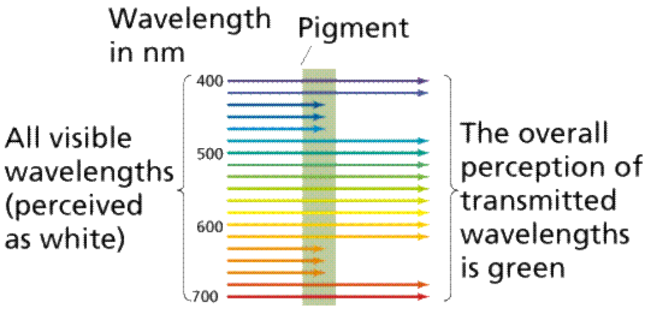
Raw materials for photosynthesis:

Raw materials for photosynthesis are:

1. *Photosynthetic pigments*
2. *Light*
3. *Carbon-dioxide*
4. *H-donor.*

Chlorophyll and Accessory Pigments

A pigment is any substance that absorbs light. The color of the pigment comes from the wavelengths of light reflected (in other words, those not absorbed). Chlorophyll, the green pigment common to all photosynthetic cells, absorbs all wavelengths of visible light except green, which it reflects to be detected by our eyes. Black pigments absorb all of the wavelengths that strike them. White pigments/lighter colors reflect all or almost all of the energy striking them. Pigments have their own characteristic absorption spectra, the absorption pattern of a given pigment.



Absorption and transmission of different wavelengths of light by a hypothetical pigment.

Chlorophyll is a complex molecule. Several modifications of chlorophyll occur among plants and other photosynthetic organisms. All photosynthetic organisms (plants, certain protistans, prochlorobacteria, and cyanobacteria) have chlorophyll a. Accessory pigments absorb energy that chlorophyll a does not absorb. Accessory pigments include chlorophyll b (also c, d, and e in algae and protistans), xanthophylls, and carotenoids (such as beta-carotene). Chlorophyll a absorbs its energy from the Violet-Blue and Reddish orange-Red wavelengths, and little from the intermediate (Green-Yellow-Orange) wavelengths.

Distribution of photosynthetic pigments:

Photosynthetic pigments (chlorophylls and carotenoids) are distributed in chloroplasts of eukaryotes and chromatophores of prokaryotes. These pigments are not throughout the *chloroplasts but are restricted to thylakoids. These pigments are found in grana lamella (grana thylakoids) as well as stroma lamella (fret thylakoids or stroma thylakoids) thylakoids are shaped membranous sacs enveloped by a unit membrane structure thylakoid membrane. Molecular organization of the thylakoid membrane is based on "fluid mosaic model" of membrane. Thylakoid membrane is ion permeable. The space enclosed by a thylakoid is called loculus or lumen. The area between two thylakoids is called partition. The end portion of each thylakoid facing the stroma is called margin. Unlike mitochondria, inner membrane of chloroplast has no continuity with thylakoids. Thylakoids get arranged to form stack and look like pile of coins. Each stack is called granum (Granum- singular, Grana- plural). Each granum consists of 10-100 thylakoids. In a granum, packing of thylakoids may be loose or tight (close). In higher plants, thylakoid in a granum are closely packed while in red algae these thylakoids are loosely packed (i.e. widely separated). There is no stacking of thylakoids in chloroplasts of algae, hence grana are not found there. Here the thylakoids (lamellae) are uniformly distributed throughout the chloroplasts.*

Red drop and Emerson effect:

In red light, there is more photosynthesis in comparison to any light of single colour. This is due to location of both pigment systems (PS1 and PSII) in this very region. One is located in short red while the other is in far red light. Both system operate in red light (in short red PSII is active while in far red light, PSI is active). Photosynthesis is maximum in white light (which comprises of all seven colours followed by red light and then by blue light. There is no photosynthesis in green light.

Emerson and lewis (1943), while working on chlorella plants, found sharp decrease in yield in red region of spectrum, when it is exposed to monochromatic light i.e. only one wavelength of light. The fall of photosynthetic yield beyond red region of spectrum is called "red drop" or decrease in photosynthetic yield in red region, can overcome by supplying short red wavelength of light along far-red light (above 680 nm of light to 700 nm of light) simultaneously.this enhancement of photosynthesis is called "enhancement effect or emerson second effect" after the name of the discoverer. Hence, emerson proposed two pigment system one is active in short red while other in far-red. In both kinds of lights, photosystem I and II operate simultaneously increasing photosynthetic yields. Wavelength of light shorter than 680nm affect PSI and PSII while wave length longer than 680nm affect only PSI. hence there is drop in far – red light.

Carbon di-oxide:

The source of carbon-di-oxide is air for terrestrial plants and water for aquatic plants. However, some amount of CO_2 *is also supplied by internal respiration. Atmospheric* CO_2 *is absorbed in gaseous state through stomata of green tissues and diffuses to intercellular spaces in the same state. Further movement of* CO_2 *i.e. from intercellular spaces to mesophyll* *cells occurs in dissolved state (CO_2 in water). Enzyme carbonic anhydrase catalyses the dissolution of CO_2 in water. Some amount of CO_2 also diffuses through cuticle.*

Hydrogen donor:

Hydrogen donor molecules for photosynthesis are water (H_2O), hydrogen sulphide (H_2S), aliphatic alcohols (isopropyl alcohol) and fatty acids. The most important hydrogen donor is H_2O. water gets oxidized by light to yield O_2 and hydrogen. Oxygen is liberated and hydrogen is utilized for the production of reducing power.

$$4H_2O \rightarrow 4H^+ + 4e^- + 2H_2O + O_2$$

In purple sulphur bacteria and green sulphur bacteria, H_2S is hydrogen donor in place of H_2O as reported by van niel(1930): $H_2S = 2(H) + S$

Mechanism of photosynthesis

It is a complicated oxidation-reduction process where water is oxidized and CO_2 is reduced to carbohydrates. The mechanism of photosynthesis consists of two parts.

1. *Light reactions / Primary photochemical reaction*
2. *Carbon reactions / Black man's reaction / Stromal reactions*

Light reaction

The basic role of light reaction is to synthesize assimilatory powers ATP and NADPH necessary for reduction of CO_2 into carbohydrates. The light energy is used to split the water into electrons which enters into electron transport chain (ETC). There are two photosystems involved namely PSI and PSII. Each consists of a light harvesting system (also called the antennae) essentially to transfer the light energy and a reaction center. Light travels from outer antennae to reaction center. Reaction center contains specialized chlorophyll a involved in charge separation and electron transfer. In PSII, the reaction center is P_{680} and P_{700} for PSI. The subscripts refer to their absorption maxima, wavelength at which maximum absorption takes place.

The light absorption by reaction centre in PSII results in excitation and under excitation state, it loses an electron into electron transport chain. Photosystem in turn derives electron from water (ultimate donor of electron) leading to splitting up of water into electrons and protons. When an electron is removed from a molecule, it is said to be oxidized. Thus, the reaction center chlorophyll becomes Photo-oxidized. This is a fundamental part of the photosynthetic process.

The excited states denoted as P 700 and P680* and electron from PSII transferred to pheophytin, plastoquinones, Cytochrome b_6f complex, plastocyanin which is mobile carrier transfer electron to PS I. The PS I transfer electron to phyllaquinone, iron sulfur protein (FeS), Ferridoxin (Fd) which finally to NADP and converted into NADPH.*

Each of the components of the electron transport chain has the ability to transfer an electron from a donor to an acceptor. In addition, Plastoquinone also transfers a proton. That is, each carrier undergoes successive rounds of oxidation and reduction. The electron moves through the electron transport chain and ultimately transferred to NADP. The product, NADPH, is a source of photochemical reducing power used to convert CO_2 to carbohydrate. NADPH is also used in many other biosynthetic reactions besides photosynthesis.

Photolysis of water or Hill reaction (oxidation of water)

Splitting of water molecule into OH^- and H^+ ions in the presence of light is called as photolysis of water. The OH- ions unite to form water molecules again and release O_2 and electrons (e). While, the H^+ is used in the formation of energy-rich molecules, $NADPH_2$ during light reaction. It explains that water is used as a source of electrons for CO_2 fixation and O_2 is evolved as a by-product. It is believed that photolysis of water involves a strong oxidant, designated as " Z" Primary phytochemcial reaction is faster than the dark reaction. It takes place only in the presence of light in the grana portions of the chloroplasts It is complex process with several important events.

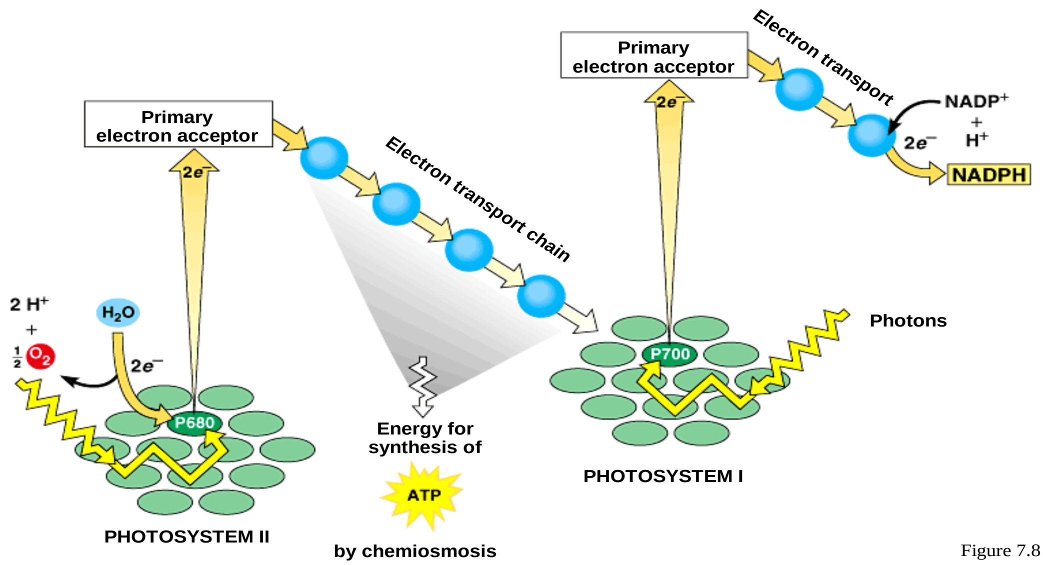


Figure 7.8

There is vectorial translocation of protons into the lumen from stroma as electrons moves from water to NADP. This sets up a transmembrane electrochemical proton gradient, or delta pH. This creates a force called proton motive force (PMF). This PMF is required to synthesize ATP. The complex that carries out this reaction is a multi-subunit protein complex *known as the ATP synthase. When proton moves through ATP synthase complex because of PMF, ATP is formed from the ADP and Pi. Thus, the products of the light reactions are NADPH and ATP.*

Photophosphorylation

The production of ATP using the light energy is called photophosphorylation. The above series of reaction is referred to as non-cyclic photophosphorylation since it involves the transfer of electrons from a donor molecule (water) to an acceptor molecule (NADP).

The non-cyclic photophosphorylation takes the shape of Z and hence it is called by the name Z-scheme. The other type is cyclic photophosphorylation which involves electrons from PSI reaches back to PSI leading to production of ATP without NADPH. The purpose of cyclic electron flow is to maintain a pH gradient to supply the extra ATP required in the dark reaction.

Inhibitors of Photosynthetic Electron Transport Chain

1. *Several urea derivatives that are used a weedicide like monouron(CMU), diuron (DCMU), simazine, atrazine, bromacil block the electron between Q_A and Q_B.*

2. *Diquat and paraquat are common photosynthetic inhibitors. These compounds referred as viologen dyes accept electron from PSI before Ferridoxin.*

Comparison of cyclic and non cyclic electron transport and photophosphorylation

Cyclic phosphorilation	***Non-Cyclic phosphorilation***
The photophosphorylation process which results in the movement of the electrons in a cyclic manner for synthesizing ATP molecules is called cyclic photophosphorylation.	*The photophosphorylation process which results in the movement of the electrons in a non-cyclic manner for synthesizing ATP molecules using the energy from excited electrons provided by photosystem II is called non-cyclic photophosphorylation.*
It involves only PS I. electrons from PS I cycled back to PS I	*It involves PS I and PS II. The electron expelled from PSII is transferred to PS I and hence it is a non cyclic electron transport*
Photolysis of water and evolution of O_2 do not take place	*Photolysis of water and evolution of O_2 take place*
Translocation of proton takes place at one site (PQ)	*Translocation of proton takes place at two sites (water and PQ)*
Only ATP is produced	*Both ATP and NADPH are produced*

*****

Lecture 11- Dark reactions of photosynthesis - C_3, $C_4$and CAM pathways and

differences

Dark reaction or Blackman's reaction or Path of carbon in photosynthesis

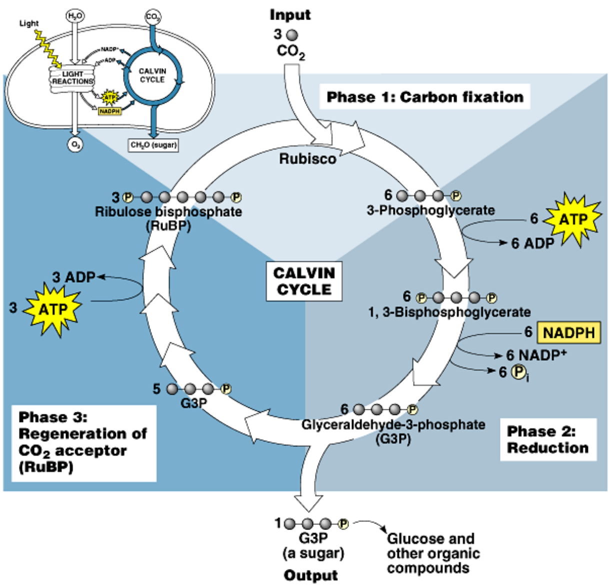
This is the second step in the mechanism of photosynthesis. The chemical processes of photosynthesis occurring independent of light is called dark reaction. *It takes place in the stroma of chloroplast. The dark reaction is purely enzymatic and it is slower than the light reaction. The dark reactions occur also in the presence of light. In dark reaction, the sugars are synthesized from CO_2. The energy poor CO_2 is fixed to energy rich carbohydrates using the energy rich compound, ATP and the assimilatory power, $NADPH_2$ of light reaction. The process is called carbon fixation or carbon assimilation. Since Blackman demonstrated the existence of dark reaction, the reaction is also called as* Blackman's reaction. *In dark reaction two types of cyclic reactions occur*

1. *Calvin cycle or C3 cycle*
2. *Hatch and Slack pathway or C4 cycle*

Calvin cycle or C_3 cycle

It is a cyclic reaction occurring in the dark phase of photosynthesis. In this reaction, CO_2 is converted into sugars and hence it is a process of carbon fixation. The Calvin cycle was first observed by Melvin Calvin in chlorella, unicellular green algae. Calvin was awarded Nobel Prize for this work in 1961. Since the first stable compound in Calvin cycle is a 3 carbon compound (3 phosphoglyceric acid), the cycle is also called as C_3 cycle. The reactions of Calvin's cycle occur in three phases.

1. *Carboxylative phase*
2. *Reductive phase*
3. *Regenerative phase*



1. Carboxylative phase

Three molecules of CO_2 are accepted by 3 molecules of 5C compound viz., ribulose diphosphate to form three molecules of an unstable intermediate 6C compound. This reaction is catalyzed by the enzyme, carboxy dismutase

3 CO_2 + 3 Ribulose diphosphate $\xrightarrow{\text{Carboxy dismutase}}$ 3 unstable intermediate 6 carbon compound

The three molecules of the unstable 6 carbon compound are converted by the addition of 3 molecules of water into six molecules of 3 phosphoglyceric acid. This reaction is also catalyzed by the enzyme carboxy mutase.

3 unstable intermediate 6 C compound + 3 H_2O $\xrightarrow{\text{Carboxy dismutase}}$ 3 phosphoglyceric acid

3 phosphoglyceric acid (PGA) is the first stable product of dark reaction of photosynthesis and since it is a 3 carbon compound, this cycle is known as C3 cycle.

2. Reductive phase

Six molecules of 3PGA are phosphorylated by 6 molecules of ATP (produced in the light reaction) to yield 6 molecules of 1-3 diphospho glyceric acid and 6 molecules of ADP. This reaction is catalyzed by the enzyme, Kinase

3 Phospho glyceric acid + ATP $\xrightarrow{\text{Kinase}}$ 1,3 diphospho glyceric acid + ADP

Six molecules of 1, 3 diphosphoglyceric acid are reduced with the use of 6 molecules of $NADPH_2$ (produced in light reaction) to form 6 molecules of 3 phospho glyceraldehyde. This reaction is catalysed by the enzyme, triose phosphate dehydrogenase.

1,3 diphospho glyceric acid + $NADPH_2$ $\xrightarrow{\text{Triose phosphate Dehydrogenase}}$ 3 phospho glyceraldehyde + NADP + H_3PO_4

3. Regenerative phase

In the regenerative phase, the ribose diphosphate is regenerated. The regenerative phase is called as pentose phosphate pathway *or* hexose monophophate shunt. *It involves the following steps.*

1. *Some of the molecules of 3 phospho glyceraldehyde into dihydroxy acetone phosphate. Both 3 phospho glyceraldehyde and dihydroxy acetone phosphate then unite in the presence of the enzyme, aldolase to form fructose, 1-6 diphosphate.*

   3 phospho glyceraldehyde $\xrightarrow{\text{Triose phosphate isomerase}}$ Dihydroxy acetone PO_4 (DHAP)

   3 phospho Aldolase Fructose 1,6 diphosphate *glyceraldehyde + DHAP* $\longrightarrow$

2. *Fructose 6 phosphate is converted into fructose 6 phosphate in the presence of phosphorylase*

   Fructose 1,6 diphosphate $\xrightarrow{\text{Phosphorylase}}$ *Fructose 6 phosphate*

3. *Some of the molecules of 3 phospho glyceraldehyde instead of forming hexose sugars are diverted to regenerate ribulose 1-5 diphosphate*

   3 phospho glyceraldehyde $\longrightarrow$ *Ribulose 1,5 diphosphate*

4. *3 phospho glyceraldehyde reacts with fructose 6 phosphate in the presence of enzyme transketolase to form erythrose 4 phosphate (4C sugar) and xylulose 5 phosphate(5C sugar)*

   3 phospho glyceraldehyde + Fructose 6 phosphate $\xrightarrow{\text{Transketolase}}$ *Erythrose 4 phosphate + Xylulose 5 phosphate*

5. *E rythrose 4 phosphate combines with dihydroxy acetone phosphate in the presence of the enzyme aldolase to form sedoheptulose 1,7 diphosphate(7C sugar)*

   Erythrose 4 phosphate + DHAP $\xrightarrow{\text{Aldolase}}$ *Sedoheptulose 1 ,7 diphosphate*

6. *Sedoheptulose 1, 7 diphosphate loses one phosphate group in the presence of the enzyme phosphatase to form sedoheptulose 7 phosphate.*

   Sedoheptulose 1 ,7 diphosphate + ADP $\xrightarrow{\text{Phosphatase}}$ *Sedoheptulose 7 phosphate + ATP*

7. *Sedoheptulose phosphate reacts with 3 phospho glyceraldehyde in the presence of transketolase to form xylulose 5 phosphate and ribose 5 phosphate (both % c sugars)*

   Sedoheptulose 7 phosphate + 3 phospho glyceraldehy de $\xrightarrow{\text{Transketolase}}$ *Xylulose 5 phosphate + Ribose 5 phosphate*

8. *R ibose 5 phosphate is converted into ribulose 1, 5 diphosphate in the presence of enzyme, phosphopentose kinase and ATP. Two molecules of xylulose phosphate are also converted into one molecule of ribulose monophosphate. The ribulose monophosphate is phosphorylated by ATP to form ribulose diphosphate and ADP, thus completing Calvin cycle.*

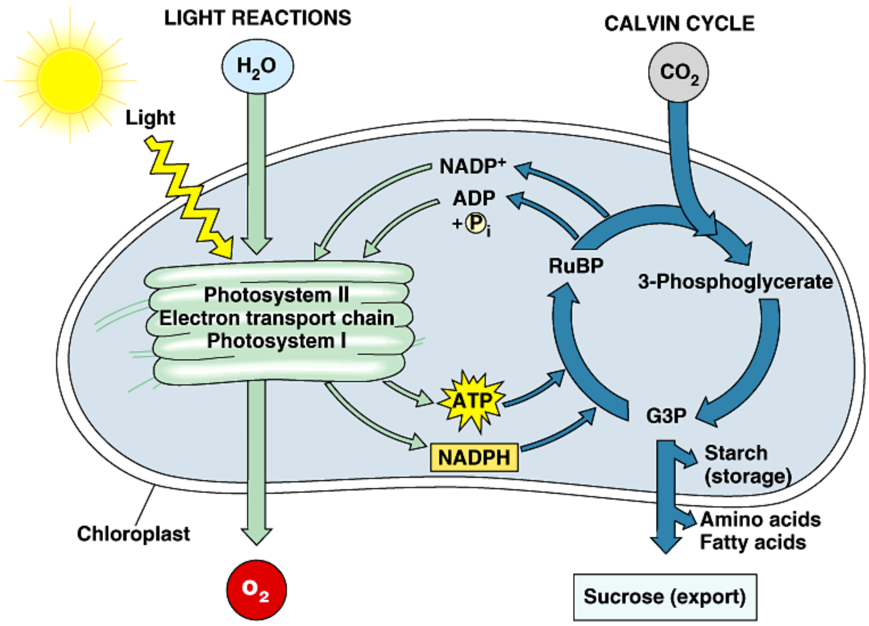
Ribose 5 phosphate + ATP $\xrightarrow{\text{Phophopentokinase}}$ Ribulose 1,5 diphosphate + ADP

Ribulose mono phosphate + ATP $\xrightarrow{\text{Phophopentokinase}}$ Ribulose diphosphate + ADP

2 mols of Xylulose phosphate $\xrightarrow{\text{Phophopentokinase}}$ 1 mol of Ribulose mono phosphate

In the dark reaction, CO_2 is fixed to carbohydrates and the CO_2 acceptor ribulose diphosphate is regenerated. In Calvin cycle, 12 $NADPH_2$ and 18 ATPs are required to fix 6 CO_2 molecules into one hexose sugar molecule (fructose 6 phosphate).

6 CO_2 + 12 NADPH2 +18 ATP $\longrightarrow$ Fructose 6 phosphate +2 NADP+18 ADP+17 Pi



Schematic diagram of light reaction and Calvin cycle

C4 cycle or Hatch and Slack pathway

It is the alternate pathway of C3 cycle to fix CO_2. In this cycle, the first formed stable compound is a 4 carbon compound viz., oxaloacetic acid. Hence it is called C4 cycle. The path way is also called as Hatch and Slack as they worked out the pathway in 1966 and it is also called as C4 dicarboxylic acid pathway. This pathway is commonly seen in many grasses, sugar cane, maize, sorghum and amaranthus.

The C4 plants show a different type of leaf anatomy. The chloroplasts are dimorphic in nature. In the leaves of these plants, the vascular bundles are surrounded by bundle sheath of larger parenchymatous cells. These bundle sheath cells have chloroplasts. These chloroplasts of bundle sheath are larger, lack grana and contain starch grains. The chloroplasts in mesophyll cells are smaller and always contain grana. This peculiar anatomy *of leaves of C4 plants is called Kranz anatomy. The bundle sheath cells are bigger and look like a ring or wreath. Kranz in German means wreath and hence it is called Kranz anatomy. The C4 cycle involves two carboxylation reactions, one taking place in chloroplasts of mesophyll cells and another in chloroplasts of bundle sheath cells. There are four steps in Hatch and Slack cycle:*

1. *Carboxylation*
2. *Breakdown*
3. *Splitting*
4. *Phosphorylation*

1. Carboxylation

It takes place in the chloroplasts of mesophyll cells. Phosphoenolpyruvate, a 3 carbon compound picks up CO_2 and changes into 4 carbon oxaloacetate in the presence of water. This reaction is catalysed by the enzyme, phosphoenol pyruvate carboxylase.

$$\underset{(3C)}{\text{Phosphoenol Pyruvate}} + CO_2 + H_2O \xrightarrow{\text{PEP carboxylase}} \underset{(4C)}{\text{Oxaloacetate}} + H_3PO_4$$

2. Breakdown

Oxaloacetate breaks down readily into 4 carbon malate and aspartate in the presence of the enzyme, transaminase and malate dehydrogenase.

$$\underset{(4C)}{\text{Oxaloacetate}} \xrightarrow[\text{Malate dehydrogenase}]{\text{Transaminase +}} \text{Aspartate (4C)} \quad \text{Malate (4C)}$$

These compounds diffuse from the mesophyll cells into sheath cells.

3. Splitting

In the sheath cells, malate and aspartate split enzymatically to yield free CO_2 and 3 carbon pyruvate. The CO_2 is used in Calvin's cycle in the sheath cell.

$$\text{Malate} \xrightarrow{\text{Decarboxylation}} CO_2 + \text{Pyruvate}$$

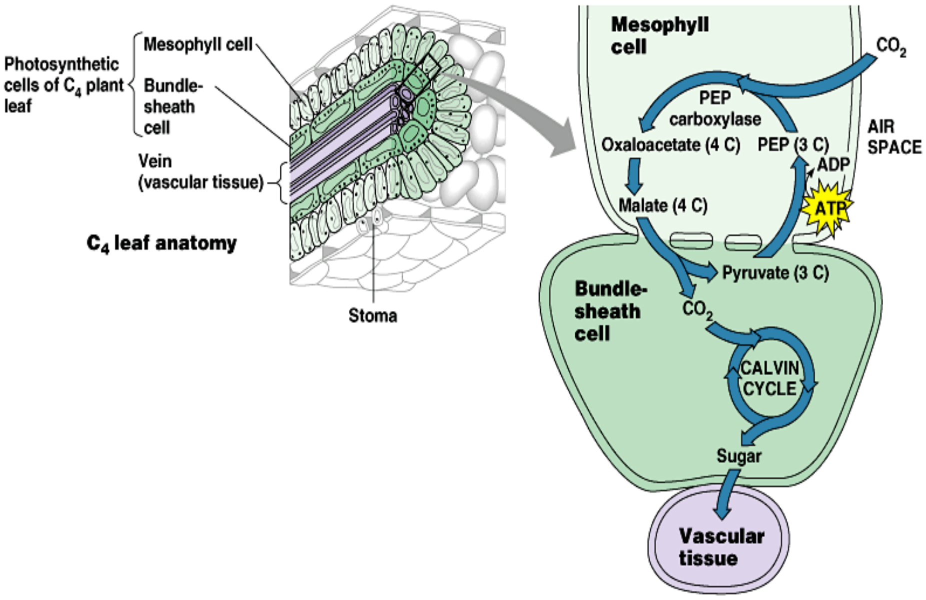
The second Carboxylation occurs in the chloroplast of bundle sheath cells. The CO_2 is accepted by 5 carbon compound ribulose diphosphate in the presence of the enzyme, carboxy dismutase and ultimately yields 3 phosphoglyceric acid. Some of the 3 phosphoglyceric acid is utilized in the formation of sugars and the rest regenerate ribulose diphosphate.

4. Phosphorylation

The pyruvate molecule is transferred to chloroplasts of mesophyll cells where, it is phosphorylated to regenerate phosphoenol pyruvate in the presence of ATP. This reaction is catalysed by pyruvate phosphokinase and the phophoenol pyruvate is regenerated.

Pyruvate + ATP + Pi $\xrightarrow{\text{Pyruvate phosphokinase}}$ *Phosphoenol pyruvate +AMP + Pyrophosphate*

In Hatch and Slack pathway, the C_3 and C_4 cycles of carboxylation are linked and this is due to the Kranz anatomy of the leaves. The C_4 plants are more efficient in photosynthesis than the C_3 plants. The enzyme, phosphoenol pyruvate carboxylase of the C_4 cycle is found to have more affinity for CO_2 than the ribulose diphosphate carboxylase of the C_3 cycle in fixing the molecular CO_2 in organic compound during Carboxylation.



The C_4 pathway

Crassulacean Acid Metabolism (CAM) cycle or the dark fixation of CO_2 in succulents

CAM is a cyclic reaction occurring in the dark phase of photosynthesis in the plants of Crassulaceae. It is a CO_2 fixation process wherein, the first product is malic acid. It is the third alternate pathway of Calvin cycle, occurring in mesophyll cells. The plants exhibiting CAM cycle are called CAM plants. Most of the CAM plants are succulents e.g., Bryophyllum, Kalanchoe, Crassula, Sedium, Kleinia etc. It is also seen in certain plants of Cactus e.g. Opuntia, Orchid and Pine apple families.

CAM plants are usually succulents and they grow under extremely xeric conditions. In these plants, the leaves are succulent or fleshy. The mesophyll cells have larger number of chloroplasts and the vascular bundles are not surrounded by well defined bundle sheath cells.

In these plants, the stomata remain open during night and closed during day time. The CAM plants are adapted to photosynthesis and survival under adverse xeric conditions. CAM plants are not as efficient as C4 plants in photosynthesis. But they are better suited to conditions of extreme desiccation.

CAM involves two steps:

1. *Acidification*
2. *Deacidification*

Acidification

In darkness, the stored carbohydrates are converted into phophoenol pyruvic acid by the process of Glycolysis. The stomata in CAM plants are open in dark and they allow free diffusion of CO_2 from the atmosphere into the leaf. Now, the phosphoenolpyruvic acid carboxylated by the enzyme phosphoenol pyruvic acid carboxylase and is converted in to oxalaoacetic acid.

$$\text{Phosphoenol Pyruvate} + CO_2 \quad H_2O \xrightarrow[\text{carboxylase}]{\text{PEP}} \text{Oxaloacetic acid} + H_3PO_4$$

The oxaloacetic acid is then reduced to malic acid in the presence of the enzyme malic dehydrogenase. The reaction requires NADPH2 produced in Glycolysis.

$$\text{Oxaloacetic acid} + NADPH_2 \quad \text{Malic dehydrogenase} \longrightarrow \text{Malic acid} + NADP^+$$

The malic acid produced in dark is stored in the vacuole. The malic acid increases the acidity of the tissues.

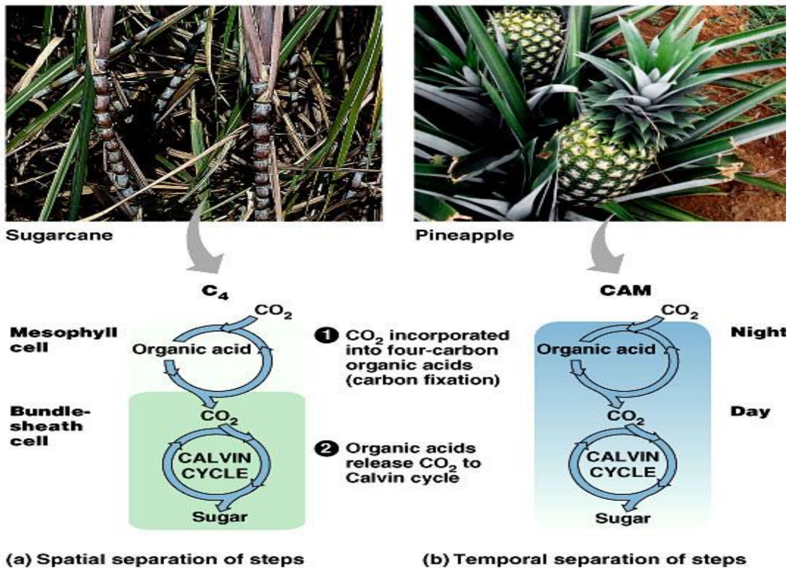
Deacidification

During day time, when the stomata are closed, the malic acid is decarboxylated to produce pyruvic acid and evolve carbon dioxide in the presence of the malic enzyme. When the malic acid is removed, the acidity decreases the cells. This is called deacidification. One molecule of $NADP^+$ is reduced in this reaction.

$$\text{Malic acid} + NADP^+ \xrightarrow{\text{Malic enzyme}} \text{Pyruvic acid} + NADPH_2 + CO_2$$

The pyruvic acid may be oxidized to CO_2 by the pathway of Kreb's cycle or it may be reconverted to phosphoenol pyruvic acid and synthesize sugar by C3 cycle. The CO_2 released by deacidification of malic acid is accepted by ribulose diphosphate and is fixed to carbohydrate by C3 cycle.

CAM is a most significant pathway in succulent plants. The stomata are closed during day time to avoid transpiration loss of water. As the stomata are closed, CO_2 cannot enter into the leaves from the atmosphere. However, they can carry out photosynthesis during the day time with the help of CO_2 released from organic acids. During night time, organic acids are synthesized in plenty with the help of CO_2 released in respiration and the CO_2 entering from the atmosphere through the open stomata. Thus, the CO_2 in dark acts as survival value to these plants.



Major differences in C_3, C_4 and CAM plants

S No	Parameter	C_3	C_4	CAM plants
1	*Primary carboxylating enzyme*	*Rubisco and is having more affinity towards O_2 than CO_2*	*PEPCase and is having more affinity towards CO_2 than O_2*	*PEP Case*
2	*Primary carboxylation product*	*3-phospho glyceric acid (3-carbon compound)*	*Oxalo acetic acid (4 carbon compound)*	*In light: PGA In dark: OAA*
3	*CO_2 fixation Pathways*	*C_3 pathway only*	*Both C_3& C_4 path way present*	*Both C_3& C_4 path way present*
4	*Leaf anatomy*	*Diffuse mesophyll*	*Compact mesophyll*	*Diffuse mesophyll*
5	*Bundle sheath Chloroplast*	*Absent*	*Present Kranz anatomy*	*Absent*
6	*Chloroplast dimorphism*	*Absent*	*Grana absent in Bundle sheath Chloroplast and present in mesophyll chloroplast*	*Absent*
7	*Net Photosynthetic capacity*	*Slight to high*	*High to very high*	*In light: slight In dark : medium*

8	*Photorespiration*	*Present*	*Absent*	*Present*
9	*CO_2 compensation point*	*High*	*Low*	*In dark – low* *In light – high*
10	*Optimum temperature (° C)*	*Low to high*	*High*	*High*
11	*Dry matter production*	*Medium*	*High*	*Low*
12	*Water use efficiency (g of water per g of DMA)*	*Low*	*High*	*High*

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Lecture 12- Factors affecting photosynthesis – photorespiration – pathway - significance

Photosynthesis factors:

Like other physiological processes, photosynthesis depends upon internal as well as external factors. Internal factors include photosynthetic pigments, protoplasmic factor, anatomy and age of the leaf, accumulation of photosynthetic products and hormones. External factors include light, temperature, carbon-dioxide concentration, oxygen concentration, water and mineral status of soil and air pollutants.

Internal factors:

Internal factors are some metabolic activity can be created inside the cell that affect the normal plant growth. Those factors include photosynthetic pigments, protoplasmic factor, anatomy and age of the leaf, accumulation of photosynthetic products and hormones.

Internal factors:

1. *Photosynthetic pigments*
2. *Protoplasmic factors*
3. *Anatomy and age of the leaf*
4. *Accumulation of photosynthetic products*
5. *Leaf resistance*
6. *Chlorophyll concentration*
7. *Hormones*

1. Photosynthetic pigments:

Solar energy of the sun is trapped by photosynthetic pigments and is converted into chemical form. The rate of photosynthesis is directly proportional to the concentration of photosynthetic pigments. No photosynthesis occurs in absence of these pigments

(E.g. heterotrophs) in the non-green part of variegated leaves of croton.

2. Protoplasmic factors:

Protoplasmic factors are probably enzymes. In dehydrated protoplasm the enzyme activity is reduced, thereby, photosynthesis rate is also reduced. In well hydrated protoplasm, protoplasmic factors are more active, thereby more photosynthesis.

3. Anatomy and age of the leaf:

Photosynthesis depends upon the anatomy of leaves.

C_3 and C_4 plants have the basic difference in their leaf anatomy. In C_4 plants "Kranz type" of anatomy is found. Chloroplasts are distributed in mesophyll cells and bundle sheath cells in C_4 plants unlike C_3 plants, where chloroplasts are distributed only in mesophyll cells. Therefore, double fixation of carbon occurs in plants due to their peculiar leaf anatomy leading to more yield.

Photosynthesis does not take place in etiolated or just opened leaves. Maximum photosynthesis occurs in matured leaves. It might be due to increased chlorophyll pigment as well as shift in CO_2 compensation point. Photosynthesis decreases with age of the leaf.

- ✓ *Leaf age of young, mature leaves have greatest rate and output of PS.*
- ✓ *Young, immature leaves have high rate of PS but use more of what they produce for their own growth.*
- ✓ *Mature leaves have slower photosynthetic rates.*
- ✓ *Defoliation of young or young and mature leaves of a plant drains the plant.*

4. Accumulation of photosynthetic products:

Accumulation of photosynthetic products, at the site of photosynthesis leads to retardation/ inhibition of photosynthesis. This is caused due to "Feed back inhibition mechanism". Accumulation of photosynthetase occurs when the rate of utilization and translocation of food substances to storage region/ consumption region decreases.

5. Leaf resistance:

The rate of photosynthesis and transpiration regulated too much by leaf resistance, probably because the exchange of CO_2 and H_2O occurs through stomatal aperture. The external environmental factors which affect the stomatal opening includes light, CO_2, photoperiod and humidity. Soil moisture also affects the rate of photosynthesis.

6. Chlorophyll concentration

The concentration of chlorophyll affects the rate of reaction as they absorb the light energy without which the reactions cannot proceed. Lack of chlorophyll or deficiency of chlorophyll results in chlorosis or yellowing of leaves. It can occur due to disease, mineral *deficiency or the natural process of aging (senescence). Lack of iron, magnesium, nitrogen and light affect the formation of chlorophyll and thereby causes chlorosis.*

7. Mineral nutrients

The deficiency of essential nutrients produce chlorosis, yellowing, browning, reddening and purple colour pigmentation and all these factors reduce the photosynthesis.

8. Hormones:

Gibberellic acid and cytokines increases the photosynthetic yield and carboxylating activity of enzyme RuBisCo. Meidner (1967) found 12% increase in photosynthetic yield/ hour by applying 3µ molar kinetin.

Blackman's principle of limiting factors:

For the study of internal factors of chlorophyll, protoplasmic factors, product of photosynthesis, leaf resistance these all are factors is essential to have a knowledge of Blackman's law of limiting factors. Before 1905, it was customary to study the effect of individual factor on the rate of photosynthesis in terms of minimum (at which photosynthesis starts), optimum (at which photosynthesis is at its best) and maximum (above which photosynthesis does not occur).

Many workers obtained different values of these cardinal points under different conditions.e.g. They found optimum CO_2 concentration to be greater at high intensity than at low intensity.F.F.Blackmancritized the above concept, and established law of limiting factor. He stated that when a process is conditioned as to its rapidly by a number of separate factors, the rate of the process is limited by the pace of the slowest factor.(i.e. factor present in minimum amount).

External factors:

Some external environment is affected the plant growth in that plant surround places or microenvironment. External factors include light, temperature, carbon-dioxide concentration, oxygen concentration, water and mineral status of soil, air pollutants and pollution.

External factors:

1. *Light*
2. *Temperature*
3. *Carbon-dioxide concentration*
4. *Oxygen concentration*
5. *Water*

6. *Mineral status of soil*
7. *Air pollutants*
8. *Osmotic relation*
9. *Pollution*

1. Light :

Light is a raw material for photosynthesis; in absence of light, no photosynthesis takes place. However, photosynthesis is reported to take place in marine algae by trapping moon light.

Light has direct as well as indirect affect on photosynthesis. Directly, it is involved in the photo oxidation of water and excitation of chlorophyll molecules. Indirectly, it controls the stomatal movement, leading to diffusion of CO2 inside the cell from outside. Quantity and quality of light affects photosynthesis.

a. Light intensity:

Light intensity is directly proportional to the photosynthetic yield upto a limit (when other factor are not limiting). Under low light intensity the photosynthetic rate is low but increases with increases in light intensity.

Light intensity requirement varies as per nature of the plant. Heliophytes require more light intensity in comparison to sciophytes, in bean plant, optimum photosynthesis takes place at 2000 ft.candles of light intensity.

At very high light intensity, photosynthesis is inhibited due to solarization of photosynthetic cell constituents as well as stomatal closure. The later leads to storage of CO2 supply.

Intermittent light exposure yields more photosyntythetase than continuous light. It might be due to utilization of the product of light reaction immediately. No accumulation of product leads to forward reaction, thereby, increase in yield, hence photosynthetic yield is increased.

b. Quantity:

Photosynthesis is greater in plants exposed to weak light of longer duration than in plants exposed to strong light for shorter period. That is why plants growing in places where days are longer & produce early fruits than short day places. Ten to twelve hours light exposure results into better photosynthesis. Photosynthesis may occur continuously throughout the 24 hours in arctic region. Total quantity of light is the product of light intensity and exposure time.

c. Light quality:

Photosynthesis takes place in visible part of solar electromagnetic spectrum. It does not occur in infrared and ultraviolet light. More photosynthesis occur in blue and red light; In green light there is not photosynthesis in green plants. In red algae photosynthesis is maximum in green light. Photosynthesis in green plants in maximum in red light followed by blue light. In brown algae maximum photosynthesis occurs in blue light.

Because of having more energy, blue light is able to reach submerged plants and pass through clouds. Red light favours more carbohydrate formation while blue light favours more protein formation.

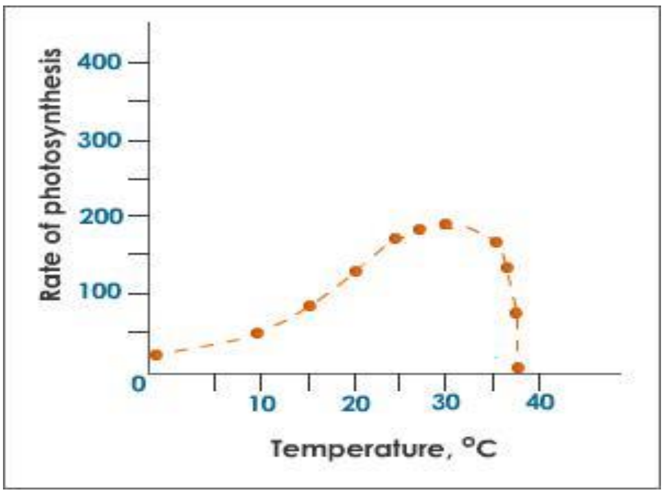
(2) Temperature:

Temperature requirement for the optimum photosynthetic yield varies with the plant species and nature of their environment. For temperate plants it is 10 – 25ºC, for tropical plants it is 40 to 45ºC. Beyond this temperature range (either low or high), photosynthesis decreases.

In temperate region, photosynthesis occurs even at 0ºCor far below in some conifers (upto-35ºC). Lichen can start photosynthesis at -20ºC. Algae of hot spring photosynthesis at 75ºC or even more.

Normally, stomata get closed at high as well as at low temperature as enzymes are inactivated at the latter and denatured at the former. Thereby, photosynthesis is reduced/inhibited.

An optimum temperature ranging from 25°C to 35°C is required for a good rate. At temperatures around 0°C the enzymes stop working and at very high temperatures the enzymes are denatured. Since both the stages of photosynthesis require enzyme activity, the temperature has an affect on the rate of photosynthesis.



Graph Showing Effect of Temperature on Rate of Photosynthesis

Increasing the temperature will move the molecules in the cells at a faster rate, increasing the speed of photosynthesis.Increasing temperature will increase rate of photosynthesis.

(3). Carbon-dioxide concentration:

CO_2 concentration in atmosphere is 330 ppm. Increases in concentration up to 700 ppm would increase photosynthetic yield, if other factors are not limiting. This increased yield is for short period; if it intended for 10-15 days, it becomes toxic to plants and plants face injury.

High level of CO_2 helps to overcome the inhibitory effect of O_2 on photosynthesis in C_3 plants carboxylase activity of RuBisCO is increased and oxygenase activity is suppressed.

High concentration of CO_2get dissolved in water of plasm and lower pH of cell sap, which favours stomatal closure, thereby inhibiting transfusion of CO_2/O_2 which leads to reduction/inhibition of photosynthesis.

In the atmosphere, the concentration of carbon dioxide ranges from .03 to .04 %. However, it is found that 0.1% of carbon dioxide in the atmosphere increases the rate of photosynthesis significantly. This is achieved in the greenhouses which are enclosed chambers where plants are grown under controlled conditions. The concentration is increased by installing gas burners which liberate carbon dioxide as the gas burns. Crops like tomatoes, lettuce are successfully grown in the greenhouses. These greenhouse crops are found to be bigger and better-yielding than their counterparts growing in natural conditions.

(4). Oxygen concentration:

When oxygen concentration is decreased from normal 21 percent to about 2 percent, the rate of photosynthesis increases by 1.5 to 2 folds.

Increases in O_2 concentration decreases the rate of photosynthesis in C_3 plants and CAM plants; as oxygenase activity of enzyme RuBisCO is increased in carboxylase activity is decreased. This results into loss of newly produced photosynthesis, by the process of photorespiration.

(5) Water:

Water is raw material for photosynthesis. The chief role of water in photosynthesis is to supply electrons and protons for reduction of $NADP^+$ by splitting in light. Here, water is directly involved in photosynthesis. Water is indirectly involved in stomatal movement and hydration of cell plasm / protoplasm.

Water stress result into decrease in photosynthesis. During stress, water is not available or less available for production of reducing power. Short supply of reducing power leads to reduction in photosynthesis as CO_2 is not carbohydrate sufficiently. Water stress also alters *the hydration of enzymatic proteins thereby, reducing photosynthetic rate. C_4 plants are more sensitive to leaf dehydration than C_3 plants.Water is one of the raw materials in photosynthesis and shortage of water can slow or even stop photosynthesis. If the plant doesn't have enough water, its stomata will shut and it will be deprived of carbon dioxide.Plants in deserts have a waxy coating on their leaves to reduce water loss*

(6). Mineral status of soil:

Mineral elements are directly involved in plant growth processes. The important mineral elements in the process of photosynthesis Mg, Mn, Cl, Cu, Fe, P etc. these elements can inhibit photosynthesis by affecting chlorophyll synthesis, light harvesting complex and other processes.

Certain elements like Mg and N are the constituent of chlorophyll. Fe is not the constituent of chlorophyll but deficiency of these elements causes chlorosis in leaves. Chlorophyll is not formed in absence of Fe. Phosphorus is needed for phosphorylation reaction of photosynthesis. Zn is used as cofactor of the enzyme carbonic anhydrase. Mn is needed by plastocyanin- a constituent of ETS of chloroplast.

(7). Air pollutants:

Air pollutants decreases photosynthetic rate. Certain gases in higher concentration (lethal doze) inhibit photosynthesis for a time without causing any permanent damage.

Air pollutants include SO_2, NO_2, O_3, smog atc. They get dissolved in atmospheric moisture as well as in cell plasm; consequently, the sap becomes acidic, thereby, hindering metabolic processes, as enzymes are highly specific to pH. Enzymes acting at high pH get inactivated; stomata also get closed at low pH. Hence transfusion of gases through stomata gets demised / inhibited. NO_2 and SO_2 compete with CO_2 for the same ($NADPH + H^+$) for their reduction. Hence there would be shortage of reducing power which ultimately leads to reduction in photosynthetic yields.

Ozone, because of its strong oxidizing action, damages the permeability of members of photosynthesizing cells and cell organelles and thereby photosynthetic yields.

8. Osmotic relation:

Osmotic potential of the plants affects the rate of photosynthesis indirectly by affecting the availability of water and mineral ions. It also affects the turgidity of leaves.

9. ***Pollution***

Pollution of the atmosphere with industrial gases has been found to result in as much as 15% loss. Shoot can block stomata and reduce the transparency of the leaves. Some of the other pollutants are ozone and sulphur dioxide. In fact, lichens are very sensitive to sulphur *dioxide in the atmosphere. Pollution of water affects the hydrophytes. The capacity of water to dissolve gases like carbon dioxide and oxygen is greatly affected.*

Compensation Point

The rate of photosynthesis is not constant throughout the day. It's rate is affected by the intensity of light. The actual requirement of the light intensity for maximum photosynthesis in a plant depends on the type of plant and also on its habitat. Generally, average sunlight intensity is sufficient for photosynthesis except on rainy or cloudy days.

The rate of photosynthesis increases with increasing intensity of light and decreases with decreasing intensity of light. During early morning or late evenings when the rate of photosynthesis becomes equal to the rate of respiration, there will not be any net exchange of gases (CO_2 and O_2) between the plant and the surrounding environment. The light intensity, at which the photosynthetic intake of carbon dioxide is equal to the respiratory output of carbon dioxide is called the compensation point.

Photorespiration

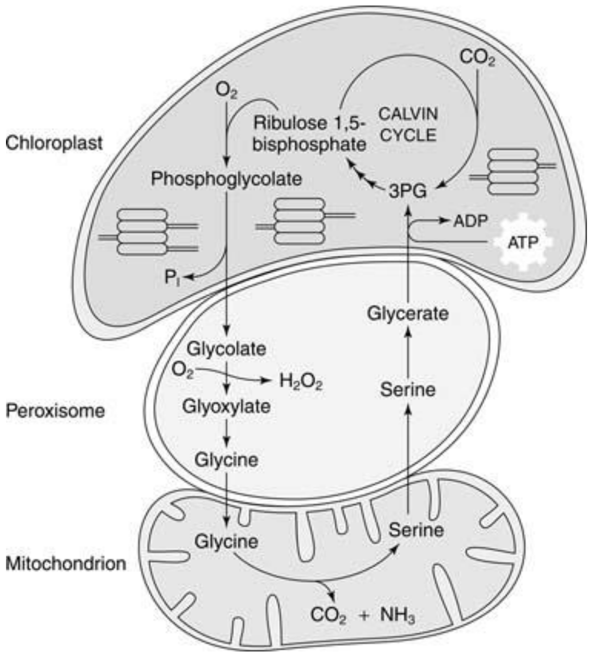
The excessive respiration that takes place in green cells in the presence of light is called as photorespiration. Decker (1955) discovered the process and it is also called as C_2 cycle as the 2 carbon compound glycolic acid acts as the substrate in photorespiration. In general, respiration takes place under both light and dark conditions. However in some plants, the respiration is more in light than in dark. It is 3-5 times higher than the rate of respiration in dark. Photorespiration is carried out only in the presence of light. But the normal respiration is not light dependent and it is called dark respiration.

In photorespiration, temperature and oxygen concentration play an important role. Photorespiration is very high when the temperature is between 25 and 30ºC. The rate of photorespiration increases with the increase in the concentration of oxygen. Three cell organelles namely chloroplast, peroxisome and mitochondria are involved in the photorespiration. This kind of respiration is seen in plants like cotton, pulses, capsicum, peas, tomato, petunia soybean, wheat, oats, paddy, chlorella etc and it is absent in grasses.

Mechanism:

1. In the presence of excess oxygen and low CO_2 , ribulose 1,5 diphosphate produced in the chloroplast during photosynthesis is split into 2 phospho glycolic acid and 3 phospho glyceric acid by the enzyme, ribulose 1,5 diphosphate oxygenase
2. The 3 phospho glyceric acid enters the Calvin cycle.
3. In the next step, phosphate group is removed from 2 phosphoglycolic acid to produce glycolic acid by the enzyme, phosphatase.
4. Glycolic acid then it come out of chloroplast and enter the peroxisome. Here, it combines with oxygen to form glyoxylic acid and hydrogen peroxide. This reaction is catalyzed by the enzyme, glycolic acid oxidase. Hydrogen peroxide is toxic and it is broken down into water and oxygen by the enzyme, Catalase. Photorespiration is an oxidation process. In this process, glycolic acid is converted into carbohydrate and CO_2 is released as the by product. As glycolic acid is oxidized in photorespiration, it is also called as glycolate metabolism.
5. The glyoxylic acid converted into glycine by the addition of one amino group with the help of the enzyme, amino transferase.

6. Now, the glycine is transported from the peroxisome into the mitochondria. In the mitochondria, two molecules of glycine condense to form serine and liberate carbon dioxide and ammonia.
7. Amino group is removed from serine to form hydroxyl pyruvic acid in the presence of the enzyme, transaminase.
8. Hydroxy pyruvic acid undergoes reduction with the help of NADH to form glyceric acid in the presence of enzyme alpha hydroxyl acid reductase.
9. Finally, regeneration of 3 phosphoglyceric acid occurs by the phosphorylation of glyceric acid with ATP. This reaction is catalyzed by the enzyme, Kinase.
10. The 3 phosphoglyceric acid is an intermediate product of Calvin cycle. If it enters the chloroplast, it is converted into carbohydrate by photosynthesis and it is suppressed nowadays with the increased CO_2 content in the atmosphere.



Significance of photorespiration

1. Important for energy dissipation to prevent photo oxidative damage of chloroplast
2. Photorespiration helps in classifying the plants for C_3 and C_4 pathway. Generally, photorespiration is found in C_3 plants and absent in C_4 plants.
3. Carbon dioxide is evolved during the process and it prevents the total depletion of CO_2 in the vicinity of chloroplasts and helps in continuous processes of photosynthesis.
4. The process causes oxidation of glycolic acid which arises as an unwanted by product of photosynthesis. The glycolic acid after oxidation is converted into carbohydrate but the remainder is converted into CO_2.
5. Glycine and serine are the intermediates of photorespiration. Glycine and serine (both are inter convertible) are essential for synthesis of glutathione. Since glutathione is a component of the antioxidative system in plants, it may provide additional protection against oxidative damage in high light and temperature stress.

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Lecture 13- Phloem transport – Munch hypothesis - Phloem loading and unloading - Source and sink strength and their manipulations

The movement of organic food materials or the solutes (the end product of photosynthesis) in soluble form from source to sink through phloem cells in higher plants is called as phloem transport. Translocation of organic solutes is essential in higher plants because in higher plants, only the green parts can manufacture food and it must be supplied to other non-green parts for consumption and also for storage.During the germination of the seeds, the insoluble reserve food material of the seed is converted into soluble form and is supplied to the growing regions of young seedling till it developed its own photosynthetic system i.e. leaves.

Translocation of organic solutes always takes place from the regions of higher concentration of soluble form i.e. the supply end (source i.e. leaves) to the region of lower concentration of its soluble form i.e. the consumption end(sink i.e. young leaves, stems and roots, fruits and seeds).

Directions of translocation

Translocation of organic solutes may take place in the following directions.

1. Downward translocation

Mostly, the organic material is manufactured by leaves and translocated downward to stem and roots for consumption and storage.

2. Upward translocation

It takes place mainly during the germination of seeds, tubers etc. When stored food after being converted into soluble form is supplied to the upper growing part of the young seedling till it has developed green leaves.

Upward translocation of solutes also takes place through stem to young leaves, buds and flowers which are situated at the tip of the branch.

3. Radical translocation

Radical translocation of organic solutes also takes place in plants from the cells of the pith to cortex.

Path of the translocation of organic solutes

1. Path of downward translocation

Downward translocation of the organic solutes takes place through phloem. This can be proved by the ringing experiment.

2. Path of upward translocation

Although translocation of organic solutes take place through phloem, but under certain conditions it may take place through xylem.

3. Path of radical translocation

Radical translocation from pit to cortex takes place through medullary rays.

Mechanism of translocation

Various theories have been put forward to explain the mechanism of phloem conduction. Among them Munchs' (1930) hypothesis is most convincing.

Munch's mass flow or pressure flow hypothesis

According to this hypothesis put forward by Munch (1930) and others, the translocation of organic solutes takes place as mass, through phloem along a gradient of turgor pressure from the region of higher concentration of soluble solutes (supply end) to the region of lower concentration (consumption end). Two members X and Y permeable only to water and dipping in water are connected by a tube T to form a closed system. Membrane X contains more concentrated sugar solution than in membrane Y. Due to higher osmotic pressure of the concentrated sugar solution in the membrane X, water enters into it so that its turgor pressure is increased. The increase in turgor pressure results in mass flow of sugar solution to membrane Y through the T till the concentration of sugar solution in both the membrane is equal. In the above system it could be possible to maintain continuous supply of sugars in membrane X and its utilization or conversion into insoluble form in membrane Y, the flow of sugar solution from X to Y will continue indefinitely. According to this theory, *a similar analogous system for the translocation of organic solutes exists in plants. As a result of photosynthesis, the mesophyll cells in the leaves contain higher concentration of organic food material in them in soluble form and correspond to membrane X or supply end.*

The cells of stem and roots where the food material is utilized or converted into insoluble form correspond to membrane Y or consumption end. While the sieve tubes in phloem which are placed end to end correspond to the tube T.

Mesophyll cells draw water from the xylem of the leaf due to higher osmotic pressure and suction pressure of their sap so that their turgor pressure is increased. The turgor pressure in the cells of stem and roots is comparatively low and hence, the soluble organic solutes begin to flow as massfrom mesophyll through phloem down to the cells of stem and the roots under the gradient of turgor pressure. In the cells of stem and the roots the organic solutes are either consumed or converted into insoluble form and the excess water is released into xylem through cambium.

Phloem loading and unloading

The translocation of organic solutes such as sucrose (i.e.phtosynthesis) takes place through sieve tube elements of phloem from supply end to consumption end. But before this translocation of sugars could proceed; the soluble sugars must be transferred from mesophyll cells to sieve tube elements of the respective leaves. This transfer of sugars from mesophyll cells to sieve tube elements in the leaves is called as phloem loading. On the other hand the transfer of sugars (photosynthates) from sieve tube elements to the receiver cells of consumption end.(i.e. sink organs) is called as phloem unloading. Both are energy requiring process.

Phloem loading

As a result of photosynthesis, the sugars such as sucrose produced in mesophyll cells move to the sieve tubes of smallest veins of the leaf either directly or through only 2-3 cells depending upon the leaf anatomy. Consequently, the concentration of sugars increases in sieve tubes in comparison to the surrounding mesophyll cells.

The movement of sugars from mesophyll cells to sieve tubes of phloem may occur either through symplast (i.e. cell to cell through plasmadesmata) or the sugars may enter the apoplast (i.e. cell wall and intercellular spaces).

Phloem unloading

It occurs in the consumption end or sink organs (such as developing roots, tubers, reproductive structures etc.) sugars move from sieve tubes to receiver cells in the sink involving following steps

1) *Sieve element unloading: In this process, sugars (imported from the source) leave sieve elements of sink tissues.*
2) *Short distance transport: the sugars are now transported to cells in sink by a short distance pathway which has also been called as post –sieve element transport.*
3) *Storage and metabolism : Finally sugars are stored or metabolized in the cells of the sink.*

Phloem unloading is typically symplastic in growing and respiring sinks. In storage organs such as fruits(grapes, orange), stem(sugarcane), sucrose unloading is known to occur through apoplast.

Source

It is the regions of photoassimilates production and export photoassimilates. The source are chlorophyllous tissues includes leaves, stipules, young stem, pedicel, awns, peduncle, calyx, bract etc

Sink

It is the regions of photoassimilates consumption and import photoassimilates which includes growing regions, storage organs of fruit and seeds

Source and sink regulation depends on two factors

***Source strength** have two components, source size and source activity*

Source size is leaf characters i.e. size, thickness, mesophyll size, compaction, vascular bundle. Source activity is photosynthetic activity - Carrying capacity of sieve element, RuBP and PEP carboxylase enzymes. Environmental factors influence source size and activity.

***Sink strength**have two components, sink size and sink activity. The sink strength ie.the capacity of a tissue or organ to accumulate or metabolize photosynthates and thereby to create or maintain a concentration gradient between the solutes in the sieve tubes and the surrounding cells in the sink. Concentration gradients for sucrose and other sugars can be created by the consumption of sucrose during growth (growth or utilization sink) and storage of photosynthates (storage sink), for example as sucrose (e.g., sugar beet) or as starch (e.g., cereal grains and tubers). For a given plant part (e.g., shoot apex) or organ (e.g., wheat grain, tomato fruit), sink strength is related to the growth rate, storage capacity (e.g., number of storage cells), transport rate from the phloem to the individual storage cells, or conversion rate of photosynthates (e.g., sugars to starch, amino acids to proteins). Sink strength and thus, phloem unloading are therefore closely related to the metabolic activity of the sink tissue or organ. Sink size is potential capacity of the sink to accumulate assimilates and competition among different sink. Sink activity has two components, duration of filling and rate of filling. Source sink relationship should be balanced for maximum yield and not be source or sink limitations.*

Source limitation:

Source will not able to supply enough assimilates to the developing sink. This is due to low canopy photosynthesis, low optimum LAI, slow peak LAI (lag vegetative growth), low LAD at filling, early leaf senescence, and biotic and abiotic stress. Source limitation are due to low canopy photosynthesis, low optimum LAI, slow peak LAI (lag vegetative growth), low LAD at filling, early leaf senescence, biotic and abiotic stress. Wheat, rice, pulses, oilseeds are source limitation crops.

Sink limitation:

Source supply assimilates without any limitations but sufficient sink might not be available to import assimilates. This is due to late anthesis, vegetative growth at reproductive phase, less sink number and size, hormonal imbalance, biotic and abiotic stresses, multi-sink demand (nodules). Cereals are sink limitation crop.

The following factors are essential for balanced source and sink regulation

- *Optimum LAI (4 to 6) and longer LAD*
- *Maximum photosynthesis and immediate translocation of photo assimilates from leaves*
- *Minimum distance between source and sink*
- *Small surplus source for stress*
- *High source size during sink differentiation*
- *Synchrony of sink organ development*
- *Maximum Total Dry Matter Accumulation (TDMA)*
- *Reduce photorespiration in C3 plants*
- *Reduced growth of non harvestable organ*
- *Prolonged faster storage*
- *Enhanced competition of storage organ*
- *Hormonal balance and efficient root system*
- *The mineral elements potassium, magnesium, sodium enhances the sucrose accumulation in sink cells. Boron is essential for translocation of photoassimilates, the deficiency of boron produced hen and chicken disorder in grapes, mal formation of fruit and vegetables, hallow spot in groundnut and choppy grains in cereals.*

PGRs on source sink regulations:

ABA inhibit sucrose uptake in source (loading) and involved in phloem unloading and even low concentration of ABA stimulate the sucrose efflux from phloem cells. Auxin, GA, cytokinin promotes source uptake. Cytokinin delays senescence of source and sink and increases photoassimilates import in sink. Ethylene induces senescence process.

Source- sink manipulation

Physical and chemicalmanipulation are essential for balanced source - sink relation.Physical manipulationsinclude pruning, training, thinning, desuckering and girdling in agricultural and horticultural crops. Chemical manipulation is foliar spray of plant growth regulators, growth retardants and mineral nutrients. This practices helps to regulate source sink relation through mmaximum interception of radiation thereby increases the photosynthesis, high C/N ratio, channelling its energy distribution from vegetative to *reproductive growth, increases translocation efficiency, stimulating the accumulation of secondary metabolites, increase the root activity, regulate stomatal movement, induce the enzyme activity, increase the green colour of the leaf, increase leaf thickness. The endogenous auxin and cytokinincontent are improved by source and sink manipulations. The air circulation is enhanced, thereby avoid thebuildup of micro climate for pest and disease infestation and also helps in easy to carry out cultural operations.*

Assimilates Partitioning

The product of carbon assimilation or photosynthesis such as hexoses, sucrose,starch etc(i.e. fixed carbon) are called as photosynthates or photo assimilates or simply as assimilates. These assimilates are produced in green leaves of higher plants which constitute the sources. Within various compartment of photosynthesizing cells(sources),these assimilates are i) metabolically utilized, ii) stored or iii) converted into transport sugars mainly sucrose for export to various sinks(through phloem) such as young leaves, roots,tubers,stems,fruits and seeds. At the sinks assimilates are metabolically utilized and / or stored in receiver cells of sinks. This depending up on the nature and specific requirements of the sinks, the photo assimilates are differentially in different sinks. This differential distribution of photo assimilates in different sinks of plants are called as assimilate partitioning.

Factors controlling translocation and assimilates partitioning in higher plants

1. *Competition among sink tissues for available translocated assimilates*

   The sink strength i.e. the ability of the sink to mobilize assimilates toward it depends on sink size and sink activity.

2. *Photosynthesis and sink demand*
   The rate of photosynthesis is strongly influenced by sink demand. The rate of phosynthesis decreases when rate of sink demand decrease.
3. *Long distance signals between source and sinks*
   The signals between sources and sinks may be physical such as turgor pressure or chemical such as phytohormones.
4. *Plasmodesmata*
   It play very important role in regulating all aspects of phloem translocation includes phloem loading, phloem unloading and assimilate partitioning. Closure of sieve pores by deposition of callose is overcome by presence of phloem protein (P-Protein).

Lecture 14 - Respiration - Glycolysis – TCA cycle.

The energy stored in carbohydrate molecules during photosynthesis is released during cellular oxidation of carbohydrates into CO_2 and H_2O. This is called as respiration. In respiration the oxidation of various organic food substances like carbohydrates, fats, proteins etc, may take place. Among these glucose is the commonest.

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

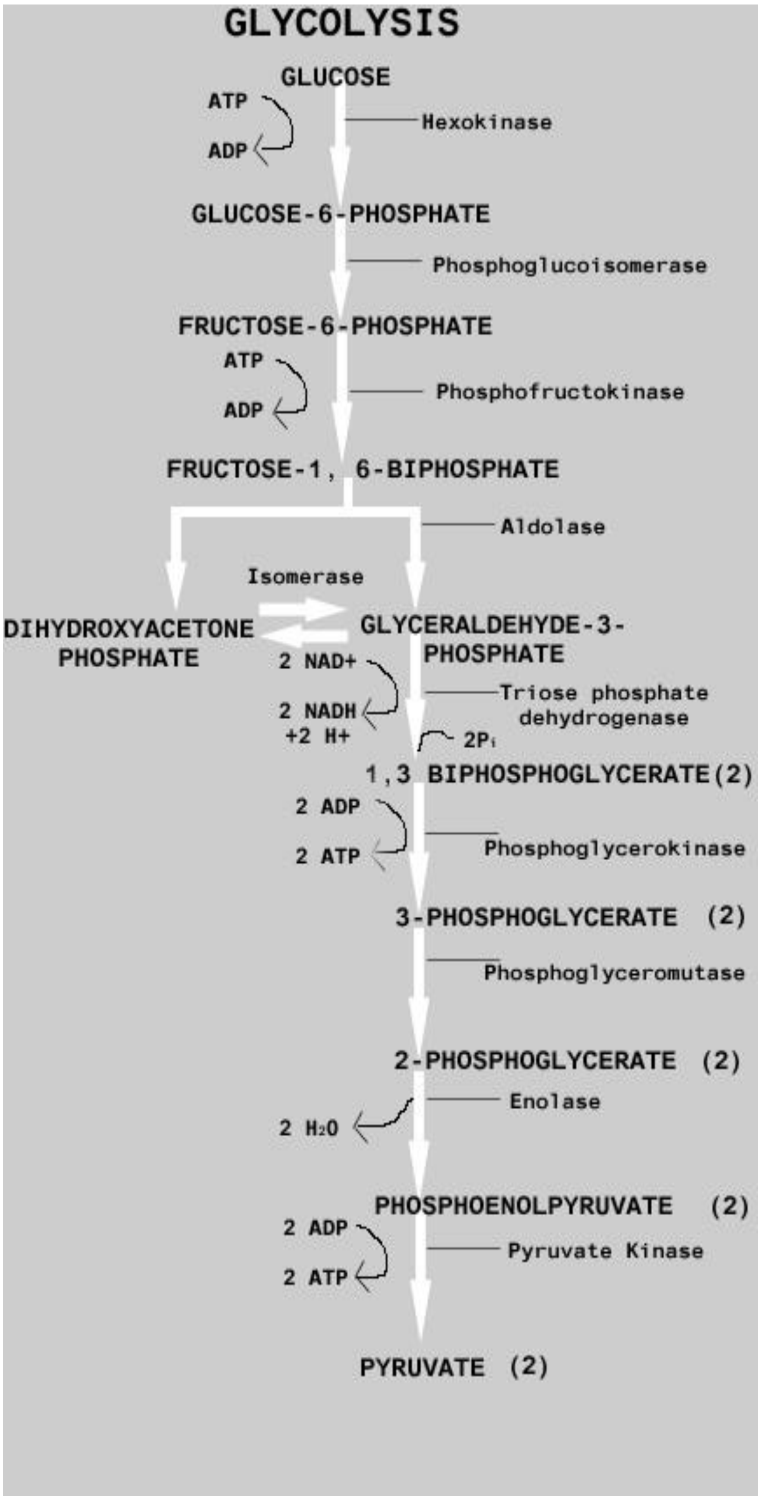
Breakdown of glucose involves many steps releasing energy in the form of ATP molecules and also forming a number of carbon compounds (intermediates).

Complete oxidation involves three steps

- *Glycolysis*
- *Kreb cycle*
- *Electron Transport Chain (ETC)*

Glycolysis (Embden – Mayer Hof-Paranas- EMP Pathway)

Glycolysis can take place even in the absence of O_2in cytoplasm. Six carbon compound glucose is broken down through a series of enzymic reactions into 3-carbon compound, the pyruvic acid.



1. Glucose molecules react with ATP molecules in the presence of the enzyme hexokinase to form glucose -6- phosphate.

2. Glucose -6-phosphate is isomerised into fructose-6-phosphate in the presence of phosphohexoseisomerase.

3. Fructose -6-phosphate reacts with one molecule of ATP in the presence of phosphohexokinase forming fructose 1, 6-disphosphate

4. Fructose 1,6-Diphosphate is converted into two trioses 3-phospho glyceraldehydes and Dihydroxy acetone phosphate in the presence of aldolase. The phosphotrioseisomerase.

5. 3-phosphoglyceraldehyde reacts with $H_3PO_4$to produce 1,3-diphoglyceraldehyde. The reaction is non –enzymatic.

6. 1, 3-Diphosphoglyceraldehyde in the presence of triose-phosphaste dehydrogenase and NAD, is oxidized to form, 1,3- diphosphoglyceric acid, NAD is reduced.

7. 1, 3-Diphosphoglyceric acid reacts with ADP is the presence of phosphoglycerictransphorylase to form one mole of ATP and 3 – phosphoglyceric acid.

8. 3-phosphoglyceric acid is isomerized into 2 phosphoglyceric acid in presence of enzyme phosphoglyceromutase

9. 2 phosphoglyceric acid is converted into 2-phosphoend-pyruvic acid in the presence of Enolase.

10. 2 phosphoenol pyruvic acid reacts with ADP to form one molecule each of ATP and pyruvic acid. The enzyme pyruvate kinase catalyases this reaction.

Glycolysis of EMP pathway is common in both aerobic and anaerobic respiration.

The overall glycolytic process can be summarized as follows.

$$C_6H_{12}O_6 + 2ATP + 2NAD + 4ADP + 2H_3PO_4$$

$$\downarrow$$

$$2\ CH_3COCOOH + 2ADP + 2NADH_2 + 4\ ATP$$

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

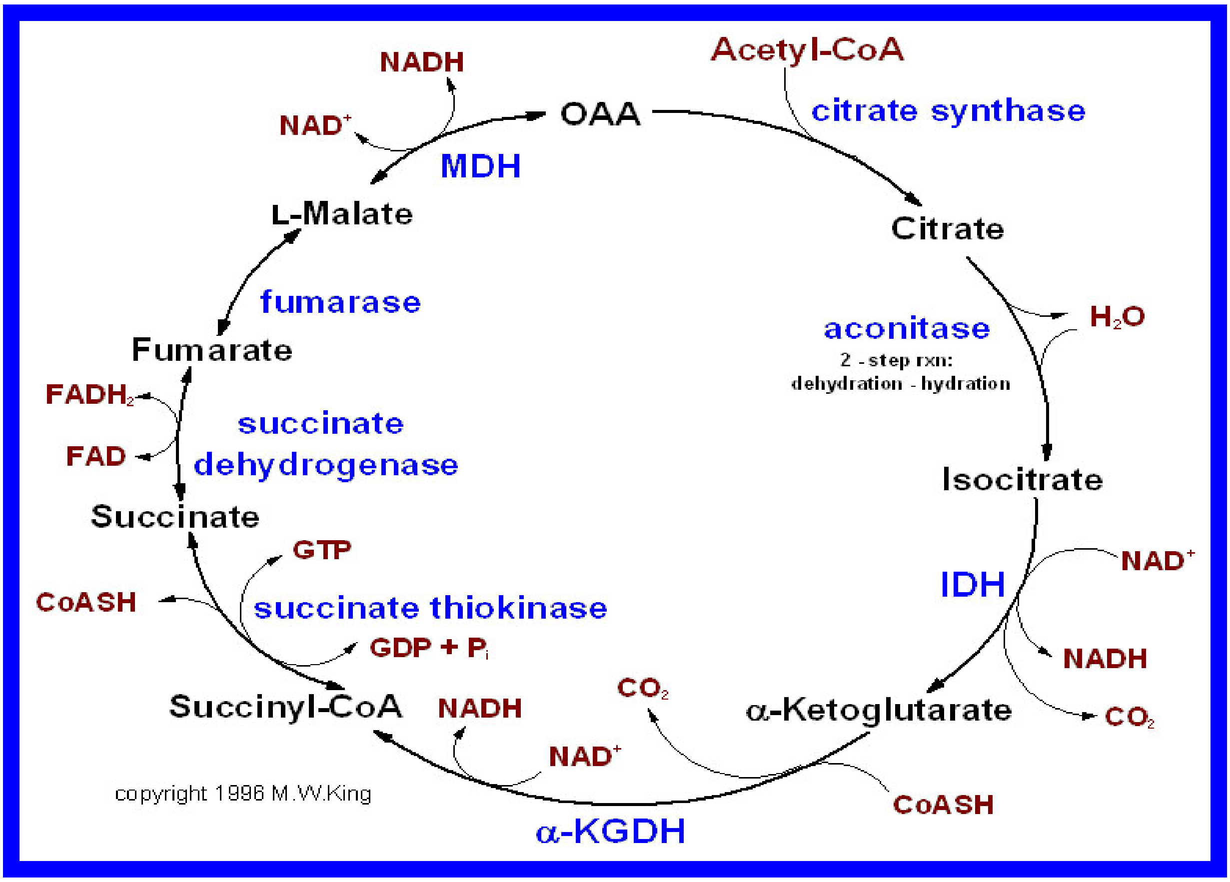
THE KREBS' CYCLE (TCA CYCLE)

The pyruvic acid produced in glycolysis enters into Krebs' cycle for further oxidation. Krebs' cycle is also known as citric acid cycle or Tri carboxylic acid cycle (TCA). It takes place in Mitochondria where necessary enzymes are present in matrix.

11. Oxidative decarboxylation of pyruvic acid

Pyruvic acid reacts with COA and NAD and is oxidativelydecarboxylated. One molecules of $CO_2$is released and NAD is reduced. Pyruvic acid is converted into acetyl COA.

$$\text{Pyruvic acid} + \text{COA} + \text{NAD} \xrightarrow{\text{Pyruvate dehydrogenase complex}} \text{Acety} - \text{COA} + CO_2 + NADH_2$$



12. Acetyl-COA condenses with oxaloacetic acid in the presence of condensing enzyme and water molecule to form citric acid. COA becomes free.

$$\text{Acetyl COA} + \text{Oxaloacetic acid} \xrightarrow{\text{Condensing enzyme}} \text{Citric acid} + \text{COA} + H_2O$$

13. Citric acid is dehydrated in the presence of aconitase to form cis – aconitic acid

Aconitase

Citric acid $\xrightarrow[H_2O]{}$ Cis – Aconitic acid

14. Cis-Aconitic acid reacts with one mole. of water to form Isocitric acid

Cis-Aconitic acid + H_2O $\rightarrow$ Isocitricacid

15. Iso-citric acid is oxidized to oxalosuccinic acid in the presence of Isocitric dehydrogenases. NADP is reduced to $NADPH_2$ in the reaction.

Isocitric acid + NADP $\xrightarrow{\text{IC dehydrogenase}}$ Oxalo succinic acid + $NADPH_2$

16. Oxalosuccinic acid is decarboxylated in the pressure of oxalo succinic decarboxylase to form α - ketoglutamic acid. A second mole of CO_2 is released

Oxalosuccinic acid $\xrightarrow{\text{Oxalosuccinic decarboxylase}}$ α-ketoglutamic + CO_2

17. α - ketoglutamic acid reacts with COA and NAD in the presence of α - ketoglutamicacid dehydrogenase complex and is oxidativelydecarboxylated to form succinyl COA and a third mole of CO_2 is released. NAD is reduced in the reaction.

α-ketoglutamicacid COA $\longrightarrow$ Succinyl-COA+CO_2+$NADH_2$

NAD NADH$_2$

18. Succinyl COA reacts with water molecule to form succinic acid. COA becomes free and one mole of GDP (Guanosinediphosphate) is phosphorylated in preserve of inorganic phosphate to form one mole. of GTP.

Succinyl-COA+GDP+ip $\xrightarrow{H_2O}$ Succinic acid + GTP

GTP may react with ADP to form one molecule of ATP

GTP + ADP $\rightarrow$ ATP + GDP

19. Succinic acid is oxidized to fumaric acid in the presence of succinic dehydrogenase, co enzyme FAD is reduced in this reaction.

$$\text{Succinic acid} + \text{FAD} \xrightarrow{\text{Succinic acid dehydrogenase}} \text{Fumaric acid} + \text{FADH}_2$$

20. One mole of H_2O is added to Fumaric acid in the presence of fumarase to form malic acid.

$$\text{Fumaric acid} + H_2O \xrightarrow{\text{Fumarase}} \text{Malic acid}$$

21. In the last step malic acid is oxidized to oxaloacetic acid in the presence of malic dehydrogenase. One mole. of coenzyme i.e NAD is reduced.

$$\text{Malic acid} + \text{NAD} \xrightarrow{\text{Malic dehydrogenase}} \text{Oxaloacetic acid} + \text{NADH}_2$$

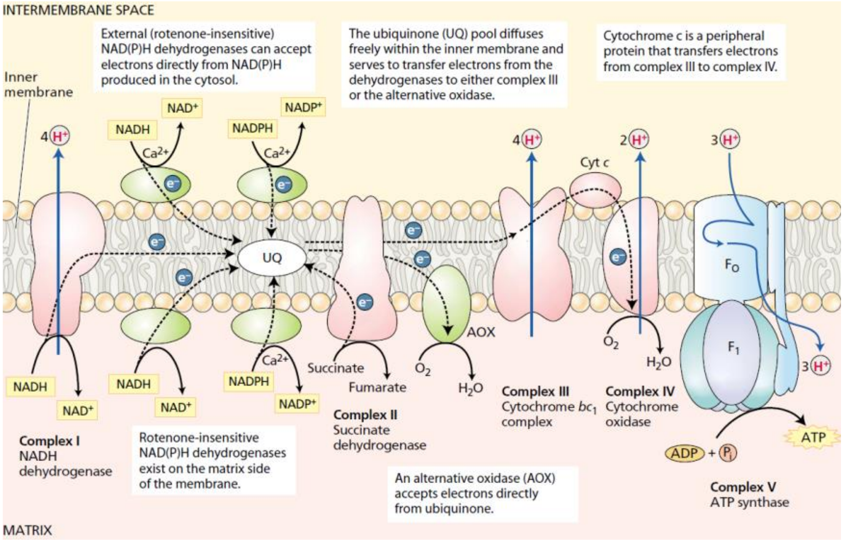
Significance ofkreb's cycle

- *The intermediates produced in the kreb's cycle are essential for biosynthesis of lipid, protein and secondary metabolites like phenol, tannin, rubber, hormone and pigments etc.,*
- *The $NADPH_2$produced during respiration is essential for the activation of glutathione (antioxidant) and also used as a reductance in biosynthetic process.*
- *It provides energy in the form of ATP molecules for various metabolic activities.*
- *It is directly related to nitrogen metabolism*
- *It is closely associated with glyoxalate cycle*

Lecture No. 15. Oxidative phosphorylation- Electron transport chain in mitochondria

ATP is the energy carrier used by cells to drive living processes. Chemical energy conserved during the citricacid cycle in the form of NADH and $FADH_2$ must be converted to ATP to perform useful work in the cell. This O_2 dependent process, called ***oxidative phosphorylation****, occurs in the inner mitochondrial membrane.*

For each molecule of sucrose oxidized through glycolysis and the citric acid cycle pathways, 4 molecules of NADH are generated in the cytosol and 16 molecules of NADH plus 4 molecules of FADH2 (associated with succinate dehydrogenase) are generated in the mitochondrial matrix. These reduced compounds must be reoxidized or the entire respiratory process will come to a halt. The electron transport chain catalyzes an electron flow from NADH (and FADH2) to oxygen, the final electron acceptor of the respiratory process. The terminal oxidation of each reduced coenzyme requires $1/2O_2$ molecule and 2H atoms (i.e. $2\ e^- + 2H^+$) to produce one H_2O molecule.



For the oxidation of NADH, the overall two-electron transfer can be written as follows:

$$\boldsymbol{NADH + H + 1/2\ O_2 \rightarrow NAD^+ + H_2O}$$

Electrons from NADH generated in the mitochondrial matrix during the citric acid cycle are oxidized by complex I. ***Complex I*** *(an NADH dehydrogenase) then transfer these electrons to ubiquinone. Four protons are pumped from the matrix to the intermembrane space for every electron pair passing through the complex. Ubiquinone, a small lipid-soluble electron and proton carrier, is located within the inner membrane and it can diffuse within the hydrophobic core of the membrane bilayer.*

Oxidation of succinate in the citric acid cycle is catalyzed by ***complex II*** *(succinate dehydrogenase), and the reducing equivalents are transferred via the FADH2 and a group of iron–sulfur proteins into the ubiquinone pool. This complex does not pump protons.* ***Complex III*** *(cytochrome* bc*1 complex) oxidizes reduced ubiquinone and transfers the electrons to cytochrome bc1 complex to cytochrome c. Four protons per electron pair are pumped by complex III.* ***Cytochrome c*** *is a small protein loosely attached to the outer surface of the inner membrane and serves as a mobile carrier to transfer electrons between complexes III and IV.* ***Complex IV (****cytochrome c oxidase) is the terminal oxidase and brings about the four-electron reduction of O_2 to two molecules of H_2O. Two protons are pumped per electon pair.*

Thus, oxidation of one molecule of reduced $NADH_2$ or $NADPH_2$ will result in the formation of 3 ATP molecules while the oxidation of $FADH_2$ lead to the synthesis of 2 ATP molecules. In eukaryotes terminal oxidation of mitochondrial NADH / NADPH results in the production of 3 ATP molecules but that of extra mitochondrial NADH / NADPH yields only 2 ATP molecules. Therefore, the two reduced coenzyme molecules (NADH) produced per hexose sugar molecule during Glycolysis will yield only 2x2:4 ATP molecules instead of 6 ATP molecules. Complete oxidation of a glucose molecule (hexose sugar) in aerobic respiration results in the net gain of 36 ATP molecules in most eukaryotes.

One glucose molecule contains about 686 Kcal. Energy and 36 ATP molecules will have 273.6 Kcal energy. Therefore about 40% (273.6/686) energy of the glucose molecule is utilized during aerobic breakdown and the rest is lost as heat. Since huge amount of energy is generated in mitochondria in the form of ATP molecules, they are called as Power Houses of the cell. ATP molecules contain energy in terminal pyrophosphate bonds. When these energy rich bonds break, energy is released and utilized in driving various other metabolic processes of the cell.

Differences between oxidative phosphorylation and Photophosphorylation

	Oxidative phosphorylation	***Photophosphorylation***
1	*It occurs during respiration*	*Occurs during photosynthesis*
2	*Occurs inside the mitochondria* *(inner membrane of cristae)*	*Occurs inside the chloroplast* *(in the thylakoid membrane)*
3	*Molecular O_2 is required for terminal oxidation*	*Molecular O_2 is not required*
4	*Pigment systems are not involved*	*Pigment systems, PSI and PSII are involved*

5	*It occurs in electron transport system*	*Occurs during cyclic and non cyclic electron transport*
6	*ATP molecules are released to cytoplasm and used in various metabolic reactions of the cell*	*ATP molecules produced are utilized for CO_2 assimilation in the dark reaction of photosynthesis*

Respiratory quotient

The ratio of the volume of CO_2 released to the volume of O_2 taken during respiration is called as respiratory quotient and is denoted as RQ

$$RQ = \frac{\text{Volume of } CO_2}{\text{Volume of } O_2}$$

Value of RQ

The value of RQ depends upon the nature of the respiratory substrate and the amount of O_2 present in respiratory substrate.

*1. When **carbohydrates** such as hexose sugars are oxidized in respiration, the value of RQ is 1 or unity because volume of CO_2 evolved equals to the volume of O_2 absorbed.*

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

$$RQ = \frac{\text{volume of } CO_2}{\text{volume of } O_2} = \frac{6}{6} = 1 \text{ or unity}$$

*2. When **fats** are the respiratory substrate, the value of RQ becomes less than one because fats are poorer in O_2 in comparison to carbon and they require more O_2 for their oxidation,*

$$\underset{\text{Tripalmitin}}{2C_{51}H_{98}O_6} + 145O_2 \longrightarrow 102CO_2 + 98H_2O$$

$$RQ = \frac{\text{volume of } CO_2}{\text{volume of } O_2} = \frac{102}{145} = 0.7$$

(Fats are oxidized in respiration usually during the germination of fatty seeds).

*3. When **organic acids** are oxidized in respiration, the value of RQ becomes more than one. It is because organic acids are rich in O_2 and require less O_2 for their oxidation.*

$$C_4H_6O_5 + 3O_2 \longrightarrow 4CO_2 + 3H_2O$$

Malic acid

$$RQ = \frac{\text{volume of } CO_2}{\text{volume of } O_2} = \frac{4}{3} = 1.3$$

Energy budgeting

Stages	***Gain of ATP***	***Consumption of ATP***	***Net gain of ATP***
Glycolysis			
1) Glucose $\longrightarrow$ *Glucose 6 PO4*		*1*	
2) Fructose 6 PO4 $\longrightarrow$ *Fructose 1,6 di PO4*		*1*	
3) 1,3 diphosphoglyceraldehyde $\longrightarrow$ *1,3 diphosphoglyceric acid*	*4*		
4) 1,3 diphosphoglyceric acid $\longrightarrow$ *3 phosphoglyceric acid*	*2*		
5) 2 phosphoenol pyruvic acid $\longrightarrow$ *Pyruvic acid*	*2*		
Total	***8***	***-2***	***6***
Kreb's cycle			
6) Pyruvic acid $\longrightarrow$ *Acetyl CoA*	*3*		
7) Isocitric acid $\longrightarrow$ *Oxalosuccinic acid*	*3*		
8) ketoglutaric acid $\longrightarrow$ *Succinyl CoA*	*3*		
9) Succinyl Co A $\longrightarrow$ *Succinic Acid*	*1*		
10) Succinic acid $\longrightarrow$ *Fumaric Acid*	*2*		
11) Malic acid $\longrightarrow$ *Oxaloacetic acid*	*3*		
Total ATP mol. produced per Pyruvic acid	*15*		
Total ATP mol. produced for 2 Pyruvic acids	*30*		*30*
Grand Total	***38***	***-2***	***6+ 30 = 36***

Lecture No. 16- Fat metabolism: Fatty acid synthesis and breakdown

Fats and oils belong to the general class termed lipids, a structurally diverse group of hydrophobic compounds that are soluble in organic solvents and highly insoluble in water. Lipids represent a more reduced form of carbon than carbohydrates, so the complete oxidation of 1 g of fat or oil (which contains about 40 kJ of energy) can produce considerably more ATP than the oxidation of 1 g of starch (about 15.9 kJ). Conversely, the biosynthesis of lipids requires a correspondingly large investment of metabolic energy.

Whereas animals use fats for energy storage, plants use them for both energy and carbon storage. Fats and oils are important storage forms of reduced carbon in many seeds, including those of agriculturally important species such as soybean, sunflower, canola, peanut, and cotton. Oils serve a major storage function in many non-domesticated plants that produce small seeds. Some fruits, such as olives and avocados, also store fats and oils.

Fats and oils exist mainly in the form of triacylglycerols(acyl refers to the fatty acid portion), in which fatty acidmolecules are linked by ester bonds to the three hydroxylgroups of glycerol.The fatty acids in plants are usually straight-chain carboxylicacids having an even number of carbon atoms. Thecarbon chains can be as short as 12 units and as long as30 or more, but most commonly are 16 or 18 carbons long.Oils are liquid at room temperature, primarily because ofthe presence of carbon–carbon double bonds (unsaturation)in their component fatty acids; fats, which have ahigher proportion of saturated fatty acids, are solid atroom temperature.

Biosynthesis of fatty acids

It was thought that fatty acid biosynthesis occurred by reversal of the β-oxidation pathway. On the contrary, it occurs by a separate pathway that differs from b-oxidation in several ways.

I. *Formation of malonyl CoA*

The synthesis of malonyl CoA from acetyl CoA is catalyzed by acetyl CoA carboxylasehavingbiotinasprostheticgroup.TheproductionofmalonylCoAisthe initialandcontrollingstepinfattyacidsynthesis.

II. *Formation of acetyl ACP*

Acetyl transacylase catalyze the formation of acetyl ACP.FormationofmalonylACP

Malonyl transacylase catalyze the formation of malonyl ACP.

III. *Formation of aceto acetyl ACP*

Acetyl ACP condenses with malonyl ACP to form acetoacetyl ACP. Carbondioxide is eliminated from malonyl ACP.

IV. *Reduction of acetoacetyl ACP to β-hydroxyl acylACP.*

The b- keto group in acetoacetyl ACP is reduced by NADPH- dependent β - ketoacyl reductase.

V. *FormationofunsaturatedacylACP.*

The hydroxyl group combines with the hydrogen atom attached to the g-carbon and a water molecule is removed to form a,b-unsaturatedacylACP.

VI. Formation of Acyl ACP

The unsaturated acyl ACP is converted to a saturated acylACP by the enzyme unsaturated acyl ACP reductase using NADPH as the coenzyme. The resultant product contains two carbon atoms more than the starting material. Addition of subsequent acetyl units through malonyl ACP leads to the formation of 16-carbonpalmitate.

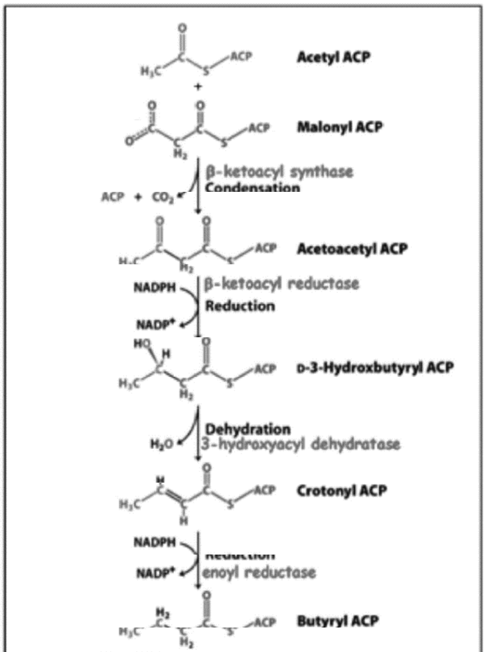


Figure 28.4
Biochemistry: A Short Course, Second Edition
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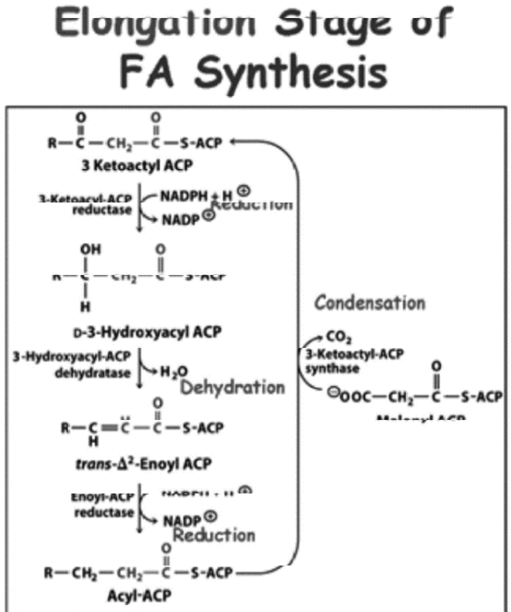

Figure 16-5 Principles of Biochemistry, 4/e
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The major product of fatty acid biosynthesis is the 16 carbon fatty acid, palmitate. Additional enzymes are required to synthesis longer chain fattya cids. Chain elongation reactions occur both in mitochondria and in microsomes. Microsomes are small membrane enclosed vesicles derived from the endoplasmic reticulum of cells. Mitochondria and microsomes carryout chain elongation by adding two carbon units to fattyacids. Chain elongation occurs by a cycle of condensation, reduction, dehydration followed by another reduction that parallels cytosolic fatty acid biosynthesis. The more active elongation system adds two carbons to palmitoyl CoA to make it steroyl CoA. The mechanism of elongation is identical with that known in the synthesis of palmitate except the enzyme systems and the acyl carrier protein.

Fatty acid breakdown

The initial step in the conversion of lipids into carbohydrates is the breakdown of triacylglycerols stored in oil bodies by the enzyme lipase, which hydrolyzes triacylglycerols into three fatty acid molecules and one molecule of glycerol. During the breakdown of lipids, oil bodies and glyoxysomes are generally in close physical association.

β–Oxidation of fatty acids

The fatty acid molecules enter the glyoxysome, where they are activated by conversioninto fatty-acyl-CoA by the enzyme fatty-acyl-CoA synthetase. *Fatty-acyl-CoA is the initial substrate for the **β**-oxidation series of reactions.*

In plant seed storage tissues, they are located exclusively in the glyoxysome, an organelle enclosed by a single membrane bilayer that is found in the oil-rich storage tissues of seeds. Acetyl-CoA is metabolized in the glyoxysome and cytoplasm to produce succinate, which is transported from the glyoxysome to the mitochondria.

1. *The first step involves the activation of fatty acid in the presence of ATP and enzyme thiokinase. COASH is consumed and CoA derivative of fatty acid is produced.*

2. *In the second step two hydrogen atoms are removed between α and β-C and a trans α,β – unsaturated fatty acyl CoA is formed. This is catalysed by FAD containing enzyme* acyl -CoA dehydrogenase.

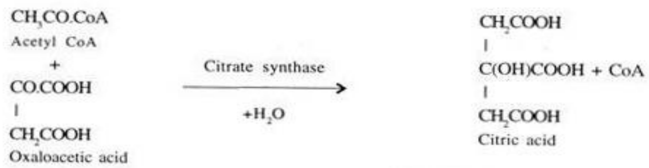
3. *The third step invloves the addition of water molecule acoross the double bond to form corresponding β-hydroxyacyl-CoA in the presence enol hydrase.*

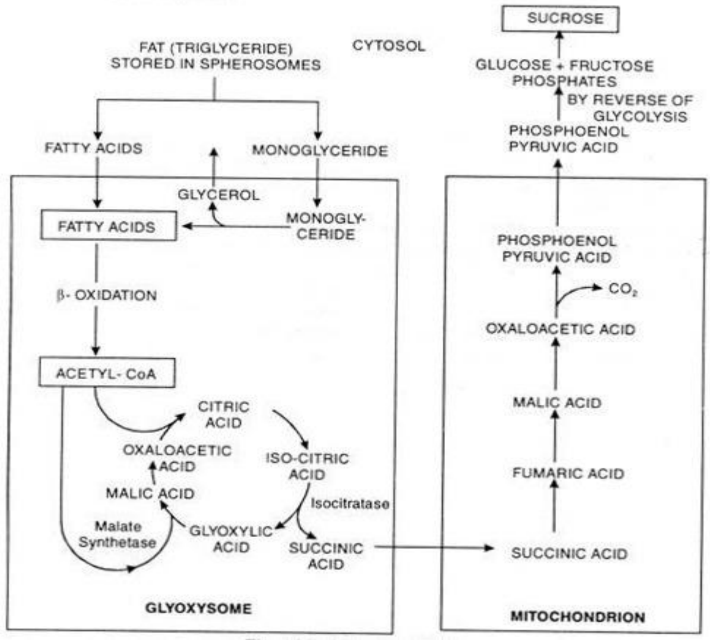
4. *In the fourth step β-hydroxyacyl-CoA is dehydrogenated in the presence of NAD specific* β-hydroxyacyl-CoA dehydrogenase*. Two hydrogen atoms are removed from the β-C atom which now bears a carbonyl function and β- Keto fatty acyl CoA is formed.*

5. *In the fifth and last step involves the thioelastic clevage of β- Keto fatty acyl CoA in the presence of enzyme* β- Keto acyl thiolase *and results in the formation of an active 2-C unit acetyl -CoA and fatty acyl CoA molecule which is shorter by two carbon atoms than when it entered the β-oxidation spiral.*

Glyoxylate cycle

The function of the glyoxylatecycle is to convert two molecules of acetyl-CoA intosuccinate. The acetyl-CoA produced by b-oxidation is further metabolized in the glyoxysome through a series ofreactions that make up the glyoxylate cycle. Initially, the acetyl-CoA reacts with oxaloacetateto give citrate, which is then transferred to the cytoplasmfor isomerization to isocitrate by aconitase. Isocitrate is reimported into the glyoxysome and converted into malateby two reactions that are unique to the glyoxylate cycle.First, isocitrate (C6) is cleaved by the enzyme isocitrate lyase to give succinate (C4) and glyoxylate (C2). The succinate is exported to the mitochondria. Next, malate synthase combines a second molecule of acetyl-CoA with glyoxylate to produce malate. Malate is then transferred to the cytoplasm and converted into oxaloacetate by the cytoplasmic isozyme of malate dehydrogenase. Oxaloacetate is reimported into the glyoxysome and combines with another acetyl-CoA to continue the cycle. The glyoxylate produced keeps the cycle operating, but the succinate is exported to the mitochondria for further processing.





Moving from the glyoxysomes to the mitochondria, the succinate is converted into malate by the two corresponding citric acid cycle reactions. The resulting malate can be exported from the mitochondria in exchange for succinate via the dicarboxylate transporter located in the inner mitochondrial membrane. Malate is then oxidized to oxaloacetate by malate dehydrogenasein the cytosol, and the resulting oxaloacetate is converted into carbohydrates by the reversal of glycolysis (gluconeogenesis).This conversion is facilitated by the enzyme PEP carboxykinase, which uses the phosphorylating ability of ATP to convert oxaloacetate into PEP and CO_2. From PEP, gluconeogenesis can proceed to the production of glucose. Sucrose is the final product of this process, and is the primary form of reduced carbon translocated from the cotyledons to the growing seedling tissues.

Lecture 18: Growth – Phases of growth – Factors affecting growth.

Growth is an irreversible increase in size. It may be evaluated by measurements of mass, length or height, surface area or volume. Growth is restricted only to living cells and is accomplished by metabolic processes involving synthesize of macromolecules, such as nucleic acids, proteins, lipids and polysaccharides at the expense of metabolic energy.

Growth at cellular level is also accompanied by the organization of macromolecules into assemblages of membranes, plastids, mitochondria ribosomes and other cell organelles. Cells do not definitely increase in size but divide, giving rise to daughter cells. An important process during cell division is synthesis and replication of nuclear DNA in the chromosomes, which is then passed into the daughter cells. Therefore, the term growth is used to denote an increase in size by cell division and cell enlargement, together with the synthesis of new cellulose materials and the organization of celluloseorganelles.Growth is also defined as a vital process which brings about a permanent change in any plant or its part in respect to its size, form, weight and volume.

Growth regions

Typical growth regions in plants are the apices of shoot and root. Such growing regions are known as apical meristems, primary meristems or regions of primary growth. These apical meristems are responsible for the increase in length, differentiation of various appendages and formation of plant tissues.

Phases of growth

Growth is not a simple process. It occurs in meristematic regions where before completion of this process, a meristematic cell has to pass through the following 3 phases.

Cell formationphase

Cell elongationphase

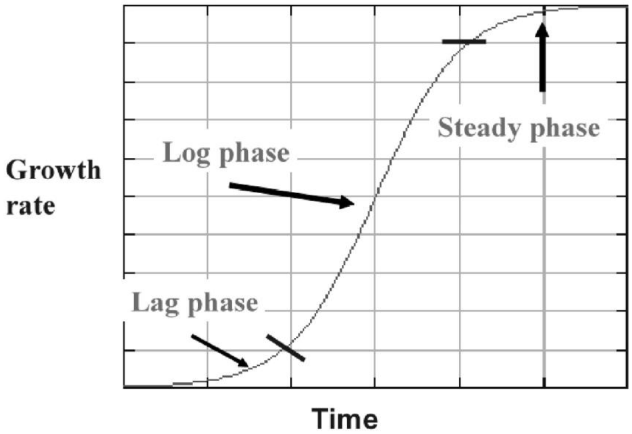
Cell differentiation (cell maturation)

The cell formation phase is represented by meristematic zone and cell enlargement phase by cell elongation zone. The dividing meristematic cells are thin walled and have dense protoplasm with a large nucleus and without or with very small vacuoles. The intercellular spaces are also absent. The newly formed cells after the first phase of cell division have to pass through the second phase of cell enlargement. During the second phase of cell elongation on account of large quantities of solutes inside the growing cell, water enters the cell due to osmotic effect resulting in the increased turgidity and expansion and dilation of the thin and elastic cell wall. This phase also results in appearance of largevacuoles.

In the last phase or cell maturation, second walls are laid down and cell matures and gets differentiated into permanent tissue.

Growth curve

Growth curve is a graph obtained by plotting the growth rate of a plant against time factor. The growth rate of a cell, a plant organ, a whole plant or the whole life cycle of plant is measured in terms of length, size, area, volume or weight .It has been found that different growth phases result in 'S' shaped curve or sigmoid curve. In initial stages during the phase of cell formation, the growth rate increases slowly while it increases rapidly during the phase of cell elongation or cell enlargement and again slows down during the phase of cell maturation.



Phases of Growth

The period during which the course of growth takes place is known as grand period of growth. Thus, in a standard growth curve, three well marked regions can be observed, the initial growth stage (lag phase), the grand period of growth (exponential or log phase) and the steady stage (maturity stage or senescence or stationaryphase).

The overall growth may be affected by external or internal factors but the S- shaped curve of grand period of growth is never influenced. This growth curve suits well to the entire life of an annual plant when measured interms of dry weight against time. Early growth of the plant is limited by the amount of food reserves in the seed. When the emerged seedlings develop an adequate root system and enough leaf surfaces to support vigorous photosynthesis and anabolism, a period of rapid increase in size is possible.

High metabolic rates are not maintained indefinitely and eventually processes are set in motion that leads to cessation of growth. The factors responsible for the decrease in growth are competition for essential metabolites, growth substances, water, light or the accumulation of inhibitors, toxic substances or waste materials. Blackman (1919) suggested that the growth of the plants can be represented by equation.

$$W_1 = Wo\ e^{rt}$$

Where, W1is the final size after time t. Wo is the initial size at the beginning of the time period. r is the rate at which plant substance is laid down during time t and e is the base of natural logarithm. Blackman pointed out that equation describes the way in which money placed at compound interest increases with time; the term compound interest law is used to describe such phenomenon. In banks, compound interest is usually applied quarterly or annually so that the increase in amount occurs as a jump. With plant system, compound interest is applied continuously and size increase follows a smoothcurve.

From the equation, the final size of an organism (W_1) depends on the initial size (W_o). Larger seed give a larger plant. In addition, equation shows that plant size also depends on the magnitude of the relative growth rate. Blackman suggested that r might be used as a measure of the ability of the plant to produce new plant material and called r as the efficiency index. The plants with high efficiency index could be expected to outperform plants with low efficiency index.

Measurement of growth

The measurement of growth is possible in terms of either increase in weight or increase in volume or area. The common and simplest method for the measurement of growth can be a direct method by which the growth is measured by a scale at regular intervals from beginning to end. The other methods that can be used are horizontal microscope, auxanometers.

Factors influencinggrowth

Growth is affected by all factors that affect the activity of protoplasm. Both physiological and environmental factors such as water, minerals, photosynthesis, respiration, climate and edaphic factors significantly influence the growth. In general, factors can be grouped into external and internal factors.

External factors

Light: *It has direct effect on photosynthesis and transpiration. Light in terms of intensity, quality and periodicity influence the growth very much.*

Light intensity: A weak light promotes shortening of internodes and affects expansion of leaf. Very weak light reduces the rate of overall growth and also photosynthesis due to poor development of chlorophyll and higher rate of water loss from the plant.

Light quality: The different wave lengths of light have different responses to growth. In blue violet radiation, the internodal growth is pronounced while green color light promotes the expansion of leaves as compared to complete spectrum of visible light. The red light favors the growth while infrared and UV is detrimental to growth.

Light duration: There is remarkable effect of the duration of light on the growth. The induction and suppression of flowering depend on duration

Temperature*: The plants have different temperature requirements based on the region where they are grown. In general, best growth takes place between 28 and 33 C and it varies from temperate to tropical conditions. The optimum temperature requirement is essential for seed germination, growth, metabolic activities, flowering and yield.*

Oxygen*: The growth of the plant is directly proportional to the amount of oxygen which is essential for respiration during which the food materials are oxidized to release energy.*

Carbondioxide*: It is one of the major factors that influence the photosynthesis. The rate of photosynthesis increases as the availability of CO2 increases while other factors are not limiting.*

Water*: Waterisanessentialfactorforgrowth.Itisessentialforuptakeof nutrients, translocation of nutrients and food materials, regulating transpiration and for various physiological processes like photosynthesis, respiration and enzymatic activities.*

Nutrients and food materials*: The rate of growth is directly proportional to the availability of nutrients and food materials. The shortage of food supply affects the growth as it provides thegrowthmaterialtothegrowingregionandalsoitprovidesthepotentialenergy to the growingregion.*

Internal factors

Internal factors includes growth hormones and their availability, Resistance to climatic, edaphic and biological stresses, Photosynthetic rate and respiration, Assimilate partitioning and nitrogen content, Chlorophyll and other pigments, Source-sink relationship and enzyme activities

Lecture 19: Hormones and plant growth regulators (PGR): physiological roles and agricultural uses - Biosynthetic pathway and role of auxins and gibberellins

Plant growth regulators or phytohormones or natural growth hormones of plants are organic substances produced naturally in higher plants, controlling growth or other physiological functions at a site remote from its place of production and active in minute amounts. Thimmann (1948) proposed the term Phytohormone as these hormones are synthesized in plants.Plant growth regulators include auxins, gibberellins, cytokinins, ethylene, growth retardants and growth inhibitors. Auxins are the hormones first discovered in plants and later gibberellins and cytokinins were also discovered.

Hormone

An endogenous compound, which is synthesized at one site and transported to another site where it exerts a physiological effect at very low concentration. But ethylene (gaseous nature), exert a physiological effect only at a near a site where it is synthesized. Classified definition of a hormone does not apply to ethylene.

Plant growth regulators

Defined as organic compounds other than nutrients, that affects the physiological processes of growth and development in plants when applied in low concentrations. It can be also defined as either natural or synthetic compounds that are applied directly to a target plant to alter its life processes or its structure to improve quality, increase yields, or facilitate harvesting.

Important groups of growth hormones in plant system are:

Auxins

Auxinsareagroupofphytohormonesproducedintheshootandrootapices and they migrate from the apex to the zone of elongation. Auxins promote the growth along the longitudinal axis of the plant and hence the name (auxeing: to grow). The term, auxin was introduced by Kogl and Haagen- Smit (1931). Went (1928) isolated auxin from the Avenacoleoptile tips by a method called Avenacoleoptileorcurvaturetestandconcludedthatnogrowthcanoccurwithoutauxin. Auxins are widely distributed throughout the plant however, abundant in the growing tips such as coleoptile tip, buds, root tips and leaves. Indole Acetic Acid (IAA) is the only naturally occurring auxin in plants. The synthetic auxinsinclude, IBA (Indole Butyric Acid),NAA:

Naphthalene Acetic acid,MCPA: 2 Methyl 4 chloro phenoxy acetic acid TIBA: 2, 3, 5 Tri iodo benzoic acid. IAA is the only naturally occurring auxin in plants. The others such as IAA, IBA, NAA, 2, 4, D and 2, 4, 5 –T are synthetic auxins.

Distribution of auxin in plants

In plants auxin(IAA) is synthesized in growing tips or meristematic regions from where it is transported to other plant parts. Therefore, the highest concentration of IAA is found in growing shoot tips, young leaves and developing auxiliary shoots. In monocot seedling, the highest concentration of auxin is found in coleoptile tip which decreases progressively towards its base. In dicot seedlings, the highest concentration is found in growing regions of shoot, young leaves, developing axillary shoots.

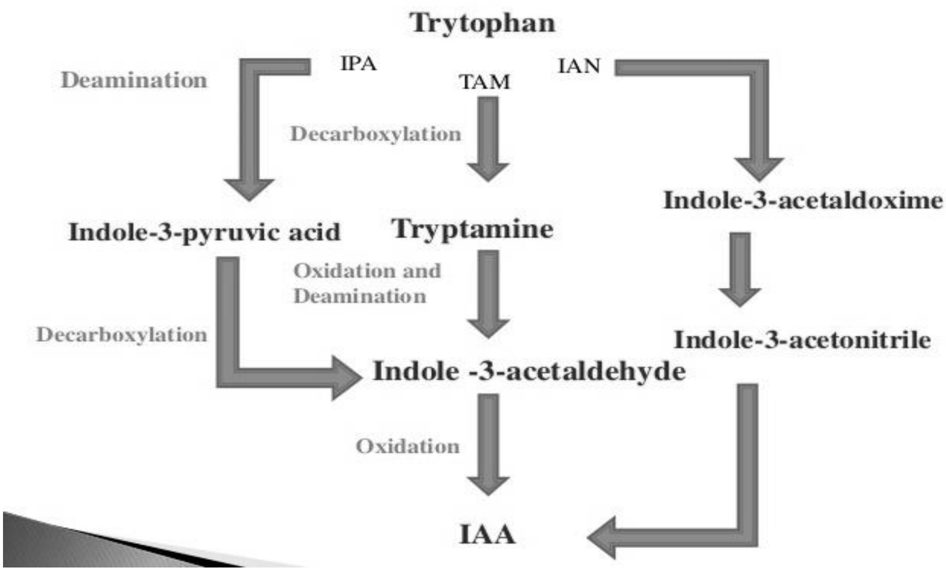
Within the plants, auxin may be present in two forms. Free auxins and bound auxins. Free auxins are those which are easily extracted by various organic solvents such as diethyl ether. Bound auxins on the other hand, need more drastic methods for their extraction from plants such as hydrolysis, autolysis, enzymolysis etc. Bound auxins occur in plants as complexes with carbohydrates such as glucose, arabinose or sugar alcohols or proteins or aminoacids such as aspartate, glutamate or with inositol.

Biosynthesis of auxin (IAA) in plants

Thimann (1935) found that an amino acid tryptophan is converted in to indole 3 aceticacid. So tryptophan is the primary precursor of IAA in plants.

Biosynthetic pathways of Auxin

IAA can be formed from tryptophan by two different pathways by deamination of tryptophan to form indole-3-pyruvic acid followed by decarboxylation to from indole-3-acetaldehyde. The enzymes involved are tryptophan deamination and indole pyruvate decarboxylase respectively. By decarboxylation of tryptophan to form tryptamine followed by deamination to form indole -3-acetaldehyde the enzymes involved are tryptophan decarboxylase and tryptamine oxidase respectively. Indole 3-acetaldehyde can readily be oxidized to indole3-aceticacid (IAA) in the presence of indole3-acetaldehydedehydrogenase.



Transport of auxin in plant

The transport of auxin is predominantly polar. In stems polar transport of auxin is basipetal i.e it takes place from apex towards base. Polar transport of auxin is inhibited by 2,3,5 Triiodobenzoicacid (TIBA) and Naphthylthalamicacid (NPA). The substances are called as ***antiauxins***.

Mechanism of Action

IAA increases the plasticity of cell walls so that the cells stretch easily in response to turgor pressure. It has been suggested that IAA acts upon DNA to influence the production of mRNA. The mRNA codes for specific enzymes responsible for expansion of cellwalls. Recent evidences indicate that IAA increases oxidative phosphorylation in respiration and enhanced oxygen uptake. The growth stimulation might be due to increased energy supply and it is also demonstrated that auxin induces production of ethylene in plants.

Physiological effects of auxin

Cell division andelongation

The primary physiological effects of auxin are cell division and cell elongation in the shoots. It is important in the secondary growth of stem and differentiation of xylem and phloem tissues.

Apicaldominance

In many plants, if the terminal bud is intact and growing, the growth of lateral buds just below it remains suppressed. Removal of the apical bud results in the rapid growth of lateral buds. This phenomenon in which the apical bud dominates over the lateral buds and does not allow the lateral buds to grow is known as apical dominance.

Skoog and Thimmann (1948) pointed out that the apical dominance might beunderthecontrolofauxinproducedattheterminalbudandwhichistransported downward through the stem to the lateral buds and hinders the growth. They removed the apical bud and replaced it with agar block. This resulted in rapid growth of lateral buds. But when they replaced the apical bud with agar block containing auxin, the lateral buds remained suppressed and did not grow.

Root initiation

In contrast to stem, the higher concentration of auxin inhibits the elongation of roots but the number of lateral roots is considerably increased i.e., higher concentration of auxin induces more lateral branch roots. Application of IAA in lanolin paste) to the cut end of a young stem results in an early and extensive rooting.This fact is of great practical importance and has been widely utilized to promote root formation in economically useful plants which are propagated bycuttings.

Prevention of abscission

Natural auxins prevent the formation of abscission layer which may otherwise result in the fall of leaves, flowers and fruits.

Parthenocarpy

Auxin can induce the formation of parthenocarpic fruits (fruit formation without pollination and fertilization).In parthenocarpic fruits, the concentration of auxin in the ovaries is higher than in the ovaries of plants which produce fruits only after fertilization. In the later cases, the concentration of the auxin in ovaries increases after pollination andfertilization.

Respiration

Auxin stimulates respiration and there is a correlation between auxin induced growth and respiration. Auxin may increase the rate of respiration in directly through increased supply of ADP by rapidly utilizing ATP in the expandingcells.

Callusformation

Besides cell elongation, auxin may also be active in cell division. In many tissue cultures, where the callus growth is quite normal, the continued growth of such callus takes place only after the addition of auxin.

Eradication ofweeds

Some synthetic auxins especially 2,4-D and 2,4,5-T are useful in eradication of weeds at higher concentrations.

Flowering and sexexpression

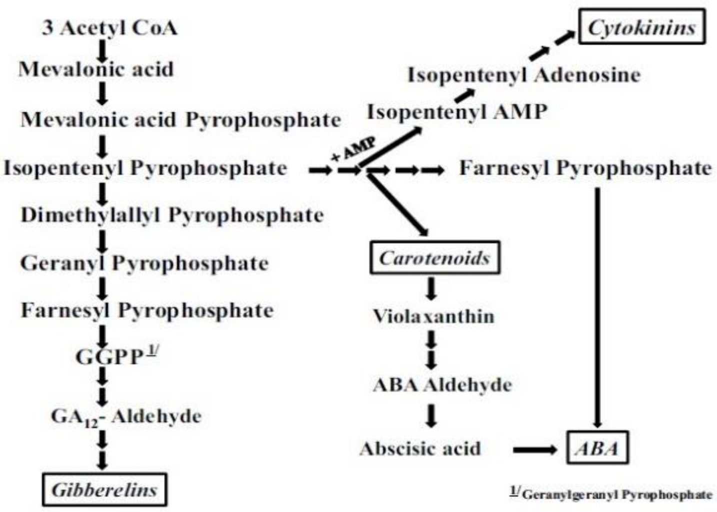
Auxins generally inhibit flowering but in pineapple and lettuce it promotes uniform flowering.

Gibberellins

A Japanese scientist Kurosawa found that the rice seedlings infected by the fungus *Gibberella fujikuroi* grow taller and turned very thin and pale. An active substance was isolated from the infected seedlings and named as Gibberellin.

Biosynthesis of gibberellins in plants

The primary precursor for the formation of gibberellin is ***acetate.***



Distribution of gibberellins in plant

Gibberellins are found in all parts of higher plants including shoots, roots, leaves, flower, petals, anthers and seeds. In general, reproductive parts contain much higher concentrations of gibberellins than the negative parts. Immature seeds are especially rich in gibberellins (10-100 mg per g fresh weight).In plants, gibberellins occur in two forms free gibberellins and bound gibberellins. Bound gibberellins usually occur as gibberellin –glycosides.

Physiological Effects of Gibberellins

Seed germination

Light or cold treatments of dormant seeds have been shown to lower the amount of ABA and increase the concentration of bioactive GA, ending dormancy and promoting germination. Treatment of seeds with bioactive GA can often substitute for the light or cold treatment needed to break dormancy. Certain light sensitive seeds eq., lettuce and tobacco, show poor germination in dark. Germination starts vigorously if these seeds are exposed to light or red light. This requirement of light is overcome if the seeds are treated with gibberellic acid in dark. During germination, GA induces the synthesis of hydrolytic enzymes, such as amylases and proteases in cereal grains. These enzymes degrade the stored food reserves accumulated in the endosperm or embryo as the seed matured. This degradation of carbohydrates and storage proteins provides nourishment and energy to support seedlinggrowth.

Dormancy ofbuds

In temperature regions the buds formed in autumn remain dormant until next spring due to severe cold. This dormancy of buds can be broken by gibberellin treatments. In potato also, there is a dormant period after harvest, but the application of gibberellins sprouts there fer vigorously. In temperature regions the buds formed in autumn remain dormant until next spring due to severe cold. This dormancy of buds can be broken by gibberellin treatments. In potato also, there is a dormant period after harvest, but the application of gibberellin stimulates sprouting vigorously.

Rootgrowth

Gibberellinshavelittleornoeffectonrootgrowth.Athigherconcentration, some inhibition of root growth may occur. The initiation of roots is markedly inhibited by gibberellins in isolated cuttings. Gas may not have dramatic effects on stem elongation in plants that are already “tall”, since bioactive GA may not to be limiting in some tall plants. However, applied Gas can promote internode elongation very dramatically in genetically dwarf mutants, in “rosette” species and in various members of the Poaceae (grass family). Exogenous GA causes such extreme stem elongationindwarfmaizeplantsthattheyresemblethetallestvarietiesofthesame species.Gibberellins arealsoimportantforrootgrowth.Extremedwarfmutantsof pea and Arabidopsis, in which GA biosynthesis is blocked, have shorter roots than wild type plants and GA application to the shoot enhances both shoot and root elongation.

Elongation ofinternodes

The most pronounced effect of gibberellins on the plant growth is the elongation of the internodes. Therefore, in many plants such as dwarf pea, dwarf maize etc., gibberellins overcome the genetic dwarfism.

Bolting andflowering

GAs can substitute for the long-day requirement for flowering in many plants, especially rosette species. In the case of the juvenile to adult transition, the nature of the effect of GA on sex determination can vary with species. In dicots such as cucumber, hemp and spinach, GAs promote the formation of staminate (male) flowers and inhibitors of GA biosynthesis promote the formation of pistillate(female) flowers. in some other plants, such as maize, GAs suppress stamen formation land promotepistilformation.

In many herbaceous plants, the early period of growth shows rosette habit with short stem and small leaves. Under short days, the rosette habit is retained while under long days bolting occurs i.e. the stem elongates rapidly into floral axis bearing flower primordia. This bolting can also be induced in such plants by the application of gibberellin even under non-inductive shortdays. In Hyoscyamusniger *(a long day plant) gibberellin treatment causes bolting and flowering under non-inductive short days. While in long day plants the gibberellins treatment usually results in early flowering. In short day plants, its effects are quite variable. It may either have no effect or inhibit or may activateflowering.*

Parthenocarpy

Germination of the pollen grains is stimulated by gibberellins, likewise, the growth of the fruit and the formation of parthenocarpic fruits can be induced by gibberellin treatment. In many cases, ex. pome and stone fruits where auxins have failed to induce parthenocarpy, the gibberellins have proven to be successful. Seedless and fleshly tomatoes and large sized seedless grapes are produced by gibberellin treatments on commercial scale.

Synthesis of the enzyme α -amylase

One important function of gibberellins is to cause the synthesis of the enzyme α-amylase in the aleurone layer of the endosperm of cereal grains during germination. This enzyme brings about hydrolysis of starch to form simple sugars which are then translocated to growing embryo to provide energy source.

GAs regulate the transition from juvenile to adultphase

Many woody perennials do not flower or produce cones until they reach a certain stage of maturity and up to that stage they are said to be juvenile. Applied Gas can regulate phase change, though whether GA hastens or retards the juvenile to adult transition will depend on the species.In many conifers, the juvenile phase, which may last up to 20 years, can be shortened by treatment with GA_3 or with a mixture of GA_4 and GA_7, and much younger plants can be induced to enter the reproductive, cone-producing phase precociously

GAs promote early seed development and germination

Some GA-deficient mutants or transgenic plants with enhanced GA inactivation, have increased seed abortion.The failure of seeds to develop normally can be attributed to reduced levels of bioactive Gas in very young seeds.Treatment with GA will not restore normal seed development, because exogenous GA cannot enter the new seeds. However, the effect of GA deficiency on seed abortion can be negated by simultaneous expression of mutations that give a constitutive GA response. Taken together, these results provide evidence for a role for GA in the early stages of seeddevelopment.

GAs promote pollen development and tube growth

Gibberellin deficient dwarf mutants (ex., in Arabidopsis and rice) have impaired anther development and pollen formation and both these defects, which lead to male sterility, can be reversed by treatment with bioactive GA. In addition, reducing the level of bioactive GA in Arabidopsis by overexpressing a GA deactivating enzyme severely inhibits pollen tube growth. Thus, GAs seems to be required for both the development of the pollen grain and the growth of the pollen tube.

Lecture 20: Plant growth regulators (PGR): physiological roles and agricultural uses - Biosynthetic pathway and role of cytokinin, ethylene and ABA

Cytokinins

Kinetin was discovered by Skoog and Miller(1950) from the tobacco pith callus in culture and the chemical substance was identified as 6-furpurcyl aminopurine. Because of its specific effect on cytokinesis (celldivision), it was called as cytokinin or kinetin. Cytokinins, besides their main effect on cell division, also regulate growth, hence they are considered as natural plant growth hormones. The term, cytokinin was proposed by Letham.Chemically cytokinins are are purine derivatives.Cytokinins,besides their main effect on celldivision, also regulate growth and hence they are considered as natural plant growth hormones. Some of the very important and commonly known naturally occurring cytokinins are **Coconut milk factor** and Zeatin. It was also identified that cytokine in as a constituent of t-RNA. Cytokinins, besides their main effect on cell division also regulate growth and hence they are considered as natural plant growth hormones.

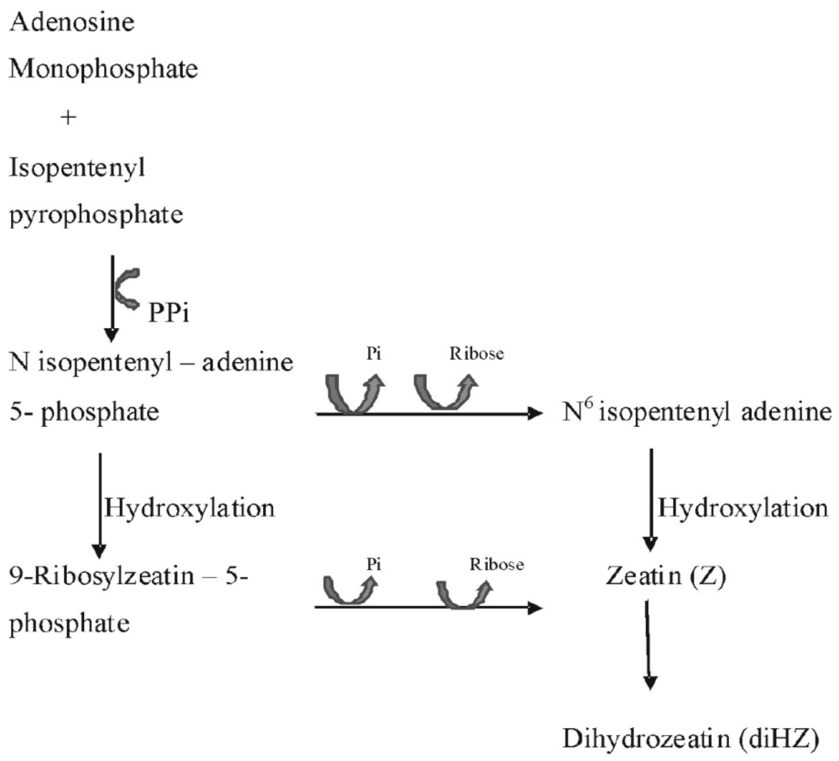
Naturally occurringcytokinins

Naturally occurring cytokinins are all adenine derivatives with either anisoprene-related side chain or an aromatic (cyclic) side chain. The former is called isoprenoid cytokinins and the latter are called aromatic cytokinins. Although there is some variation depending on species and developmental stage,the most common isoprenoid cytokinins are N6-(2-isopentenyl)-adenine (iP), trans-Zeatin (tZ), and dihydrozeatin (DZ). The aromatic cytokinins, such as benzyl adenine (BA) are less common and are found in only a fewspecies.

Zeatinisthemostabundantnaturallyoccurringfreecytokinin.Itsmolecular structure is similar to that of kinetin. Although they have different side chains, in both cases the side chain is linked to the nitrogen attached toC_6(=N_6) of adenine. Because the side chain of Zeatin has a double bond, it can exist in either the cis or the trans configuration. Since its discovery in immature maize endosperm, Zeatin has been found in many plants and in some bacteria. Zeatin and P (isopentenyl - adenine) are thought to be the most biologically active cytokinins in most plants. Reduction of the double bond in the side chain of Zeatin would give the dihydrozeatin derivative, which is particularly active in some species oflegumes.

Biosynthesis

It is assumed that cytokinins are synthesized as in the case of purinesin plants (nucleicacidsynthesis). Root tip is an important site of its synthesis. However, developing seeds and cambial tissues are also the site ofcytokininbiosynthesis. However, basipetal movement in petiole and isolated stems are alsoobserved. Cytokininsare biosynthesized from adenosine monophosphate (AMP) and isopentenyl pyro phosphate (IPP) by condensation reaction catalyzed by enzyme isopentenyl transferase, the product obtained isopentenyl–adenosinemonophosphateisprecursorforallothernaturalcytokinins.A direct cytokinin synthesis is possible via the condensation of dimethylalyl pyrophosphate (DMAPP) and 5' AMP to form isopentenyladenosine 5' - phosphate. Subsequent dephosphorylation and de-ribosylation and / or side chain hydroxylation lead to the production of cytokinins. The condensing enzyme is isopentenyl transferase.Indirect cytokinin synthesis mediated by tRNA - An alternative indirect biosynthetic rout is via isopentanylated tRNA. Cytokinins are known to be regulators ofcelldivisioninmaturecells.Themostimportanteffectofcytokininsis stimulation. Some of the very important and commonly known naturally occurring cytokininsareCoconutmilk factor, Zeatin



Cytokinins can be extracted from coconut milk (liquid endosperm of coconut), tomato juice, flowers and fruits of Pyrus malus; fruits of Pyrus communis (Pear), Prunus cerasiferae (plum) and Lycopersicum esculentum (bhendi);immature fruits of Zeamays, Juglanssp.and Musasp; female gametophytes of Ginkgobiloba; fruitlets, embryo and endosperms of Prunuspersica; seedling of Pisum sativum;root exudates of Helianthus annuus.According to Skoog and Armstrong, at least seven well established types of cytokinins have been reported from the plants. There are also synthetic cytokinin compounds, most notably of which are the diphenyl urea type cytokinins, such as thidiazuron, which is used commercially as a defoliant and as herbicide.

Cytokinins are synthesized in roots, developing embryos,young leaves, fruits. A major site of cytokinin biosynthesis in higher plants is the root. High cytokinin levels have been found in roots, especially the mitotically active root tip and in the xylem sap of roots from a variety of sources.It is generally concluded that roots are a principal source of cytokinins in most,if not all plants and that they are transported to the aerial portion of the plant through the xylem. Immature seeds and developing fruits also contain high levels of cytokinins.The first naturally occurring cytokinins were isolated from milky endosperm of maize and developing plum fruits. On the other hand, there is now evidence that cytokinins are not always a long-distance messenger. Meristematic cells in the shoot apical meristem and floral meristems in particular are under the control of locally produced cytokinins.Certain insects secrete cytokinins, which play a role in the formation of the galls these insects use as feeding sites. Root-knot nematodes also produce cytokinins, which may be involved in manipulating the host to produce the giant cells from which the nematode feeds.

Physiological effects of cytokinins

Cell division

The most important biological effect of kinetin on plants is to induce celldivision especially into baccopithcallus, carrot root tissue, soybean cotyledon,pea callus etc.

Cell enlargement

Like auxins and gibberellins, the kinetin may also induce cell enlargement. Cortical cells of tobacco root were observed to enlarge four times of their normal size in the presence of kinetin. Significant cell enlargement has been observed in the leaves of Phaseolus vulgaris, pumpkin cotyledons, tobacco pith culture,cortical cells of tobacco rootsetc.

Counteraction of apical dominance or Promotion of lateral budgrowth

External application of cytokinin promotes the growth of lateral buds and hence counteracts the effect of apical dominance.Application of cytokinins reduces apical dominance. The action of cytokinin is antagonistic to that of auxin in apical dominance. The lateral buds of intact plants which otherwise remain arrested can be made to grow by applying kinetin. It may be due to the differentiation of vascular tissue in the presence of cytokinins. The pathogen Corynebactrerium facians causesa disease called witches broom in many plants. This symptom is characterized by loss of apical dominance and emergence of numerous lateral branches which give the appearance of a broom. This effect is due to the secretion of cytokinin namely isopentenyl adenine by the pathogen.

Dormancy ofseeds

Like gibberellins,the dormancy of certain light sensitive seeds such as lettuce and tobacco can also be broken by kinetintreatment.

Delay of senescence

Cytokinins delay senescence. Generally, protein and chlorophyll content of the leaf decreases with the increase in age. Thus, when leaf becomes old, it turns into yellow, become senescent and finally shed of. Senescence of leaves can be delayed by application of kinetin. Cytokinins delay senescence by increased synthesis of proteins. The senescence of leaves usually accompanies with loss of chlorophyll and rapid breakdown of proteins. Senescence can be postponed to several days by kinetin treatment byimproving RNAsynthes is followed by protein synthesis. Richmond and Lang while working on detached leaves of Xanthium found that kinetin was able to postpone the senescence for a number of days. The delay of senescence of leaves and other organs of the plants by cytokininsis called as Richmond - Langeffect.

Morphogenesis: Root and bud differentiation

Cytokinins in interaction with auxins control morphogenesis. If the ratio of auxin to cytokinin is more in the medium, a number of roots are initiated from the callus.If the ratio is less (which means more cytokinins than auxins) a number of shoot buds are initiated. It has been shown that high auxin and low kinetin produced only roots whereas high kinetin and low auxin could promote formation of shoot buds.

Flower induction

Cytokinins can be employed successfully to induce flowering in short dayplants.

Accumulation and translocation ofsolutes

Plants accumulate solutes very actively with the help of Cytokinin and also help in solute translocation in phloem.

ChloroplastDevelopment

Cytokinins greatly enhance conversion of etioplasts into chloroplasts,when etiolated seedlings after treatment with cytokinins are exposed to light.

Cytokinins inhibit rootgrowth

Cytokinin plays a very different role in the root apical meristem than it does in the shoot apical meristem.In contrast to its effect on the shoot, overexpression of cytokinin oxidase in tobacco increases root growth, primarily by increasing the size of the root apical meristem. Similarly, mutations that partially disrupt cytokinin perception also cause enhanced root growth. The mechanism by which cytokinins negatively regulate root apical meristems has recently been explored. The size of a meristem is determined by the rate at which cells divide minus the rate at which cells exit the meristem by growth and differentiation.Cytokinins accelerate the process of vascular differentiation in the roottip.

Other effects

Cytokinins provide resistance to high temperature, cold and diseases in some plants. They also help in flowering by substituting the photoperiodic requirements. In some cases, they stimulate synthesis of several enzymes involved in photosynthesis.

Commercialapplications

Cytokinins have been used for increasing shelf life of fruits, quickening of root induction and producing efficient rootsystem, increasing yield and oilcontents of oil seeds like groundnut.

Ethylene

The scientific studies to understand phenomenon and technology of fruit ripening were initiated as early as1900s,understanding the process of fruit ripening and identification of the causative factor was possible only in 1924. Since then, detailed studies have been made on ethylene and its effect on plants. He observed that dark grown pea seedlings growing in the laboratory exhibited symptoms that were later termed as triple response includes reduced stemelongation,increased lateral growthandabnormal horizontalgrowth.

Ethylene, another class of hormone with a single representative, is a simple gaseous hydrocarbon with the chemical structure $H_2C=CH_2$ Ethylene is apparently not required for normal vegetative growth, although it can have a significant impact on the developmentof roots and shoots. Ethylene appears to be synthesized primarily in response to stress and may be produced in large amounts by tissues undergoing senescence or ripening. It is commonly used to enhance ripening of fruits.

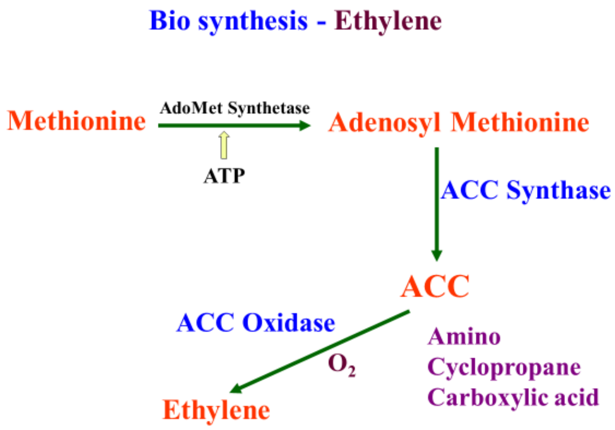
Occurrence

Ethylene can be produced by almost all parts of higher plants.Ethylene occurs in all plant organs-roots, stems,leaves,bulbs,tubers,fruits,seeds,andsoon,althoughthe rate of production may vary depending on the stage of development. Ethylene production will also vary from tissue to tissue within the organ, but is frequently located in peripheral tissues. In peach and avocado seeds, for example, ethylene production appears to be localized primarily in the seed coats, while in tomato fruit and mung bean hypocotyls it originates from the epidermal regions.

Ethylene production increases during leaf abscission and flower senescence, as well as during fruit ripening. Any type of wounding can induce ethylene biosynthesis, as that of physiological stresses such as flooding,disease and temperature or drought stress. In addition, infection by various pathogens can also elevate ethylene biosynthesis.

Biosynthesis

The ethylene biosynthesis is a three-step pathway in higherplants.The amino acid methionine is the precursor of ethylene and ACC (1-amino cyclo propane 1- carboxylic acid) serves as an intermediate in the conversion of methionine to ethylene. In the first step, an adenosine group is donated to methionine by a molecule of ATP, thus forming S-adenosyl methionine (SAM). This reaction is mediated by the enzyme SAM synthetase. In the next step, SAM breaks into 5’-methyl thio adenosine (MTA) and amino cyclo propane carboxylic acid (ACC). This reaction is carried out by the enzyme ACC synthase. ACC is oxidized to ethylene with the release of HCN and CO_2. Ethylene biosynthesis is stimulated by several factors, including developmental state,environmental conditions, other plant hormones and physical and chemical injury. Ethylene biosynthesis also varies in a circadian manner, peaking during the day and reaching a minimum at night.



Ethylene inhibitors

Ethylene inhibitors are frequently used to study biosynthes is of ethylene and ethylene activity. AVG (aminoethoxy vinyl glycine) and AOA (amino oxy-aceticacid) are inhibitors of ethylene biosynthesis. AVG andAOA block the conversion of SAM to ACC.1-methylcyclopropane (1-MCP), NBD (2,5-norbomadiene),Silvernitrate and Silver thio sulphate(STS) are the specific inhibitors of ethylene action.They inhibit an ethylene response by binding to and blocking of the ethylene receptor.

Physiological roles of ethylene

Fruitripening

Fruits can be broadly classified into two types on the basis of their respiratory pattern during ripening. In some fruits like apple and banana as the fruit matures and attains its maximum size, the rate of respiration decreases and becomes very low. After the fruit is harvested and stored for ripening, there is a great increase in the rate of respiration and the rise continues till it attains a sharp peak. This is called climacteric peak and the fruits are called climacteric fruits. In climacteric fruits ripening occurs even after harvesting. The climacteric rise is soon followed by a sharpdecline.

The non-climacteric fruits like grapes and lemon, the respiratory rate gradually decrease after the fruit is harvested without showing any abrupt rise. The peak respiratory rate in climacteric fruits usually corresponds to peak ethylene production. Application of ethylene hastens ripening of climacteric fruits such as banana, mango, apple and tomato. This is being commercially employed. In non- climacteric fruits such as lemon and orange ethylene application does not hasten ripening however rate of respiration increases greatly.

Abscission and senescence

Ethylene promotes both abscission and senescence of flowers. The flowers of orchids and roses are the most sensitive to externally applied ethylene. Ethylene also promotes leaf abscission. In general, olderleaves are more sensitive to ethylene and abscise faster than younger ones.Older leaves produce more ethylene than younger ones. This is probably responsible for the abscission of older leaves.

Root and shootgrowth

Ethylene inhibits linear growth of the stem and root of dicotyledons. The effect increases with increasing concentrations.

Flowering and sexexpression

Application of ethylene causes flowering in pine apple and shift the sex ratio of flowers towards femaleness in several cucurbits and cannabis.

Epinasty

Ethylene causes swelling of cells on the upper part of the petiole of the leaf resulting in drooping of leaves (down ward curvature). This is termed as epinasty. It is best exhibited in leaves of tomato, potato and pea etc.

Thinning in apple

Thinning of fruits in apple eliminates biennial bearing and also improves fruit size and quality.Application of ethephon at100 to300ppm reduces fruitset in cotton also ethylene induces fruit thinning.

Exudation of sap and latex

When ethrel is applied to rubber plants, flow of latex continues for a longer duration. Ethrel probably prevents coagulation of latex and consequent blocking of latex synthesis.

Abscissic acid

In1961, Liu and Cams isolated a substance from mature cotton fruits which stimulated abscission of cotton petioles. The structure of this compound, which they called abscisin I, was never determined. Frederick T. Addicot and his coworkers (1963) in California isolated a substance from young cotton fruits which also caused abscission of cotton petioles. They partially characterized it and named as abscisin II, which proved to be ABA. In the same year, P.F. Warring in England isolated an inhibitory substance from birch leaves exposed to short days. It caused buds of growing seedlings to go dormant when applied to them and therefore, named it as dormin. In 1965, Warering working in collaboration with Shell Research Laboratory in England showed that dormin and abscisin II were the same compound named as Abscisic acid (ABA).

Occurrence and transport

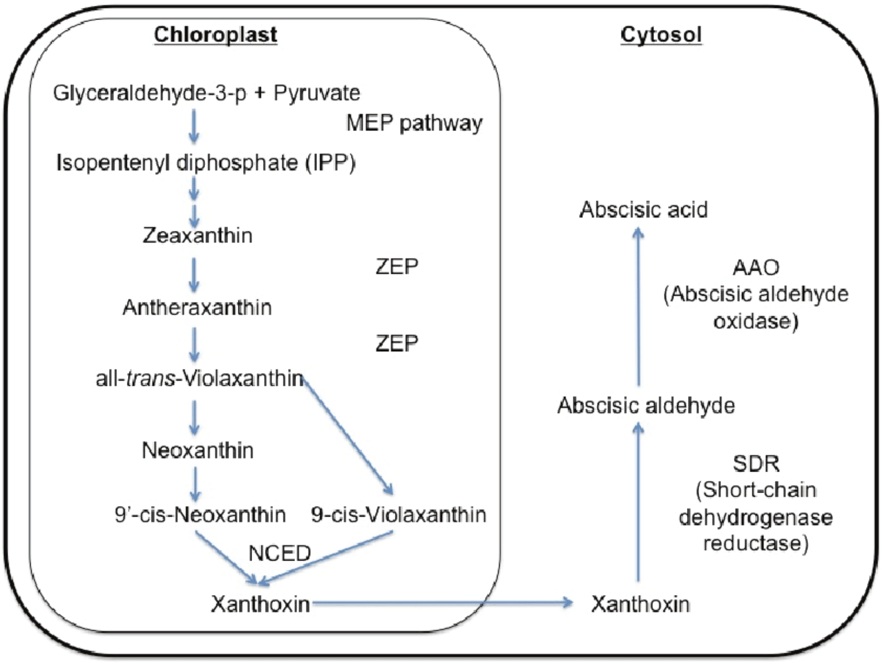
ABA is widely distributed in higher plants, mosses, green algae, fungi and recently in rat brains.. All parts of the plants such as stem, root and leaves, fruits and seeds are also capable of ABA synthesis. Chloroplasts in leaves contain the carotenoids from which ABA arises, whereas in roots, fruits, seed embryos and certain other plant parts, the necessary carotenoids are in chromoplasts, leucoplasts or proplastids. ABA is present in very low concentrations in plants. In most tissues, it varies from 10 to 50 ng g^{-1} fresh weight. In water stressed leaves, developing and mature seeds and dormant buds, its level may be higher than 10^{-6} M.

Biosynthesis

Abscisicacid (ABA) is a 15-carbon compound which is partially synthesized in the chloroplasts and other plastids via theMevalonicacid pathway with two potential routes from isopentenyl pyrophosphate.

Thus, early reactions in ABA synthesis are identical to those of isoprenoids such as gibberellins, sterols and carotenoids. The first route is via farnesyl pyrophosphate to ABA and second via carotenoids through a series of steps to ABA. In second pathway, farnesyl pyrophosphate leads to the synthesis of the C40 xanthophyll violoxanthin. It is catalysed by zeaxanthin epoxidase(ZEP).

The violoxanthinis then converted to the C40 compound 9-cis-neoxanthin, which is then cleaved to form C15 compound xanthoxal,previously called xanthoxin. The cleavage is catalysed by 9-cis- epoxycarotenoid *dioxygenase (NCED). NCED synthesis is a key regulatory step for ABA biosynthesis as its synthesis induces rapidly under stress conditions, Finally, xanthoxal is converted to ABAvia oxidative steps involving the intermediate(s) namely ABA-aldehyde and or/ possibly xanthoxioacid.*



Physiological effects of ABA

Abscisicacid plays primary regulatory roles in the initiation and maintenance of seed and bud dormancy and in the plant's response to stress, particularly water stress. In addition, ABA influences many other aspects of plant development by interacting, usually as an antagonist, with auxin, cytokinin, gibberellin, ethylene and brassinosteroids.

Synthesis of storage proteins and lipids, during seed development

The ABA content of seeds is very low during early embryogenesis, reaches a maximum at about the half way point and then gradually falls to low levels as the seed reaches maturity.Thus there is a broad peak of ABAaccumulation in the seed is noted corresponding to mid-to late embryogenesis. This early accumulation ofABA helps to suppress vivipary. During mid to late embryogenesis, when seed ABAlevels are the highest, seeds accumulate storage compounds that will support seedling growth at germination. Another Important function of ABA in the developing seed is to promote the acquisition of desiccation tolerance. As maturing seeds begin to lose water, embryos accumulate sugars and so-called late embryogenesis- abundant (LEA) proteins. Physiological and genetic studies have shown thatABA affects the synthesis of LEAs and of storage proteins and lipids.

Seed dormancy and germination are controlled by the ratio of ABA to gibberellic acid

Seed dormancy may result from coat-imposed dormancy,embryo dormancy, or both.Seed dormancy that is intrinsic to the embryo and is not due to anyinfluence of the seed coat or other surrounding tissues is called embryodormancy.Embryo dormancy is due to the presence of inhibitors, especially ABA, as wellas the absence of growthpromoters, such as GA. Loss of embryo dormancy is often associated with a sharp decrease in the ratio of ABA to GA.

ABA inhibits hydrolytic enzymes in germinating seeds

In addition to the ABA-GA antagonism affecting seed dormancy, ABA inhibits the GA-induced synthesis of hydrolytic enzymes that are essential for the breakdown of storage reserves in germinating seeds. GA stimulates the aleurone layer of cereal grains to produce a-amylase and other hydrolytic enzymes that break down the stored resources in the endosperm during germination.

ABA promotes root growth and inhibits shoot growth

ABA restricts shoot growth only under water stress conditions. When ABA levels are high, endogenous ABA exerts a strong positive effect on primary root growth by suppressing ethylene production. The overall effect is a dramatic increase in the root: shoot ratio at low water potentials, which, along with the effect of ABA on stomatal closure, helps the plant cope with water stress. Furthermore, the temporary inhibition of lateral root growth promotes exploration of new areas of soil, and permits replacement of dehydrated laterals following rehydration.

ABA accelerates senescence

Abscisicacid was originally isolated as an abscission causing factor.However, it has since become evident that ABA stimulates abscission of organs in only a few species and that the hormone primarily responsible for causing abscission is ethylene. On the other hand, ABA is clearly involved in leaf senescence and through its promotion of senescence it might indirectly increase ethylene formation and stimulateabscission.

Abscisic acid closes stomata under waterstress

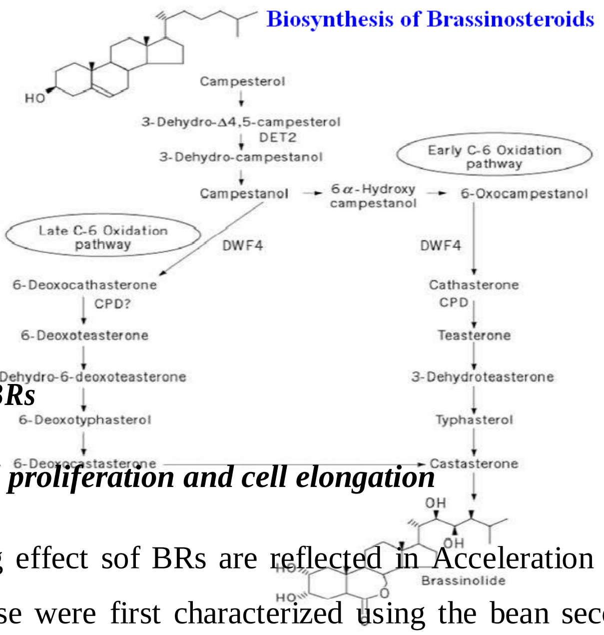
ABA accumulates in water-stressed leaves and exogenous application of ABA is a powerful inhibitor of stomatal opening. the stomata close in response to soil desiccation well before there is any measurable reduction of turgor in the leaf mesophyll cells. Furthermore,ABAis readily translocated from roots to theleaves in the transpiration stream, even when roots are exposed to dry air. These results suggest that ABA is involved in some kind of early warning system that communicates information about soil water potential to the leaves.

Lecture 21 :Novel growth regulators – Brassinosteroids and Jasmonic acid &salicylic acid

Brassinosteroids

Brassinosteroids(BRs)are steroid hormones with achemical structure similar to the steroid hormones in animals. Brassinosteroidselicit an impressive array of developmental responses, including an increased rate of stem and pollen tube elongation, increased rates of cell division (in die presence of auxin and cytokinin), seed germination, leaf morphogenesis, apical dominance, inhibition of root elongation, vascular differentiation, accelerated senescence and cell death, Brassinosteroids are also implicated in mediating responses to both abiotic and biotic stress, including salt, drought, temperature extremal and pathogens.

The study of Brassinosteroids as plant hormones dates back to the early 1970s, when a group of agricultural researchers began screening pollens, already known as a rich source of growth-promoting substances.The result was a complex mixture of lipids that stimulated elongation of bean internodes. Because the most active preparations were isolated from pollen of the rape plant (*Brassica napus*), the active substances were referred to collectively as brassins. Many of the effects of the brassins were similar to those of GA, leading many to believe the extracts were simply crude extracts of gibberellins, rather than a new class of hormones as originally proposed. Later it is identified the active component as brassinolide. Various brassinosteroids.However, the pathway for the biosynthesis of Brassinolideis best understood.The precursor to brassinolide is campesterol, aC28sterol.



Physiological roles of BRs

BRs promote both cell proliferation and cell elongation

The growth-promoting effect sof BRs are reflected in Acceleration of both cell elongation and cell division. These were first characterized using the bean second- internode bioassay. Ther ice leaf lamina inclination bioassay is dependent on BR induced cell expansion. Lamina inclination resembles the epinasty caused by ethylene. In esponse to BR, the cell s on the adaxial (upper) surface of the leaf near the joint region expand more than the cells on the abaxial(lower)surface, causing the vertically oriented leaf to bend outward.An increase in cellwall loosening is required for BR-induced cell expansion ont head axial side of the leaf.The stimulatory effect of BRs on grow this most pronounced in young, growing shoot tissues. In addition to cell elongation, BR also stimulates cellproliferation.

BRs promote root growth at low concentrations and inhibit root growth at high concentration

When applied exogenously,BRs promote rootgrowth at low concentrations and inhibit root growth at high concentrations. The threshold concentration for inhibition depends on the activity of the BR analog used. The effects of BR on root growth are independent of both auxin and gibberellin action. An inhibitor of polar auxin transport, 2,3,5-triiodobenzoic acid (TIBA), does not prevent BR-induced growth. When BR and auxin are applied simultaneously, both the promotive and inhibitory effects on root growth are additive. Observations indicate that BR inhibitionofrootgrowthdoes notinvolveinteractionswitheitherauxinorGA.On the otherhand, high concentrations of BR, like auxin, stimulate ethylene production, so it is possible that at least some of BR's inhibitory effects on root growth are due to ethylene. At low concentrations, BRs can also induce the formation of lateral roots. In these conditions, however, BRs and auxin act synergistically.BRs promote lateral root development partially by influencing polar auxintransport.

BRs promote differentiation of the xylem and suppress the phloem

BRs play an important role in vascular development by promoting differentiation of the xylem and suppressing the phloem.This is evident in the impaired vascular systems of BR mutants, which have a higher phloem to xylem ratio than thewild type. BR- deficient mutants also have a reduced number of vascular bundles with irregular spacing between the bundles. In contrast, mutants overexpressing the BR receptor protein produce more xylem than thewildtype.

BRs promote seedgermination

Seeds, like pollen grains, contain very high levels of BRsand BRs promote seed germinationas well.BRs promote seed germination by interacting with other plant hormones, although the molecular basis for these interactions is not known. It is well established that GA and abscisic acid (ABA) play positive and negative roles, respectively, in stimulating seed germination. BRs can enhance germination of tobacco seeds, independent of GA signaling. Thus, BRs can stimulate germination and are needed to overcome the inhibitory effect of

ABA. As BRs areknown to stimulate cell expansion and division, it is likely that BRs facilitate germination by stimulating the growth of theembryo.

BRs in crop production and stress tolerance

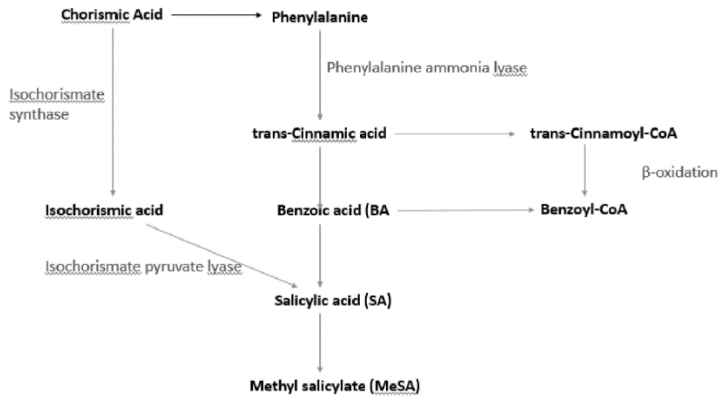
Brassinosteroids were discovered as a classofgrowth promoting hormones and researchers immediately recognized their potential applications in agriculture. For the past 20years, numerous small scale studies have been conducted to test the ability of BRs to increase yields of crop plants.BRs has been found to increase bean crop yield (based on the weight of seeds per plant) by about 45%,and to enhance the leaf weight of various ettuce varieties by 25%. Similar increase in the yields of rice, barley, wheat and lentils have been observed. BRs also promoted potato tuber growth and increased its resistance toinfections.Tomato fruitset was also enhanced by BRs. A crop grown under optimal conditions shows little effect to applied BR,while a crop grown under conditions of stress shows dramatic effects of BR application on yield.

BR plays an important role in many light and hormone regulated processes including expression of light regulated genes, promotion of cell elongation, leaf and chloroplast senescence and floral induction.

SALICYLIC ACID (SA)

Salicylicacid(SA) is known for its medicinal propertiest han for its regulatory roles in plants. Although many physiological effects of SA application have been reported, only a few have been shown to be physiologically important in plants, for example, the regulatory role of SA in thermogenesis of various species and in the response ofplants to pathogens.

Biosynthetic Pathway



Biosynthetic pathway of Salicylic acid

SA biosynthesis occurs through phenylpropanoid pathway, Phenyl alanine ammonia lyase converts Phenylalanine into trans cinnamic which is converted in to benzoic acid (BA), which undergoes 2 hydroxylation by the enzyme BA 2- hydroxylase to form SA. In another pathway, chorismate is first converted to isochorismate by isochorismate synthase (ICS), which is then converted to SA and pyruvate by isochorismate pyruvate lyase (IPL). In another pathway, conversion of trans cinnamic acid to BA, which is then converted to SA.

Physiological functions of Salicylic Acid

Its major function is in the Induction of flowering and thermogenesis regulation. It gives protection against abiotic & biotic stress condition. It gives defences against microbes, insect & pathogens through systemic acquired response (SAR). SA also essential for inducing the expression of pathogenesis related (PR) proteins.

Jasmonate (JA)

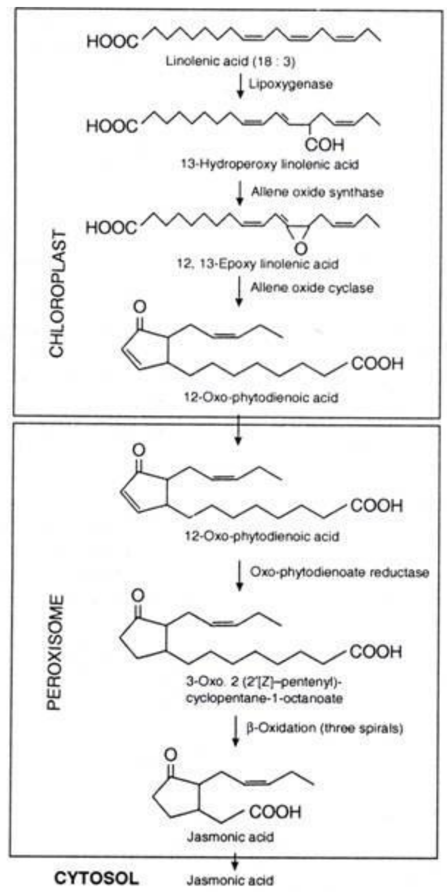
Jasmonic acid and its derivatives are lipid-based hormone signals that regulate a wide range of processes in plants, ranging from growth to reproductive development. In particular, JAs are critical for plant defense against herbivory and plant responses to adverse environmental conditions and other kinds of abiotic and biotic challenges. Some JAs can also be released as volatile organic compounds (VOCs) to permit communication between plants in anticipation of mutual dangers. The isolation of methyl jasmonate from jasmine oil derived from *Jasminum grandiflorum* led to the discovery of the molecular structure of jasmonates and their name.

JA itself can be further metabolized into active or inactive derivatives. Methyl JA (MeJA) is a volatile compound that is potentially responsible for interplant communication. JA conjugated with amino acid isoleucine (Ile) results in JA-Ile, which is currently the only known JA derivative needed for JA signaling.

Biosynthetic pathway

Jasmonate (Jasmonic acid or JA) is a 12-C unsaturated fatty acid with a cyclopentane ring structure and a keto group that occurs along with its methyl ester, methyl jasmonate in wide variety of organisms including fungi, mosses, ferns and higher plants and appears to be ubiquitous in plant kingdom.Higher concentrations of jasmonate are found in young actively growing tissues. Methyl jasmonate is chief component of oil of jasmine (Jasminum, Family Oleaceae) Jasmonates are known to accumulate in wounded plants and plants treated with elicitors.

Jasmonate is synthesized in plants from linolenic acid. Membrane lipids are rich source of linolenic acid in the form of phospholipids from where it is released from the action of the enzyme phspholipases. Linolenic acid is then converted to jasmonic acid through the pathway called octodeconoid signaling pathway.



Biosynthetic pathway of Jasmonic acid

The conversion of linolenic acid into jasmonate is completed in chloroplast and peroxisome. The first three steps of this pathway occurs in chloroplast which result in the formation of cyclized intermediate called 12-oxo-phytodienoic acid. The latter now moves to peroxisome where it is first reduced and then converted into jasmonate by β-oxidation that is repeated thrice.

Physiological roles

Role of Jasmonates

Jasmonates have been shown to inhibit growth of certain plant parts and to strongly promote senescence of leaves. In-fact, jasmonates were first recognized for their ability to promote senescence of detached barley leaf segments.

Jasmonates are believed to modulate the action of a host of genes, influencing a number of other physiological processes in plants such as, (i) seed and pollen germination, (ii) vegetative protein storage and (iii) root development and tendril coiling. In most of these *effects jasmonates appear to act in close harmony with ethylene. Wide ranging physiological effects of jasmonates on plants have prompted some scientists to suggest that they may represent another class of plant growth substances.*

Jasmonates are known to activate genes that encode defensive proteins (such as α-amylase inhibitors, lectins, and proteinase inhibitors) and promote the rapid buildup of phytoalexins that provide strong deterrents to feeding insects and other herbivores. The precise mechanism of action of jasmonates in plant defense responses is not clearly understood. However, extensive studies done by scientists in recent years on legumes, tomatoes and other plants have thrown some light on it.

Lecture No. 22.Photoperiodism - short, long and day-neutral plants – Chailakhyan's theory of flowering

Photoperiodism is the phenomenon of physiological changes that occur in plants in response to relative length of day and night (i.e. photoperiod). The response of the plants to the photoperiod, expressed in the form of flowering is also called as photoperiodism. The phenomenon of photoperiodism was first discovered by Garner and Allard (1920).

Depending upon the duration of photoperiod, the plants are classified into three categories.

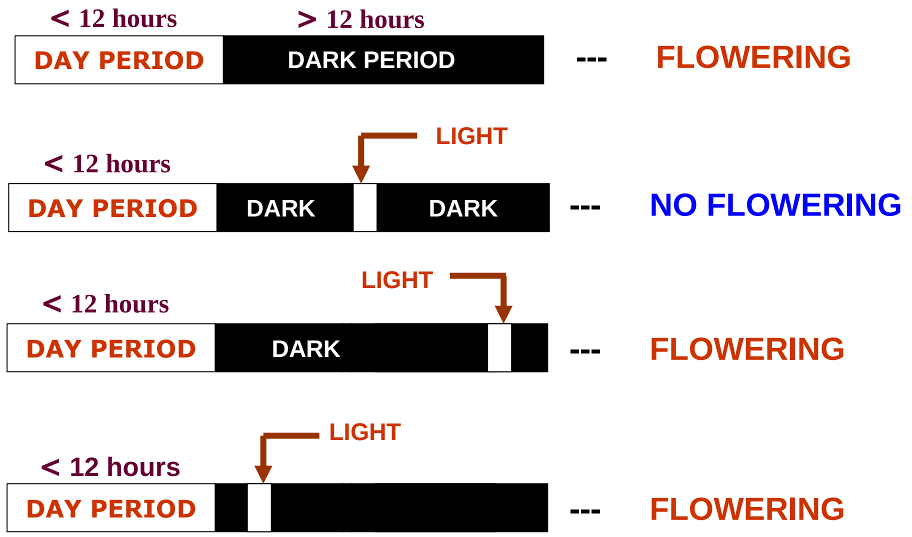
Short day plants (SDP)

Long day plants (LDP)

Day neutral plants (DNP)

Short day plants

SHORT DAY PLANTS

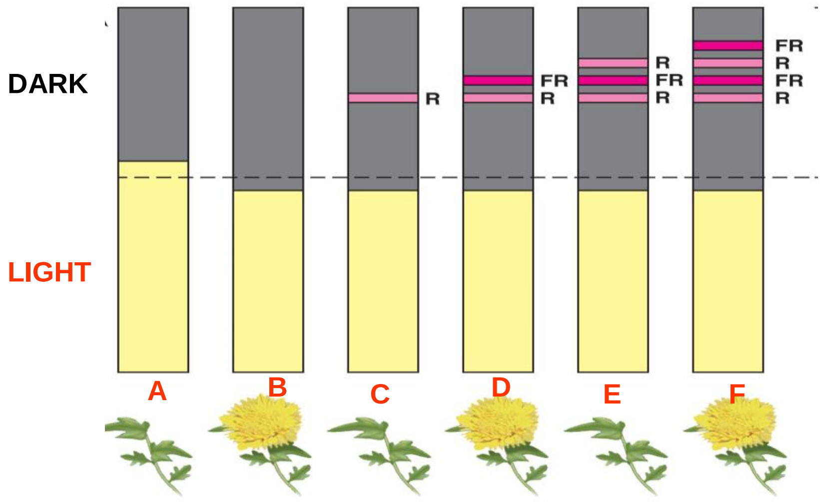


These plants require a relatively short day light period (usually 8-10 hours) and a continuous dark period of about 14-16 hours for subsequent flowering. These plants are also known as long-night plants

E.g. Rice, coffee, soybean, tobacco and chrysanthemum

In short day plants, the dark period is critical and must be continuous. If this dark period is interrupted with a brief exposure of red light (660-665 nm wavelength), the short day plant will not flower.Maximum inhibition of flowering with red light occurs at about the middle of critical dark period.

However, the inhibitory effect of red light can be overcome by a subsequent exposure with far-red light (730-735 mm wavelength).Interruption of the light period with red light does not have inhibitory effect on flowering in short day plants.Prolongation of the continuous dark period initiates early flowering.



SHORT DAY PLANTS - FLOWERING

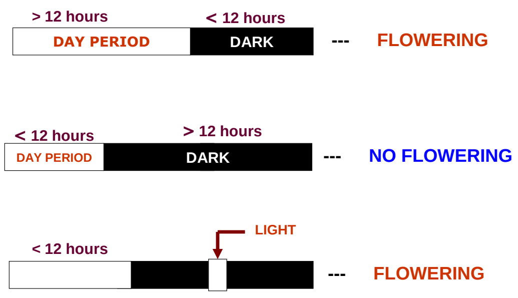
Long day plants

These plants require longer day light period (usually 14-16 hours) in a 24 hours cycle for subsequent flowering. These plants are also called as short night plants.

E.g. Wheat, radish, cabbage, sugar beet and spinach.

- *In long day plants, light period is critical*
- *A brief exposure of red light in the dark period or the prolongation of light period stimulates flowering in long day plants.*

LONG DAY PLANTS



Day neutral plants

These plants flower in all photoperiod ranging from 5 hours to 24 hours continuous exposure.

E.g. Tomato, cotton, sunflower, cucumber, peas and certain varieties of tobacco.

During recent years, intermediate categories of plants such as long short day plants and short long day plants have also been recognized.

Long short day plants

These are short day plants but must be exposed to long days during early periods of growth for subsequent flowering. E.g. Bryophyllum.

Short –long day plants

These are long day plants but must be exposed to short day during early periods of growth for subsequent flowering. E.g. certain varieties of wheat and rye.

Differences between short day and long day plants

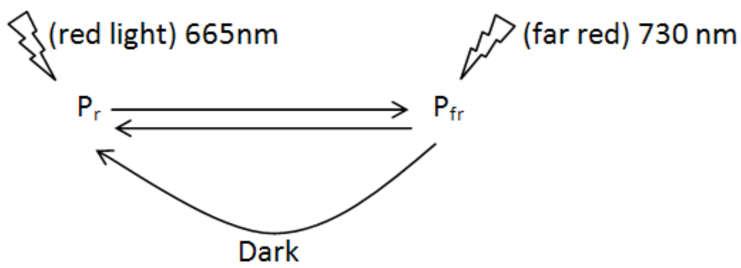
	Short day plant	***Long day plant***
1	*Plants flower when photoperiod is less than the critical day length*	*Plants flower when photoperiod is more than the critical day length*
2	*Interruption during light period with darkness does not inhibit flowering*	*Interruption during light period with darkness inhibit flowering*
3	*Flowering is inhibited if the long dark period is interrupted by a flash of light*	*Flowering occurs if the long dark period is interrupted by a flash of light*
4	*Long continuous and uninterrupted dark period is critical for flowering*	*Dark period is not critical for flowering*
5	*Flowering does not occur under alternating cycles of short day and short light period.*	*Flowering occurs under alternating cycles of short day followed by still shorter dark periods*

Phytochrome

It is observed that that a brief exposure with red light during critical dark period inhibits flowering in a short day plant and this inhibitory effect can be reversed by a subsequent *exposure with far-red light. Similarly, prolongation of the critical light period or the interruption of the dark period stimulates flowering in long-day plants.*

This inhibition of flowering in short day plant and stimulation of flowering in long day plants involves the operation of a proteinaceous pigment called phytochrome. It is present in the plasma membrane of cells and it has two components, chromophore and protein. Phytochrome is present in roots, coleoptiles, stems, hypocotyls, cotyledons, petioles, leaf blades, vegetative buds, flower tissues, seeds and developing fruits of higher plants.

The pigment, phytochrome exists in two different forms i.e., red light absorbing form which is designated as Pr and far red light absorbing form which is designated as Pfr. These two forms of the pigment are photo chemically inter convertible. When Pr form of the pigment absorbs red light (660-665 nm), it is converted into Pfr form. When Pfr form of the pigment absorbs far red light (730-735 nm), it is converted into Pr form. The Pfr form of pigment gradually changes into Pr form in dark.



It is considered that during day time, the Pfr form of the pigment is accumulated in the plants which are inhibitory to flowering in short day plants but is stimulatory in long day plants. During critical dark period in short day plants, this form gradually changes into Pr form resulting in flowering. A brief exposure with red light will convert this form again into Pfr form thus inhibiting flowering. Reversal of the inhibitory effect of red light during critical dark period in SDP by subsequent far-red light exposure is because, the Pfr form after absorbing far-red light (730-354 nm) will again be converted back into Pr form. Prolongation of critical light period or the interruption of the dark period by red-light in long day plants will result in further accumulation of the Pfr form of the pigment, thus stimulating flowering in long-day plants.

Differences between Pr and Pfr forms of phytochrome

	Pr form	*Pfr form*
1	*It is blue green in colour*	*It is light green in colour*
2	*It is an inactive form of phytochrome and it does not show phytochrome mediated responses*	*It is an active form of phytochrome and hence shows phytochrome mediated responses*
3	*It has maximum absorption in red region (about 680nm)*	*It has maximum absorption in far-red region (about 730nm)*
4	*It can be converted into Pfr form in red region (660-665nm)*	*It can be converted into Pr form in far red region (730-735nm)*
5	*It is found diffused throughout the cytosol*	*It is found in discrete areas of cytosol*
6	*The Pr form contains many double bonds in pyrrole rings*	*The Pfr form contains rearranged double bonds in all pyrrole rings*

Significance of photoperiodism

Photoperiodism is an example for physiological preconditioning. The stimulus is given at one time and the response is observed after months. Exposure to longer photoperiods hastens flowering (E.g). In wheat, the earing is hastened. During long light exposure, Pr form is converted into Pfrform and flowering is initiated. If dark period is greater, Pfr is converted into Pfr form that inhibits flowering.

The important phytochrome mediated photo responses in plants include photoperiodism, seed germination, sex expression, bud dormancy, rhizome formation, leaf abscission, epinasty, flower induction, protein synthesis, pigment synthesis, auxin catabolism, respiration and stomatal differentiation.

THEORIES OF FLOWERING

Photoperiodic Induction

The influence of the length of day and night on the initiation of flowering is called photoperiodic induction or photo induction. Plants may require one or more inductive cycle for flowering. An appropriate photoperiod in 24 hours cycle constitutes one inductive cycle. If a plant which has received sufficient inductive cycle is subsequently placed under unfavourable photoperiod, it will still flower.

Flowering will also occur if a plant receives inductive cycles after intervals of unfavourable photoperiods (i.e. discontinuous inductive cycle). This persistence of photoperiodic after effect is called as photoperiodic induction.

An increase in the number of inductive cycles results in early flowering of the plant. For instance, xanthium (a short day plant) requires only one inductive cycle and normally flowers after about 64 days. It can be made to flower even after 13 days if it has received 4-8 inductive cycle. In such case number of flowers is also increased.Continuous inductive cycles promote early flowering than discontinuous inductive cycle.

Perception of photoperiodic stimulus and presence of a floral hormone

Photoperiodic stimulus is perceived by the leaves and a floral hormone is produced in the leaves which are then translocated to the apical tip, subsequently causing initiation of floral primordia. Photoperiodic stimulus perceived by the leaves can be shown by a simple experiment on cocklebur (xanthium), a short day plant. Cocklebur plant will flower if it has previously been kept under short day conditions. If the plant is defoliated and kept under short day condition, it will not flower. Flowering will also occur if all the leaves of the plant except one leaf have been removed.

If the cocklebur plant whether intact or defoliated is kept under long day condition it will not flower. But if even one of its leaves is exposed to short day condition and the rest are under long day condition, flowering will occur. The photoperiodic stimulus is transmitted from one branch of the plant to another branch. For example, if in a two branched cocklebur plant one branch is exposed to short day and the other to long day photoperiod, flowering occurs on both the branches.

Flowering also occurs if one branch is kept under long day conditions and other branch from which all the leaves except one have been removed is exposed to short day condition. However, if one branch is exposed to long photoperiod and the other has been defoliated, under short day conditions, flowering will not occur in any of the branches.

Flowering stimulus: Florigen

The flowering stimulus is produced in leaves and translocated to apical and lateral meristems where flower formation is initiated. ***Chailakhyan (1937)*** *called the flowering stimulus or flowering hormone as Florigen. Flowering stimulus is similar in long day plants and short-day plants. This can be proved by a grafting experiment and can be translocated from one plant to another.*

Maryland mammoth tobacco, a short-day plant and Hyoscyamusniger, *a long day plant, are grafted so that the leafy shoots of both the species are available for experiment. If the grafted plants are exposed to either long day a short-day condition, both partners flower. If grafting union is not formed, the flowering stimulus is not translocated from one partner to another partner.*

Theories of Flowering

1. Bunning's hypothesis

2. Chailakhyan's hypothesis

Bunning's hypothesis:

Bunning (1958) assumes the presence of endogenous rhythms (Oscillator which consist of two half cycles. The first half cycle occurs in day and is called photophilous phase. During this, anabolic process predominates including flowering in plants. The other half cycle is dark, sensitive and is called skotophilous phase. In this, catabolic process (dehydration of starch) predominates.

SD plants have a critical day length of 9 hours. This period falls within the photophilous phase. Light during scotophil phase will inhibit photo process initiated during photophase. The L.D. plants have a critical day length of 15 hours and some light falls in the skotophilous phase. Under these conditions in L.D. plants will flower. In S.D. plants oscillator is present close to skotophilous phase, while in L.D. plants it is close to photo philous phase.

Chilakhyan's hypothesis:

This hypothesis assumes that flowering hormone – florigen is a complex of two types of substances – gibberellin and anthesins. Gibberellin is essential for growth of the plant stems and anthesins are required for flower formation. According to him, flowering in all annual *seed plants requires two phases: (i) Floral stem formation phase (ii) Flower formation phase. First phase involves increased carbohydrate metabolism and respiration with increased content of GA in leaves. Second phase requires intensive nitrogen metabolism, higher content of anthesins in leaves and nucleic acid metabolites in stem buds. Long day conditions favour the first phase while short day conditions favour second phase. In long day plants gibberellins are critical, while anthesins are critical in short day plants. However, anthesin is hypothetical; it has not been isolated as yet.*

Lecture 23: Phytochrome- Forms of phytochrome - Pr and Pfr - regulation of flowering

In1950s,BorthwickandHendricksandtheirassociatesobtainedspectacular results by exposing lettuce seeds to alternating red and far-red treatments. They observedmuchhigherpercentageofgerminationwhentheseedsreceivedredlight as the final treatment. Seed germination was markedlyinhibited when seeds received final treatment with far-red light. Borthwick and his associates also predictedexistenceofthephotoreceptorphytochromeintwodifferentformswhich was proved to be absolutely correct later on when this pigment was isolated in plantextractsforthefirsttimebyButleretal.,(1959)anditsphotoreversibilitywas confirmedinvitro.Abriefexposurewithredlightduringcriticaldarkperiodinhibitsfloweringinashortdayplantandthisinhibitoryeffectcanbereversedbyasubsequentexposurewithfar-redlight.Similarly,prolongationofthecriticallightperiodorthe interruptionofthedarkperiodstimulatesfloweringinlong-dayplants.

Thisinhibitionoffloweringinshortdayplantandstimulationoffloweringin longdayplantsinvolvestheoperationofaproteinaceouspigmentcalledphytochrome. It is present in the plasma membrane of cells. Phytochrome is present in roots, coleoptiles,stems,hypocotyls,cotyledons,petioles,leafblades,vegetativebuds,flower tissues,seedsanddevelopingfruitsofhigherplants.

The pigment, phytochrome exists in two different forms i.e.,

- *Red light absorbing form which is designated as P_r and*
- *Far red light absorbing form which is designated as P_{fr}.*

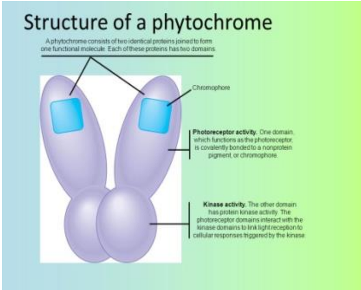
Thesetwoformsofthepigmentarephotochemicallyinterconvertible.WhenP_r form of the pigment absorbs red light (660 - 665 nm), it is converted into P_{fr}form.

WhenP$_{fr}$formofthepigmentabsorbsfarredlight(730-735nm),itisconvertedinto Prform.TheP$_{fr}$formofpigmentgraduallychangesinto P$_{r}$formindark.

Itisconsideredthatduringdaytime,theP$_{fr}$formofthepigmentisaccumulated intheplantswhichareinhibitorytofloweringinshortdayplantsbutisstimulatory inlongdayplants.Duringcriticaldarkperiodinshortdayplants,thisformgraduallychanges into P$_{r}$ form resulting in flowering. A brief exposure with red light will convert this form again into P$_{fr}$form thus inhibiting flowering. Reversal of the inhibitory effect of red light during critical dark period in SDP by subsequent far-red light exposure is because, the P$_{fr}$form after absorbing far-red light (730-354 nm) will again be converted back into P$_{r}$ form. Prolongation of critical light period or the interruption of the dark period by red- light in long day plants will result in further accumulation of the P$_{fr}$form of the pigment, thus stimulating flowering in long-day plants.

Structure of Phytochrome

Successfulpurificationofintactnativephytochromefrometiolatedoatseedlingswasfirstreportedby*VierstraandQuail*in1983.Thenativephytochromeisasoluble proteinwithamolecularweightofabout250kDa.It'sa*homodimer*oftwoidentical polypeptideseachwithamolecularweightofabout125kDa.Eachpolypeptidehasa prostheticgroupcalledaschromophorewhichiscovalentlylinkedtothepolypeptide via a sulphur atom (Thioether Linkage) in the cysteine residue of thepolypeptide.Theproteinpartofthephytochromeiscalledasapoprotein.Apoproteinalongwith chromophore constituteholoprotein.The chromophore of phytochrome is an *open tetrapyrrole*which is related to *phycocyanobilin*instructureandtherefore,morerecentlythischromophorehasbeen calledas*phytochromobilin.*Thestructuresofchromophoresortheprostheticgroups ofP$_{r}$ andP$_{fr}$formsofphytochromewhichare*cisandtransisomers*ofeachotherrespectively.Thecis-transisomerizationoccur;atcarbon-15inresponsetoredandfar-redlight.Apartfromabsorbingredandfar-redlight,thephytochromealsoabsorbs bluelight.



TheP$_r$formofphytochromeis*blue*whileP$_{fr}$formis*olive-green*incolour.But owingtoverylowconconcentrationofphytochrome,thecolourofthispigmentisnotvisiblein planttissues.Phytochromeaccountsforlessthan0.2%ofthetotalextractableprotein in etiolatedseedlings.

Noneof thetwocomponentsofphytochromei.e.,apoproteinandchromophore,canabsorblightalone.Phytochromeshavebeendetectedinwiderangeofplantsin angiosperms,gymnosperms,bryophytesandalgae.Darkgrownetiolatedseedlings arerichestsourcesofphytochromewherethispigmentisespeciallyconcentratedin apical meristems. Phytochromes have directly been detected in different parts of seedlings,inroots,cotyledons,hypocotyls,epicotyls,coleoptiles,stems,petioles,leaf blades,vegetativebuds,floralreceptacles,inflorescences,developingfruitsandseeds. Presence of phytochrome has also been shown indirectly in other plant materials. Within the cells, phytochrome exists in nucleus and throughout the cytosol. The chromophoreofphytochromeissynthesizedinplastidswhileapoproteinissynthesized onnucleargenome.Assemblyofthesetwocomponentsofphytochromeisautocatalyticand occurs in cytosol.

There are two major types of phytochromes in plants,

(i) TypeI

(ii) TypeII.

The type I predominates in etiolated seedlings while type II in green plants and seeds (such as oat seeds). There are minor differences in molecular weight and spectral properties of these two types of phytochromes.

Type I phytochrome is encoded by PHY A gene while type II is encoded by PHY B, PHY C, PHY D and PHY E genes.

Mechanism of Action

The exactmechanismoftheactionofphytochromeisnotveryclear.Theyact probablybycontrollingactivetransportofionsandmoleculesacrossmembranesbyregulatingATPase activity,bycontrollingtheactivityofmembraneboundhormonessuchasgibberellins modulatingtheactivityofmembraneboundproteinsandby regulating transcription of numerous genes involving multiple signal transduction pathways.

Phytochrome MediatedPhotoresponses

Basedontheamountoflightrequiredorthefluence(no.ofphotonsabsorbed per unit surface area), the phytochrome mediated photoresponses can be grouped into three maincategories;

(a) Very Low Fluence Responses (VLFRS)

These responses are initiated by very low fluences (0.1 to 1 n mol m^{-2}) saturating at 50 n mol $m^{-2}$and are non-photoreversible. For example, brief flash of redlightwithfluenceaslowas0.1nmolm^{-2}canstimulatethegrowthofcoleoptile and inhibit growth of mesocotyl in oat seedlings that have been grown in dark. Similarly,redlightwithfluenceofonly1-100nmol.m^{-2}isenoughtostimulateseed germinationinArabidopsis.(Inmonocots,theelongatedareaofaxisbetweencoleoptiles androotiscalledasmesocotyl)

(b) Low Fluence Responses(LFRs)

These responses require fluence of at least 1.0 nmol m^{-2}saturating at 1000 nmolm^{-2}andarephotoreversible.Mostofthered/far-redphotoresponsesincludingthelettuceseedgerminationbelongtothiscategory.

(c) High Irradiance Responses(HIRs)

Theseresponsesrequirecontinuousorprolongedexposuretolightofrelatively high irradiance saturating at much higher fluences (at least 100 times more) than LFRsandarenon-photoreversible.Examplesareanthocyaninsynthesisindicotseedlingsandinappleskin, ethyleneproductioninsorghum,inductionoffloweringinHyoscyamus(alongdayplant), openingofplumularhookinlettuce,enlargement of cotyledons in mustard,inhibitionof hypocotyls elongationinmanydicotseedlingsetc.

Theories of Flowering

1. *Bunning'shypothesis*
2. *Chailakhyan'shypothesis*

Bunning's hypothesis:

Bunning(1958)assumedthepresenceofendogenousrhythms(Oscillatorwhichconsistoftwohalfcycles).Thefirsthalfcycleoccursindayandiscalledphotophilous phase.Duringthis phase anabolicprocesspredominatesincludingfloweringinplants.The otherhalfcycleisdarksensitiveandiscalledskotophilousphase.Inthis,catabolic process (dehydration of starch)predominates.

Shortdayplantshaveacriticaldaylengthof9hours.Thisperiodfallswithin thephotophilousphase.Lightduringscotophilousphasewillinhibitphotoprocess initiated during photophase. The long day plants have a critical day length of 15 hoursandsomelightfallsintheskotophilousphase.Under this condition, long day plants will flower. In short day plants oscillator is present close to skotophilousphase, whilein long dayplantsitisclosetophotophilousphase.

Chilakhyan's hypothesis:

This hypothesis assumes that flowering hormone - florigenis a complex of twotypesofsubstances–gibberellinandanthesins.Gibberellinisessentialforgrowth oftheplantstemsandanthesinsarerequiredforflowerformation.Accordingtohim, floweringinallannualseedplantsrequirestwophases:

Floral stem formationphase

Flower formationphase.

Firstphaseinvolvesincreasedcarbohydratemetabolismandrespirationwith increased content of GA in leaves. Second phase requires intensive nitrogen metabolism, higher content of anthesinsinleaves and nucleic acid metabolites in stem buds. Long day conditions favour the first phase while short day conditions favour second phase. In long day plants gibberellins are critical, while anthesins are critical in short day plants. However, anthesin is hypothetical; it has not been isolated asyet.

Gibberellins and the Flowering Response

Itis nowwellknownthatthe gibberellins caninducefloweringinlong-day plants even under non-inductive short days. It is also definite that the gibberellins alone do not constitute the 'florigen', but it is usually held that the gibberellins are in some way connected with the overall process offlowering.

Lecture 24: Vernalisation - theories of vernalisation – Lysenko and Hormonal theories – devernalization

The cold treatment given to plant buds, seeds or seedlings for promoting early flowering is known as Vernalisation. In short, the chilling treatment for induction of early flowering is called Vernalisation.Besides an appropriate photoperiod, certain plants require a low temperature treatment during their early stages of the life for subsequent flowering in the later stages. This low temperature treatment requirement was termed vernalization by Lysenko (1928). Due to vernalization, the vegetative period of the plant is cut short resulting in an early flowering. In nature, vernalisation takes place in the seed stage in annuals like winter rye (Secale cereale). The biennials and many perennials respond to cold treatment at a very late stage. E.g. Henbane, apples etc.

Perception of cold stimulus and presence of floral hormone

The cold stimulus is perceived by the apical meristems. The perception of the cold stimulus results in the formation of a floral hormone which is transmitted to other parts of the plant. In certain cases, the cold stimulus may even be transmitted to another plant across a graft union. For instance, if a vernalized henbane plant is grafted to an unvernalized henbane plant, the later also flowers. This is due to the induction of the plant to produce a hormone named as Vernalin by Melchers (1939).

Conditions necessary for vernalization

1. Age of the plant

The age of the plant is an important factor in determining the responsiveness of the plant to the cold stimulus and it differs in different species. In cereals like winter wheat, the *vernalization is effective only if the germinating seeds have received cold temperature treatment for sufficient time.*

While in the case of biennial variety of henbane (Hyoscyamusniger), the plant will respond to the cold treatment, only if they are at rosette stage and completed at least 10 days of growth.

2. Appropriate low temperature and duration of the exposure

Most suitable temperature for vernalizing the plants ranges between 1-6°C. The effectiveness of low temperature treatment decreases from 0 to 4°C. Low temperature at about -6°C is completely ineffective. Similarly at high temperatures from 7°C onwards, the response of the plants is decreased. Temperature of about 12-14°C is almost effective in vernalizing the plant. Besides an appropriate low temperature, a suitable duration of the cold treatment is essential for vernalization. Depending upon the degree of temperature and in different species this period may vary, but usually the duration of the chilling treatment is about one and half months or more.

3. Oxygen

The vernalization is an aerobic process and requires metabolic energy. In the absence of O_2, cold treatment becomes completely ineffective.

4. Water

Sufficient amount of water is also essential for vernalization. Vernalization of the dry seed is not possible.

Mechanism of Vernalization

There are two main theories to explain the mechanism of vernalisation.

1. Phasic developmental theory

This theory was proposed by Lysenko (1934) as follows.

(i) The growth (increase in size) and development (i.e. progressive change in the characteristic of the new organs) are two distinct phenomenons.

(ii) According to this theory, the process of the development of an annual seed plant consists of a series of phases which must occur in some predetermined sequence.

(iii) *Commencement of any of these phases will take place only when the preceding phase has been completed.*

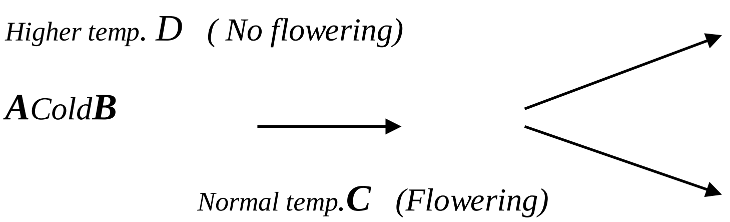
(iv) *The phases require different external conditions for the completion such as light and temperature.*

(v) *Vernalization accelerates the thermo phase i.e. that phase of development which is dependent upon temperature.*

Thus, in winter wheat, low temperature is required for the completion of first thermo phase. After this, the next phase that is dependent upon light (photo phase) starts. Vernalization of winter wheat accelerates the first thermo phase so that there is an early swing from vegetative to reproductive phase or flowering.

2. Hormonal theories

It has already been described that vernalization probably involves the formation of a floral hormone called as vernalin.Based on this fact, many hypothetical schemes have been proposed by different workers from time to time. The first hormonal theory proposed by Long and Melchers (1947) is schematically shown below.



According to this scheme, the precursor A is converted into a thermo labile compound B during cold treatment. Under normal conditions B changes into C which ultimately causes flowering. But at higher temperature B is converted into D and flowering does not take place (devernalization).

Devernalization

The positive effect of the low temperature treatment on the vernalization of the plant can be counteracted by subsequent high temperature. This is called devernalization. The devernalized plant can again be vernalized by subsequent low temperature treatment.

Vernalization and Gibberellins

The gibberellins are known to replace the low temperature requirement in certain biennial plants such as henbane, where the plant normally remains vegetative and retains its rosette habit during the first growing season and after passing through the winter period flowers in the next season. The gibberellins cause such plants to flower even during the first year.

Significance of vernalization

1. *Vernalization shortens the vegetative period of the plant*
2. *It increases cold resistance of the plants*
3. *Vernalization increases the resistance of plants to fungal diseases.*
4. *It is a physiological process that substitutes or compensates the effect of thermo phase.*
5. *In biennials, vernalisation induces early flowering and early fruit setting.*
6. *A non vernalised shoot apex can be induced to flower by grafting the plant with a vernalised plant.*

Lecture 25: Physiological aspects of growth and development of major crops

Plant development

Plant development is an overall term which refers to the various changes that occur in a plant during its life cycle. Development consists of both growth and differentiation involving quantitative and qualitative changes (Hopkins 1999). It is characterized by change in size, shape, form, degree of differentiation and state of complexity (Abellanosa and Pava 1987). The interactions of the environment and the genetic instructions inherited by the cells determine how the plant develops.However, there can be growth without differentiation and likewise there can be differentiation without growth.

Plant growth

Plant growth is the irreversible, quantitative increase in size, mass, and/or volume of a plant or its parts. It occurs with an expenditure of metabolic energy. Therefore, the events leading to leaf formation and the increase in height of a plant are growth, but the increase in volume of a seed due to uptake of water or imbibition is not growth. Growth in general is a combined effect of cell division and cell enlargement. Enlargement necessitates a change in the elasticity of the cell walls together with an increase in the size and water content of the vacuole.

In some instances, growth can occur even without cell division and the reverse is also true. Likewise, early growth of the embryo in the flower can be quantified by the increase in cell number although these cells being small do not increase the size of the embryo. There are various ways of quantifying plant growth. These include cell number, fresh weight, dry weight, plant height, length, width, area, and volume. Each one has limitations.

Growth can be **determinate**—when an organ or part or whole organism reaches a certain size and then stops growing—or **indeterminate**—when cells continue to divide indefinitely. Plants in general have indeterminate growth.

Differentiation of cells into tissues and organs

Differentiation is an orderly processes of change in which structurally simple and genetically identical cells become different by becoming specialized for certain functions and produce the various tissues and organs of a plant. Since all of the cells produced by division in the meristems have the same genetic makeup, differentiation is a function of which particular genes are either expressed or repressed. The kind of cell that ultimately develops also is a result of its location: Root cells don't form in developing flowers, for example, nor do petals form on roots. Mature plant cells can be stimulated under certain conditions to divide and differentiate again, i.e. to **dedifferentiate.** This happens when tissues are wounded, as when branches break or leaves are damaged by insects. The plant repairs itself by *dedifferentiating* parenchyma cells in the vicinity of the wound, making cells like those injured or else physiologically similar cells.

Systems in developmental process

In agriculture, the term plant growth and development is often substituted with crop growth and yield. This is so because plant agriculture is mainly concerned with crops and their economic products. All but a relatively few plants are ***multicellular****and the majority have* ***bodies****composed of two major systems: the* ***root system*** *and the* ***shoot system.*** *The former is usually underground, and the latter above ground. To succeed and grow simultaneously in two such entirely different environments—air and soil—requires a myriad of adaptations, starting with cellular modifications into specialized kinds of* ***tissues*** *(groups of similar cells that are organized in a structural and functional unit) followed by development of* ***organs (****structures composed of several kinds of tissues grouped in a structural and functional unit). The acquisition of form and structure is called* ***morphogenesis*** *and is a highly orchestrated procedure controlled by the DNA of the plant cells but influenced as well by the environment.*

With a few exception in plants, a single-celled zygote forms after fertilization, or syngamy, in which a haploid nucleus in the egg cell within the embryo sac fuses with a haploid sperm nucleus from a germinated pollen. This zygote soon undergoes growth through a series of cell division and cell enlargement and ultimately transforms into a multicellular embryo in the seed. This embryo is likened to a miniature plant within the seed. It possesses all the potential for developing into a mature plant, but is temporarily in dormant or arrested growth. Just like a mature plant, it has a root and shoot. Called the radicle, this root is described as an embryonic root because it is a part of the embryo. It is also referred to as a rudimentary root. The embryonic shoot, is likewise described as epicotyl.

With all the necessary environmental requisites, a small and relatively simple seed germinates. With time, it completes various stages of development and transforms into a complex mature plant having multiple organs. Finally, its overall size and weight could be several hundred or thousand times more than that of the seed, or even more.

Stages of development

In broad terms, the stages of development in plants can be divided into the following: **vegetative**, **reproductive**, **ripening**, and **senescence**. Each stage can be subdivided into various component substages or phases. Although plant development is cyclical, the seed is considered as the starting point for the sequencial events leading to a mature plant, the formation of seed, and finally death.

Vegetative Stage

This is generally a lengthy period of development in plants, starting from seed germination until prior to reproductive stage. In seed germination the young, quiescent plant (embryo) within the seed initiates active growth and ultimately the embryonic root (radicle) and the embryonic shoot (epicotyl) extend outward from the seed.

The seedling emerges from the soil and soon assumes independent growth. Subsequently, the plant grows bigger with more roots and more aerial parts such as tillers (as in rice), nodes and internodes, branches and leaves. This stage terminates immediately before it reaches reproductive stage at which time it starts to initiate the formation of inflorescences (i.e., panicle in rice) and flower primordia.

In trees and other perennial crops, this stage of development is also called the juvenile stage during which the tree progressively increases in root mass and size of trunk, branches, canopy and height, but not reproductive parts. Thus a tree which cannot be induced to flower is referred to as a juvenile while another which has reached fruit-bearing age is called mature or adult. In seed-grown mango (*Mangiferaindica* L.), juvenility may last for about 5 years or more and coconut for about 3 years or more, depending on the variety, care, and environmental conditions. Stated another way, it takes mango about 5 years or more and coconut about 3 years or more to start producing flowers, fruits and seeds.

Reproductive Stage

This stage of development in plants occurs after the vegetative or juvenile stage is completed. At this stage the plants are considered mature, that is, they are physiologically capable of commencing the production of reproductive parts: the flowers, fruits and seeds. This stage consists of the period from the time that the plant starts to form inflorescence or flower primordia (called booting in rice) until flowering, pollination, and fertilization. According to Ryugo (1988), in fruit trees the reproductive stage commences with a *transition phase* during which few flowers are produced.

Ripening stage

In annual crops, this is the developmental stage during which botanical fruits and seeds are formed. But for horticultural crops, ripening has been defined as "the composite of the processes that occur from the latter stages of growth and development through the early stages of senescence and that results in characteristic aesthetic and/or food quality, as evidenced by changes in composition, color, texture, or other sensory attributes" (Watada et al. 1984).

In rice, ripening starts after fertilization (syngamy) and ends when the grains (commonly called seeds) become mature. A seed is physiologically mature when it has achieved maximum accumulation of dry matter (and dry weight). However, at this stage of maturity the seeds would have high moisture content of about 30-35%. To accelerate drying of seeds even while still attached to the plant, it is a common practice to drain the paddy field of water about 1-2 weeks before the harvest schedule.

Senescence

This is the final stage of development in plants during which physical and chemical changes occur leading to the death of the whole plant. In annual plants, senescence may start during the reproductive stage and plant death sets in soon after seed maturity which marks the end of irreversible growth. Progressive change in color from green to yellowish (chlorosis) is a major indicator of leaf senescence and the start of whole-plant death.

In the following session, five major crops were such as rice, maize, sunflower, cotton and sugarcane were discussed with reference to their physiological limitations prevent the yield improvement of crops during their growth and development. The physiological ways and means for the yield improvement were also discussed.

RICE

Improving the physiological efficiency of the new rice varieties is contemplated for achieving the breakthrough in the yield potential. The opportunities for increasing the efficiency of potential photosynthetic capacity for better plant growth and yield of rice need to remove the internal limitation to carbohydrate metabolism and increasing the functional life span of leaves. Physiological studies to identify the processes that can be manipulated to increase the net photosynthetic rate, particularly during the reproductive growth stages in *order to increase the total biomass production are important. The specific targets for such investigation include:*

1) *Increasing specific leaf N and the photosynthetic rate per unit specific leaf N:*
2) *Increasing leaf photosynthesis at each stratum in the canopy by manipulating the canopy characteristics*
3) *Increasing panicle photosynthesis*
4) *Reducing respiration*
5) *Increasing leaf area duration and delaying leaf senescence*
6) *Incorporating c-4 characteristics into rice leaf and further*

- *Sink limitation needs to be quantified under a wide range of environment.*
- *Efforts should be made to improve the harvest index through increased sink size and greater partitioning of dry matter to grains, particularly long duration varieties.*
- *The recent advances in the genetic transformation should be used in exploring and validating the yield limiting processes and also in removing the constraints they impose on the yield.*
- *Among the conventional approaches, improving agronomic practices for realizing yield potential should be an important strategy.*
- *This would include the proper timing and method of crop establishment, optimization of space utilization within the crop stand, adequate supply of nutrients and water effective weed control.*
- *Research on hybrids should be pursued more vigorously for development of*
- *Increasing starch biosynthesis and its translocation to endosperm. The transfer of ''nif'' genes to rice is also worth attempting*

Maize

- *In maize, sucrose is the primary transport form of carbon. The import and utilization of soluble carbohydrates, along with amino acid as a source of reduced N, is essential to vegetative growth and reproductive development.*

- *Assimilate partitioning controls transfer of organic nutrients between grains and non grain plants components.*

- *Strategies to improve grain yield include increased partitioning to the grain (HI), delayed leaf senescence and extended duration of grain filling period.*

- *The vegetative parts are mined efficiently, to an extent that the harvest index for nitrogen changes from 50 to 64%.*

- *Manipulation of source strength and sink size is an important control mechanism in grain filling for higher grain yields.*

- *Increase in dry matter accumulation may be in part attributable to stay-green" and an increased leaf angle, most of the yield improvement appears to be the result of increased stress tolerance.*

- *The adoption of low input management practices in maize production will increase the need for more stress-tolerant genotypes and stress tolerance will continue to be the most important trait in maize breeding for grain yield.*

SUNFLOWER

- *Sunflower phenotypic plasticity is responsible to varying environments of both leaf expansion and the pattern of phenological development for fixing plant population.*

- *Leaf area is critical in determining light interception and crop growth. Before anthesis, leaf expansion rate is the main determinant of leaf area.*

- *Particularly interactions between hydraulic and non-hydraulic mechanisms of control of leaf expansion need to be investigated.*

- *Research at various organization levels - viz. cell, tissue, organ, plant is necessary to advance present knowledge of the physiology of nitrogen effects on leaf expansion.*

- *Understanding of thermal and photoperiodic control of reproductive development is also quite limited. Role of temperature and water on the control of seed-filling duration are necessary in sunflower.*

- *Experimental work is required to characterize the juvenile phase, photoperiod ranges for both the pre- and post floral initiation*

COTTON

- *Early maturity is desired because it can drastically reduce input costs (eg, pesticides), especially in irrigated areas.*

- *Morphological development data may help to determine the fertility and water needs accordingly the rate and timing of growth retardant and defoliant applications can be determined.*

- *Physiological reasons for cotton's have greater susceptibility to pest is unclear. Three factors that contribute to cotton's greater sensitivity to Insect pests are:*

- *Many cotton insects attack fruit, rather than leaves*

- *Many of these pests are small and difficult to detect, and Cotton's extended fruiting cycle that can last nearly 70 days from visible floral bud (pinhead square) to open boll.*

- *Establishing a canopy with an LAI of 3.5 to 4 is needed to produce an optimal yield in many environments*

- *Numerous studies supported the importance of producing many flowers during early and mid- bloom.*

- *Obtaining good fruit set and flower production is essential for obtaining high yield.*

- *Combinations of new genotypes and management practices that increase utilization of PAR and increase flowering may increase cotton yield in the near future.*

SUGARCANE

- *When sucrose is the desired product of sugarcane production, sucrose yield per hectare is of ultimate concern.*
- *Greater sucrose yield can be achieved by increasing total biomass produced by the crop, increasing sucrose concentration in the crop, or both.*
- *Increasing cane yield has been a successful means in increasing sucrose yield. Increasing cane yield might be accomplished by increasing the efficiency of photosynthesis in sugarcane.*

- *Limiting factors, such as temperature, water availability, and nitrogen fertilization, suppresses photosynthesis.*

- *Fields yielding the highest biomass achieve high leaf area index quickly after planting or ratooning, and retain that leaf area until harvest. This can be accomplished by increasing plant population, decreasing row spacing, and maintaining high soil nitrogen contents to encourage rapid growth.*

- *Selection of plants that begin growth when soil temperature are below the current optimum may increase biomass accumulation by increasing the duration of maximum leaf area index.*

- *The capacity of sugarcane stalks to accumulate sucrose it is limited by the translocation, partitioning, and metabolism of sucrose. Selection for high stalk sucrose by traditional plant breeding gas resulted in increasing sucrose yield.*

- *The differences in sucrose biochemistry between Saccharum species with extremely high or low sucrose concentration are not clear. Genetic modification through biotechnology offers hope for insight and improvement in this area.*

- *Many of the key genes of sucrose metabolism have been isolated and cloned. This makes it possible both to examine gene expression related to sucrose accumulation and to manipulate enzyme activity by transforming plants. In time, this may lead to sugarcane genotypes with enhanced capability to store sucrose.*

Lecture 26: Growth analysis – Role of physiological growth parameters in crop productivity

Growth analysis can be used to account for growth in terms that have functional or structural significance. The type of growth analysis requires measurement of plant biomass and assimilatory area (leaf area) and methods of computing certain parameters that describe growth. The growth parameters that are commonly used in agricultural research and the name of the scientists who proposed the parameters are given below.

LAI - Williams (1946)

LAR - Radford (1967)

LAD - Power et al. (1967)

SLA - Kvet et al. (1971)

SLW - Pearce et al. (1968)

NAR - Williams (1946)

CGR - Watson (1956)

RGR - Williams (1946)

HI - Nichiporovich (1951)

i. Leaf Area

This is the area of photosynthetic surface produced by the individual plant over a period of interval of time and expressed in cm^2 $plant^{-1}$.

ii. Leaf Area Index (LAI)

Williams (1946) proposed the term, Leaf Area Index (LAI). It is the ratio of the leaf of the crop to the ground area over a period of interval of time. The value of LAI should be optimum at the maximum ground cover area at which crop canopy receives maximum solar radiation and hence, the TDMA will be high.

Total leaf area of a plant

LAI = ______________________

Ground area occupied by the plant

iii. Leaf Area Ratio (LAR)

The term, Leaf Area Ratio (LAR) was suggested by Radford (1967), expresses the ratio between the area of leaf lamina to the total plant biomass or the LAR reflects the leafiness of a plant or amount of leaf area formed per unit of biomass and expressed in cm^{-2} g^{-1} of plant dry weight.

$$LAR = \frac{\text{Leaf area per plant}}{\text{Plant dry weight}}$$

iv. Leaf Weight Ratio (LWR)

It was coined by (Kvetet al., 1971) Leaf weight ratio is expressed as the dry weight of leaves to whole plant dry weight and is expressed in $g\ g^{-1}$.

$$LWR = \frac{\text{Leaf dry weight}}{\text{Plant dry weight}}$$

v. Leaf Area Duration (LAD)

To correlate dry matter yield with LAI, Power et al. (1967) integrated the LAI with time and called as Leaf Area Duration. LAD takes into account, both the duration and extent of photosynthetic tissue of the crop canopy. The LAD is expressed in days.

$$LAD = \frac{L_1 + L_2}{2} \times (t_2 - t_1)$$

L_1 = LAI at the first stage

L_2 = LAI at the second stage, $(t_2 - t_1)$ = Time interval in days

vi. Specific Leaf Area (SLA)

Specific leaf area is a measure of the leaf area of the plant to leaf dry weight and expressed in $cm^2 g^{-1}$ as proposed by Kvetet al. (1971).

$$SLA = \frac{\text{Leaf area}}{\text{Leaf weight}}$$

Hence, if the SLA is high, the photosynthesizing surface will be high. However no relationship with yield could be expected.

vii. Specific Leaf Weight (SLW)

It is a measure of leaf weight per unit leaf area. Hence, it is a ratio expressed as g cm^{-2} and the term was suggested by Pearce et al. (1968). More SLW/unit leaf area indicates more biomass and a positive relationship with yield can be expected.

$$SLW = \frac{\text{Leaf weight}}{\text{Leaf area}}$$

viii. Absolute Growth Rate (AGR)

AGR is the function of amount of growing material present and is influenced by the environment. It gives Absolute values of biomass between two intervals. It is mainly used for a single plant or single plant organ e.g. Leaf growth, plant weight etc.

$$AGR = \frac{h_2 - h_1}{t_2 - t_1} \quad cm\ day^{-1}$$

Where, h1 and h2 are the plant height at t_1 and t_2 times respectively.

ix. Net Assimilation Rate (NAR)

The term, NAR was used by Williams (1946). NAR is defined as dry matter increment per unit leaf area or per unit leaf dry weight per unit of time. The NAR is a measure of the average photosynthetic efficiency of leaves in a crop community.

$$NAR = \frac{(W_2 - W_1)}{(t_2 - t_1)} \times \frac{(\log_e L_2 - \log_e L_1)}{(L_2 - L_1)}$$

Where, W_1 and W_2 is dry weight of whole plant at time t_1 and t_2 respectively

L_1 and L_2 are leaf weights or leaf area at t_1 and t_2 respectively

$t_1 - t_2$ are time interval in days

NAR is expressed as the grams of dry weight increase per unit dry weight or area per unit time (g $g^{-1} day^{-1}$ or g cm^{-2} day^{-1})

x. Relative Growth Rate (RGR)

The term was coined by Williams (1946). Relative Growth Rate (RGR) expresses the total plant dry weight increase in a time interval in relation to the initial weight or Dry matter increment per unit biomass per unit time or grams of dry weight increase per gram of dry weight and expressed as unit dry weight / unit dry weight / unit time (g g $^{-1}$day^{-1})

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

Where, W_1 and W_2 are whole plant dry weight at t_1 and t_2 respectively
t_1 and t_2 are time interval in days

xi. Crop Growth Rate (CGR)

The method was suggested by Watson (1956). The CGR explains the dry matter accumulated per unit land area per unit time (g m^{-2} day^{-1})

$$CGR = \frac{(W_2 - W_1)}{\rho\,(t_2 - t_1)}$$

Where, W_1 and W_2 are whole plant dry weight at time $t_1 - t_2$ respectively
ρ is the ground area on which W_1 and W_2 are recorded.

CGR of a species are usually closely related to interception of solar radiation

xii. Total dry matter production (TDMP) and its distribution

The TDMP is the biomass accumulated by the whole plant over a period of interval of time and its distribution (allocation) to different parts of the plant such as roots, stems, leaves and the economic parts which controls the sink potential.

xiii. Translocation percentage (TP)

The term translocation percentage indicates the quantum of photosynthates translocated from source (straw) to the grain (panicle/grains) from flowering to harvest.

$$TP = \frac{\text{Straw weight at flowering – straw weight at harvest}}{\text{Panicle weight at flowering – panicle weight at harvest}}$$

xiv. Light extinction coefficient

It is the ratio of light intercepted by crop between the top and bottom of crop canopy to the LAI.

$$K = (\log_e I / I_o) / LAI$$

Where, I_o and I are the light intensity at top and bottom of a population with LAI

xv. Light Transmission Ratio (LTR)

It is expressed as the ratio of quantum of light intercepted by crop canopy at top to the bottom. Light intensity is expressed in K lux or W m^{-2}

$$LTR = I / I_o$$

Where, I : light intercepted at the bottom of the crop canopy
I_o: light intercepted at the top of the crop canopy

xvi. Dry Matter Efficiency (DME)

It is defined as the percent of dry matter accumulated in the grain from the total dry matter produced over the crop growth period.

$$DME = \frac{\text{Grain yield}}{\text{TDMP}} \times \frac{100}{\text{Duration of crop}}$$

xvii. Unit area efficiency (UAE)

It is expressed as the quantum of grain yield produced over a unit land area for a specified crop growth period.

$$UAE = \frac{\text{Grain yield}}{\text{Land area}} \times \frac{1}{\text{Duration of crop}}$$

xviii) Harvest Index

The harvest index is expressed as the percent ratio between the economic yield and total biological yield and was suggested by Nichiporovich (1951).

$$HI = \frac{\text{Economic yield}}{\text{Total biological yield}} \times 100$$

Lecture 27: Seed germination - physiological and biochemical changes - seed dormancy and breaking methods

The process of seed germination starts with the imbibition of water by seed coat and emergence of growing root tip of embryo. The process ends with the development of embryo into a seedling.

Physiological and biochemical changes during seed germination

1. Water uptake

Seed germination starts with the imbibition of water by dry seed coat. Due to imbibition of water, the seed coats become more permeable to O_2 and water and less resistant to outward growth of embryo.

2. Respiration

Rapid increase in respiration rate of embryo occurs. Sucrose is probably the respiratory substrate at this stage which is provided by endosperm.

3. Mobilization of reserve materials

As germination progresses, there is mobilization of reserve materials to provide

- *building blocks for the development of embryo*
- *energy for the biosynthetic process and*
- *nucleic acids for control of protein synthesis and embryonic development*

Changes in these components are as follows

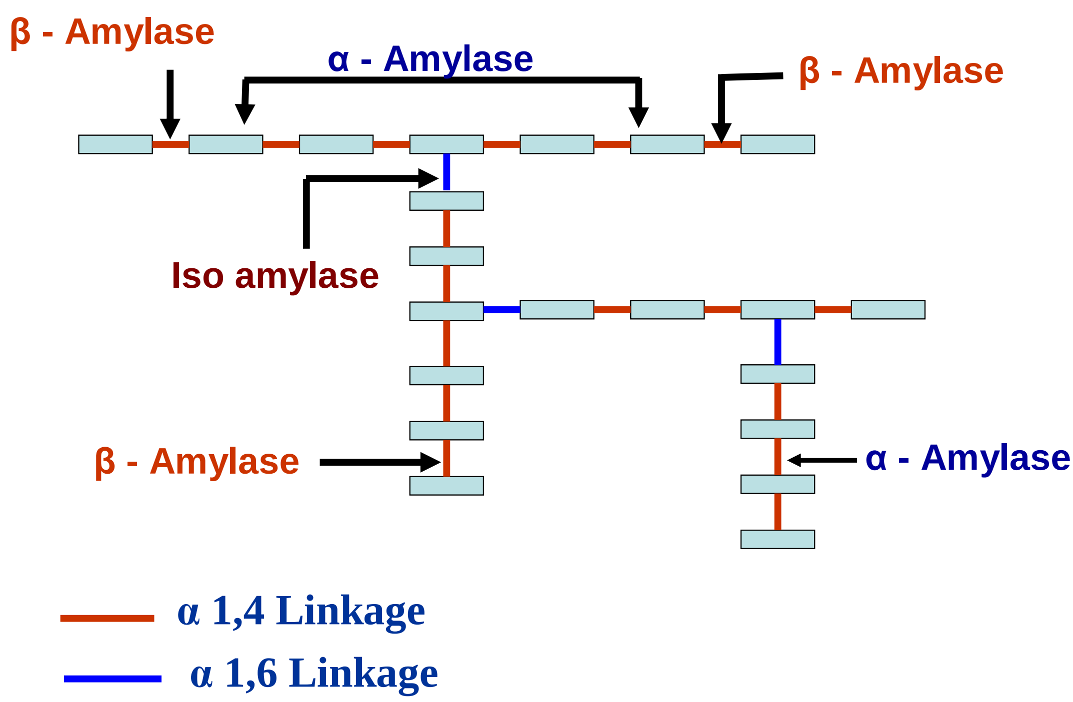
i) Nucleic acids

In monocots, during imbibition, there is a rapid decrease of DNA and RNA content in the endosperm with a simultaneous increase in the embryonic axis probably due to their transportation as such. High concentration of RNA in the embryonic axis precedes cell division. Due to more cell division, DNA content is increased.

ii) Carbohydrates

Insoluble carbohydrates like starch are the important reserve food of cereals in the endosperm. During germination, starch is hydrolyzed first into maltose in the presence of α-amylase and β - amylase and then maltose is converted into glucose by maltase. The glucose is further converted into soluble sucrose and transported to growing embryonic axis. During germination, the embryonic axis secretes gibberellic acid into the aleurone layer which causes synthesis of α-amylase.

ENZYME ACTION ON STARCH



iii) Lipids

Plants like castor bean, peanut etc., store large amount of neutral lipids or fats as reserve food in their seeds. During germination, the fats are hydrolyzed into fatty acids and glycerol by lipase enzyme. Fatty acids are further converted into acetyl – CoA by the process, β - oxidation. The acetyl CoA is further converted into sucrose via glyoxylate cycle and is transported to the growing embryonic axis.

iv) Proteins

Some plants store proteins as reserve food in their seeds in the form of aleurone grains. Proteins are hydrolyzed into amino acids by peptidase enzyme. The amino acids may either provide energy by oxidation after deamination (removal of amino group) or may be utilized in the synthesis of new proteins.

v) Inorganic materials

A number of inorganic materials such as phosphate, calcium, magnesium and potassium are also stored in seeds in the form of phytin. These stored materials are liberated during germination due to the activity of various phosphatases including phytase.

Emergence of seedling out of the seed coat

All the changes described above gradually result in splitting of seed coat and emergence of the growing seedling. The radical comes out first and grows downward, and then plumule comes out and grows upward. Due to the continued growth of this seedling, the plumule comes out of the soil, exposed to light and develops its own photosynthetic apparatus.

Splitting of seed coat may take place either by imbibition pressure or by internal pressure created by the growing primary root or by hydrolytic enzymes which act on cell wall contents of seed coat and digest it (e.g. cellulose and pectinase). Sometimes the seed coat may be extensively rotted by the activity of micro-organisms in the soil.

DORMANCY OF SEEDS

All the viable seeds have capacity to germinate if placed under suitable conditions necessary for germination. But, some seeds fail to germinate sometimes even if placed under the condition favourable for germination. This may be due to some internal factors or due to *specific requirement for some environmental factors. During this period, the growth of the seed remains suspended and they are said to be in rest stage or dormant stage and this phenomenon is called as dormancy of seeds.*

Factors causing dormancy of seeds

1. Seed coats impermeable to water

The seeds of certain plants especially those belonging to the family's leguminaceae, solanaceae, malvaceae, etc. have very hard seed coats which are impermeable to water. The seeds remain dormant until the impermeable layer decay by the action of soil micro-organisms.

2. Seeds coats impermeable to oxygen

In many plants such as cocklebur and many grasses, the seed dormancy is due to the impermeability of the seed coat to oxygen. However, during the period of dormancy, the seed coat gradually becomes more permeable to oxygen so that they may germinate.

3. Immaturity of the Embryo

In certain orchids, the seed dormancy is due to the immaturity of the embryos which fail to develop fully by the time the seeds are shed. In such cases, the seeds germinate only after a period or rest during which the development of embryo inside the seed is completed.

4. Germination Inhibitors

In certain seeds, the dormancy of the seeds is due to the presence of certain germination inhibitors like coumarin, ferulic acid, abscissic acid, etc. These may be present in endosperm, embryo, testa or juice or pulp of fruit.

5. Chilling or low temperature requirement

In certain plants such as apple, rose, peach etc, the seeds remain dormant after harvest in the autumn as they have a low temperature or chilling requirement for germination. In nature, this requirement is fulfilled by the winter temperatures. In such case the seeds remain dormant throughout the winter season and germinate only in the following spring.

6. Light sensitive seeds

In many species, the germination of the seeds is affected by light resulting in seed dormancy. Such light sensitive seeds are called photo blastic. Seeds of lettuce, tomato and tobacco are positively photo blastic and germinate only after they have been exposed to light. On the other hand, the seeds of certain plants are negatively photo blastic and their germination is inhibited by light.

Advantages of dormancy

1. *In temperature zones, the dormancy of seeds helps the plants to tide over the severe colds which may be injurious for their vegetative and reproductive growth.*
2. *In tropical regions, the dormancy of seeds resulting from their impermeable seed coats ensures good chances of survival.*
3. *Dormancy of seeds in many cereals is of utmost importance to mankind. If these seeds germinate immediately after harvest in the field, they will become useless to man for consumption as food.*

Methods of breaking seed dormancy

A. Scarification

Any treatment i.e. physical or chemical that weakness the seed coat, is known as scarification. The various ways to tear the seed coat can be done by rubbing the seeds on a sand paper or by any physical means. Care should be taken that not to damage the axis of the seed(eg. green gram and subabool). When seed coat is too hard i.e. of woody nature, the seed coat has to be removed completely by breaking it(e.g. rubber seed and india teak wood seed.) Soaking of seeds in concentrated or diluted solution of sulphuric acid for 1 to 60 minutes removes the seed coat impermeability (eg. cotton seeds, india teak wood seeds etc.).

B. Temperature treatments

When the dormancy is due to embryo factor , the seeds can be incubated at low temp. (0- 5^{o} C) over a substratum for 3 to 10 days (eg. Mustard). Some seeds require a brief period of incubation (from a few hours to one to five days) at 40 to 50 ^{o}C before germinating at required temperature (eg.Paddy). Hot water treatment is also an effective method of breaking hard- seediness in legumes. In this method the seeds are soaked in water at 80^{o}C for1 – 5 minutes (depending up on the type of seed) before putting for germination.

C. Light treatments

Some seeds do not germinate in dark and require continuous or periodic exposure of light (e. g. Lettuce - require red light (660nm) or white light for germination to occur).

D. Treatments with growth regulators &chemicals

Endogenous dormancy may be due to presence of germination inhibitors. Application of low level of growth regulators (i.e. Gibberellins, cytokinins and ethylene etc) may break the seed dormancy. Seeds of sorghum crop presoaked with GA_3 at a concentration of 100 ppm have been used for breaking seed dormancy. Among other chemicals, Potassium nitrate (0.2%) and thio – urea (0.5 to 3%) are widely used for breaking seed dormancy in oat , barley and tomato.

Lecture 28: Senescence and abscission – physiological and biochemical changes

Like human beings, plants a1so grow old and undergo aging and then they die. Aging is the sum total of changes in the total plant or its organs. Duringaging, the plants undergo chemical and structural changes. Aging leads to senescence and later phase of development that u1timately terminates to death.

Senescence

The deteriorative process which naturally terminates the functional life of an organ, organism or other life unit is collectively called senescence. Senescence is a phase of the aging process. The major characteristic of senescence is that the metabolic processes are catabolic and eventually become irreversible and terminate to death.Senescence is not confined only to whole plant. It may be limited to a particular plant organ such as leaf and flowers or cells or cell, organelles. Senescence is closely associated with the phenomenon of aging. Aging leads to senescence. Wheat plant dies after the development of fruit. This is the senescence of an entire plant. Leaf fall in a coconut tree is an example of senescence.

Types of senescence

Leopold (1961) has proposed types of senescence patterns in plants which are as follows.

(a) Overall Senescence

This type of senescence occurs in annuals where whole plant is affected. It is also called whole plant senescence. The entire plant dies after the development of fruit and seeds. E.g. Paddy, wheat, soybean etc.

(b) Top Senescence

In top senescence, the parts remaining above the ground or (shoot system) may die, but the root system and underground system remain viable. It is also called shoot senescence. E.g. Dock, perennial herbs.

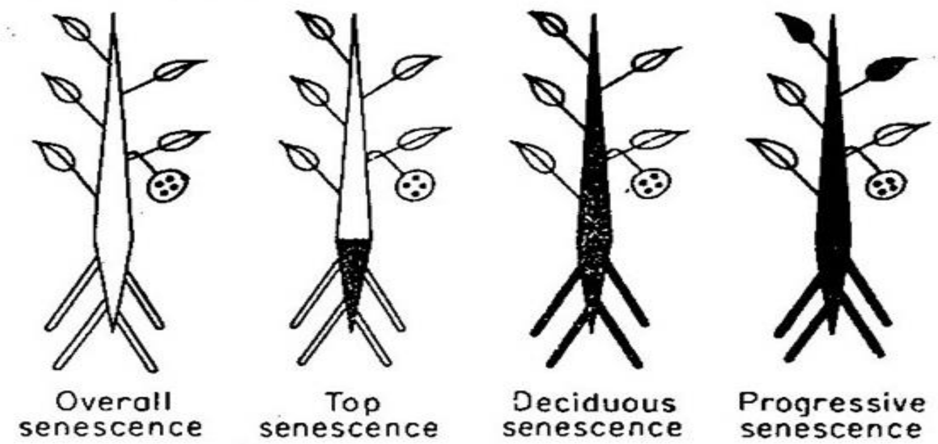
(c) Deciduous Senescence

In deciduous woody plants, all the leaves die but the bulk of the stem and root system remains viable. It is called deciduous senescence or simultaneous or synchronous senescence. E.g. Leaf fall in deciduous trees.

(d) Progressive Senescence

It is a gradual death of old leaves from the base to the top of the plants. It may occur at any time. It is also called sequential senescence. E.g. Leaf fall in a coconut tree.

Fig. 11-13 Senescence may develop in several patterns in plants, such as overall death of the plant (*left*), the senescence of only the aboveground parts, the deciduous habit of leaf senescence, or the progressive senescence of leaves up the stem (*right*) (Leopold, 1961).



Causes of Senescence

1. *Leaf senescence is accompanied by early loss in chlorophyll, RNA and enzymes.*

2. *Cellular constituents are decreased due to slower synthesis or faster break down.*
3. *Competition between vegetative and reproductive organs for nutrients.*
4. *A senescence factor (a hormone) is produced in soybean fruits that move to leaves where it causes senescence.*
5. *Short-day and long-night conditions induce flowering and leaf senescence.*
6. *Degradation of food reserves and loss of integrity in food storage cells of seeds.*
7. *Senescence is also hormonally controlled.*

Physiology of Senescence

The following physiological changes occur during senescence.

1. Photosynthesis stops.

2. Chlorophyll degradation: The colour of leaf changes from green to yellow.

3. Anthocyanin pigments accumulation in the leaves causing reddening in leaves.

4. The vacuoles function as lysosomes and digest the cellular materials.

5. The starch content decreased.

6. RNA and proteins are decreased.

7. DNA molecules are degraded by the enzyme DNase.

8. Growth promoting hormones such as cytokinin decrease.

9. The deteriorative hormones such as ethylene and abscisic acid (ABA) contents are increased.

Senescence Promoters

Senescence is promoted by hormones such as abscisic acid and ethylene. The senescence accelerating ability of abscisic acid is well documented. The function of ABA as a promoter of flower tissue senescence including initiaton of colour fading or blueing has been established. The ABA content of aging leaves increases markedly as senescence is initiated. Ethylene plays a very important role in the senescence of certain plant parts, particularly fruit and petals and in the abscission process. It is an inducer in the senescence of flower tissue.

Senescence Retardants:*The primary plant hormones involved here are auxin, gibberellin and cytokinin.*

Significance of Senescence

1. *The whole plant senescence occurs in monocarpic plants coinciding the seed setting and seed dispersal.*
2. *Due to the formation of abscission layer, the older leaves tend to fall down so that the nutrients will be diverted to the next young leaf.*
3. *The senescence process helps the mobilization of nutrients from the vegetative parts of the plant into the fruits.*
4. *Plants escape the influence of seasonal adversity by undergoing senescence of its organs. Leaf fall in deciduous trees reduces the rate of transpiration to survive under adverse conditions.*

Abscission

Shedding of leaves, flowers and fruits is called abscission. Abscission is distinct in deciduous trees and shrubs. In autumn, all the leaves of deciduous plants fall, at about the same time giving the plants a naked appearance. In evergreen plants there is gradual abscission of leaves. The older leaves fall while new leaves are developed continuously throughout the year. In most of the herbaceous species, however the leaves are not shed even after they die. In many cases leaves are retained in withered dry condition even after the whole shoot is dead.

Abscission is a complex physiological process. During abscission, the colour of the leaves, flowers and fruits changes due to degradation of chlorophyll and the synthesis of anthocyanin pigment.

Leaf abscission takes place at the base of the petiole. The site of abscission is internally marked by a distinct zone called abscission zone. This zone is made up of one or more layers of cells arranged transversely across the petiole base. This is called abscission layer. The abscission zone is pale or brown in colour. The cells of the abscission layer separate from each other due to the dissolution of middle lamellae and the primary cellulose walls under the influence of the activity of enzymes, pectinase and cellulase.

At this stage, the petiole remains attached to the stem by vascular elements only. But due to its own weight and the wind force, the leaf is detached from the stem. The broken *vascular elements are soon plugged with tylosesor gums. Wound healing in cells proximal to the breaking point involves formation of a corky layer that protects the plant from pathogen invasion and excess water loss. Suberin and lignin are synthesized during healing.*

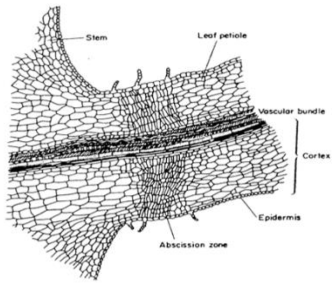
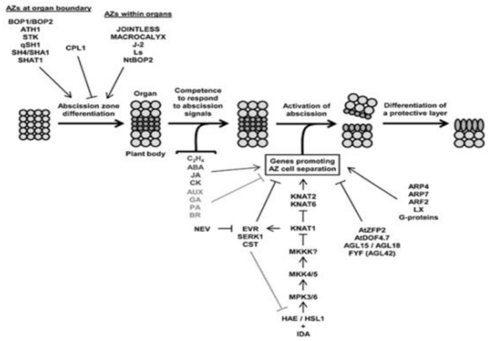


FIG. 12.12. Longitudinal section through the abscission region of the base of the petiole of a leaf of a typical dicotyledonous plant. (From J. Torrey, *Development in Flowering Plants*, Macmillan, New York, 1967.)



Several environmental factors such as drought, high and low temperature stress and high and low light intensity andalso nutrient deficiency promote abscission. Auxin is synthesized in growing leaf blades and it strongly retards senescence and abscission. Abscission starts when the amount of auxin begins to decrease. Cytokinins and gibberellins arriving from the roots also delay senescence and abscission. Abscission is caused by the formation of cell wall degrading enzymes in the abscission zone, due to ethylene production.

Changes during abscission

There is an increase in respirationrate and protein synthesis of plants and the nucleus become enlarged. Cell wall breakdown occurs especially in middle lamella –which increases turgor and pushes cells apart. Xylem vessels shows some lysis and the activity of the enzymes namely catalase, pectinase and cellulase increase.

Significance of Abscission

1. *It helps in diverting water and nutrients to the young leaves*
2. *It is a self pruning process through which fruits and injured organs are shed from the parent plant.*
3. *It helps in disseminating fruits and vegetative propagates.*
4. *Abscission serves as function in removing plant parts containing waste materials.*

Lecture 29: Physiology of fruit ripening- climacteric and non climacteric fruits - factors affecting ripening and manipulations

Ripening is defined as the summation of changes in tissue metabolism rendering the fruit organ attains its full colour, flavour, aroma and other characteristics attractive for consumption.

Ripening is a process in fruits that causes them to become more edible. In general, a fruit becomes sweeter, less green, and softer as it ripens. However the acidity as well as sweetness rises during ripening, but the fruit still tastes sweeter regardless. The reason for this is the Brix-Acid Ratio. Depending upon plucking, fruits are classified as climacteric (ripening hapens after plucking of fruits from tree) and nonclimacteric fruits (which ripen in the tree itself).

After the period of growth, fruit undergoes some characteristic qualitative changes. These changes are collectively referred to as fruit ripening. Some important events in fruit ripening are as follows:

Changes with Ripening

The general changes that occur during the process of ripening of fruits are,

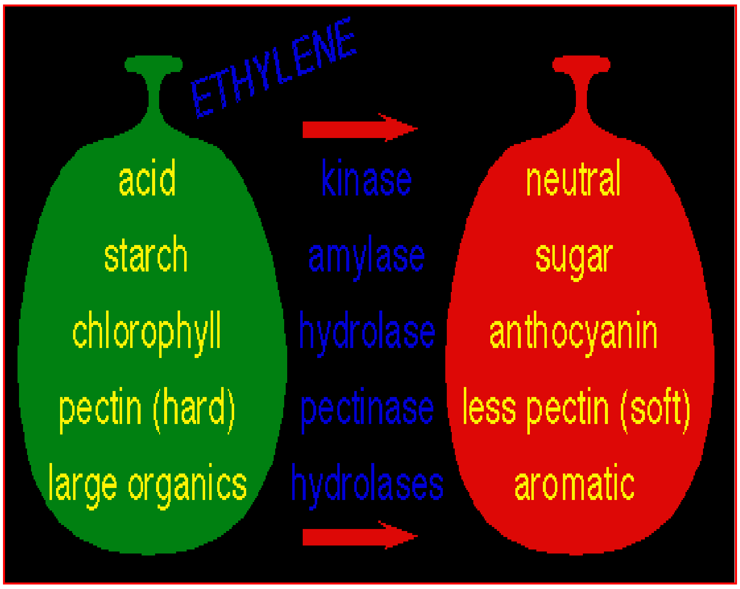
(1) Softening of fruit flesh,
(2) Hydrolytic conversion of storage material in the fruit, and
(3) Changes in pigment and flowers.

Softening is an important change with the ripening of fruits. the major role played in this process is that of cell wall degrading enzymes. But in squashes and avocado, the softening is associated with hydrolysis of cell contents. As such pectolytic enzyme activities induces solubilization of pectic substances found in middle lamellae. The solubilization may occur through an increase in methylationof the galacturonic acid or through reduction in the size of a chain of polygalacturonic or both.

Hydrolytic changes in the fruit during ripening usually lead to the formation of sugars. Such changes show different rates in different fruits. e. g. banana ripens extremely fast; apple shows gradual ripening and citrus fruits show very slow changes, sometimes *taking months. During ripening of fruits some qualitative changes occur such as change in pigmentation, production of flavour material and depletion of astringent substances.*

The changes in pigments in fruits are normally the loss of chlorophyll (green colour) and development of carotenoids (various colour). These changes in colour may be due to loss of chlorophyll with little or no formation of carotenoids as in banana or due to formation of carotenoids, as in oranges. The pigment changes occur mainly in the chloroplasts with grana into chromoplasts with loosely dispersed thylakoid membranes. Electron microscopic studies have revealed that new thylakoid memberanes are synthesized in conversion. Chlorophyll is lost due to chlorophyllase (enzyme) activities. The newly developed pigments may be carotenes as in papaya or anthocyanins as in strawberry and these are synthesized in the presence of sun light and with the involvement of phytochrome.

Very little work has been done on development of flavour substances. in apples, numerous esters, aldehydes, ketones etc. have been identified. The loss of astringent materials such as phenolics is commonly found in pomaceous fruits.



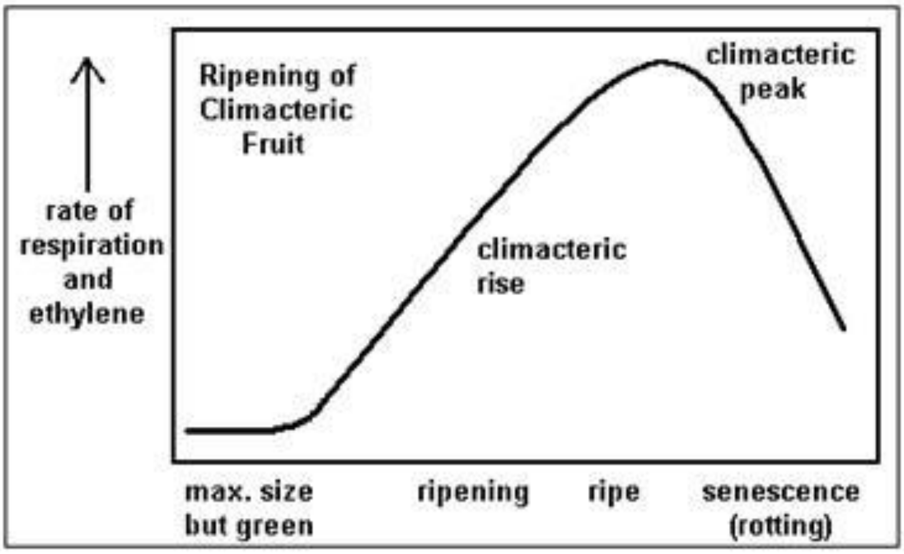
Changes During Fruit Ripening

2. The respiratory climacteric

Kidd and west (1930) observed that in apple fruits, a major change occurs in respiration rates during their ripening. They found lowering of respiration rate in maturing *fruits followed by a large increase in respiration during ripening. And after reaching a climacteric peak, the rate of respiration again falls.*

The period of occurrence of climacteric peak in fruits shows variation in different fruits, e. g. at the time of optimum eating quality as in pears, it slightly precedes this optimum in apple and banana or just before the fruit if fully ripe in tomatoes. Earlier it was suggested that climacteric is associated with the hydrolysis of food reserves, but it has not been found true in all cases, e. g. orange, lemon and fig in which case hydrolytic activities are not found. This climacteric may occur rapidly as in banana or at relatively slower rates as in pear, mango and apples. As regards occurrence of climacteric, fruits may be divided into two types.

1. ***Climacteric fruits**, e. g. banana, apple, papaya, pear, mango, tomatoes, guava etc.*
2. ***Non-Climacteric fruits**, e. g. oranges, lemons, citrus, pepper, pea nut, pineapple, cashew, grapes etc.*



In non-climacteric fruits, the rate of respiration remains steady during their ripening. Climacteric rise has been found affected by low oxygen and increased concentration of carbon dioxide. Both these factors prevent climacteric rise and thus improve storage quality of fruits. An increase in climacteric rise has been found in an atmosphere rich in unsaturated carbohydrates. Gane (1937) established that the ethylene stimulates climacteric rise and that ripe banana stimulates it in the same manner as ethylene.

Some further researchers established that there is a marked rise in ethylene formation in fruits either just at or just before the onset of climacteric rise. It has also been found that the *ability of ethylene to induce climacteric rise is found in both climacteric and non climacteric fruits. Strawberry is one single exception where ethylene has no effect on the rate of respiration and ripening.*

3. Ripening Mechanism:

The mechanism of fruit ripening mainly comprises respiratory source of energy, synthesis of new sets of enzymes and action of these enzymes inducing softening, pigmentation, and qualitative changes. A modern concept of ripening of fruits is based on the synthesis of new enzymes required for the ripening process. It was found that with the blocade of production of energy (respiratory energy) with dinitrophenol, arsenite, or fluoroacetate, the ripening process is terminated and also that ripening can proceed even though the climacteric has been remarkably suppressed. Based on these findings it seems reasonable to believe that respiration provides the energy for the synthesis of new enzymes and for the action of these enzymes in bringing about the ripening changes.

4. Hormonal controls of ripening

Ethylene has been established as a ripening hormone. Massive doses of ethylene can bring about ripening changes in immature fruits. So, it is the hormone which has the most powerful regulatory role in ripening. Burg and Burg (1962) observed that a rise of ethylene occurs at the onset of the climacteric rise and can be assigned the role of the trigger of ripening. They proposed that on set of ripening is associated not only with a rise in the ability to biosynthesise ethylene but also a marked increase in ethylene responsiveness. It has also been found that ethylene can induce a respiratory climacteric in some leaves and develop many of the pigments commonly developed in fruit ripening. In general, ethylene may be bringing about the formation of new types of enzymes in fruits.

However, ethylene is not a universal ripening hormone. Some non-climacteric fruits like strawberry and citrus have no effect on their ripening by the ethylene treatment.

Leopold and Kriedemann (1975) concluded that ripening appears as an unveiling enzyme system which brings about the alteration of the fruit, and respiratory energy must be provided both for the synthesis of the new enzyme systems and for their actions in ripening. Hormonal regulation may be involved in the change from a mature fruit resistant to a ripening to one which becomes receptive to ripening signals.

Factors affecting ripening

1. *Temperature – 13º to 30ºC*
2. *Light – Light essential for ripening in most crops also important in de-greening process.*
3. *Oxygen – Ripening is an aerobic process*
4. *CO_2 – Inhibits the ripening*
5. *Atmospheric Pressure – Enhances the ripening*

Role of PGR's & Nutrients on Shelf life of fruits & Flowers

1. *Low ethylene*
2. *Low O_2*
3. *High CO_2*
4. *Cytokinin treatment*

Ethylene Biosynthesis Inhibitor

AVG – Aminoethoxy Vinyl Glycine and AOA – Amino Oxy Acetic acid

Both of these inhibit the ACC Synthase activity

Ethylene action inhibitor*-STS – SILVER THIO SULPHATE*

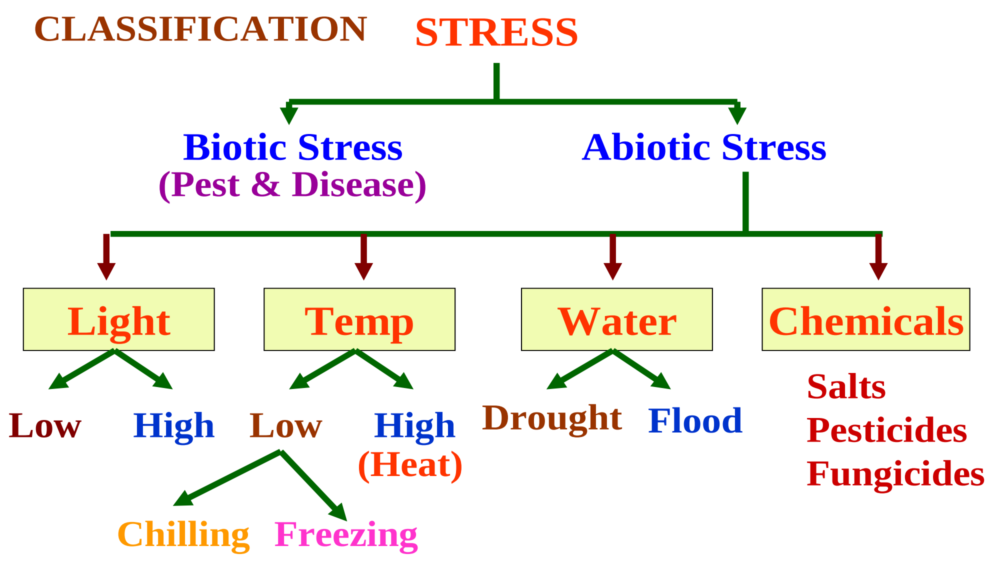
Ethylene receptor inhibitor *-1-MCP –1- METHYL CYCLO PROPENE*

Ethylene absorber - *$KMNO_4$ – POTASSIUM PERMANGANATE*

Lecture 30 :Drought - physiological changes - adaptation – compatible osmolytes – alleviation

*In both natural and agricultural conditions, plants are frequently exposed to environmental stresses.**Stress** is usually defined as an external factor that exerts a disadvantageous influence on the plant. The concept of stress is intimately associated with that of **stress tolerance**, which is the plant's fitness to cope with an unfavorable environment.*

Any change in Environmental conditions that might adversely change the Growth and Development of a plant



Types of drought

Soil Drought:

It often leads to atmospheric drought; mainly resulting due to soil

Physical Soil Drought

due to limited or non-availability of water from various sources like rainfall and irrigation.

Physiological Soil Drought-

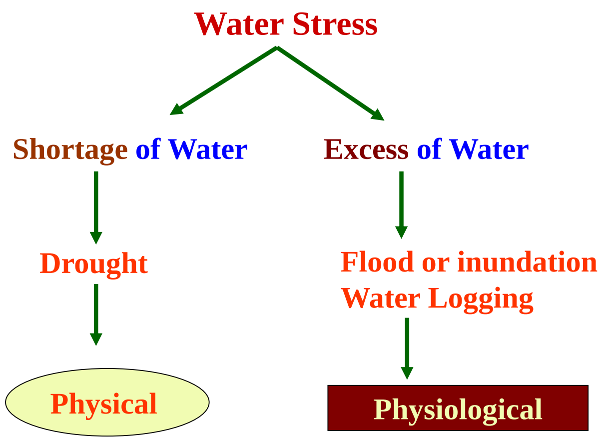
due to the physiological reasons such as presence of excessive salts, pH alterations etc.

Atmospheric Drought

due to low atmospheric humidity, high wind velocity and high temperature which cause a plant to lose most of its water by transpiration, thus resulting in water deficit situations.

DROUGHT (Water stress)

Drought is defined as the deficiency of water severe enough to reduce the plant growth. Drought has been classified into two broad categories viz., soil drought and atmospheric drought. Soil drought leads to atmospheric drought. Atmospheric drought occurs due to low atmospheric humidity, high wind velocity and high temperature which cause a plant to lose most of its water.



Drought resistance

- *The ability of the plant to grow satisfactorily when exposed to periods of water stress is called drought resistance (May &Miltthrope, 1962)*
- *The ability of plants to withstand drought and to overcome easily prolonged wilting with the slightest damage both to the plant and the yield.*

Drought resistance can be classified into three types.

I. Drought escape: *The ability of the plant to complete its life cycle before solid and plant water deficits develop. Developmental plasticity and rapid phenological development comes under this.*

II. Drought Avoidance: *The ability of the plant to bears the periods of drought by maintaining a high plant water status either by saving the water or getting more water. Having less transpiration by waxiness or by deep roots*

III. Drought tolerance :*The ability of a plant to survive under low water potential or ability of the plant to function all metabolic activity under low water potential.*

Physiological changes occur due to drought

1. Functioning of stomata

In general, stomata lose their function and may die, because wilting after certain limit denatures the starch in the guard cells and also in the mesophyll cells.

2. Carbohydrates metabolism in green leaves

The very first effect of drought on carbohydrates metabolism is that starch disappears from the wilted leaves and sugar accumulates simultaneously.

3. Photosynthetic activity

CO_2 diffusion into the leaf is prevented due to decrease in stomatal opening and there by reduces photosynthetic activity in green cells.

4. Osmotic pressure

The reduced amount of water during drought causes an increase in the osmotic pressure of plant cell. This increase in osmotic pressure permits the plant to utilize better soil moisture.

5. Permeability

The permeability to water and urea increases during drought.

6. Biochemical effects

Water shortage alters the chemical composition. For example, starch is converted to sugar, besides this, there is a considerable increase in nitrate nitrogen and protein synthesis is adversely affected.

Adaptations or Mechanism

- *Drought Tolerance-Desiccation tolerance- at low water potential*

- *Desiccation Postponement-at high water potential-maintain tissue dehydration*
  a. *Water Savers-**Ability of plants to reduce the water loss by stomatal control during drought condition (Non Succulents)***

  b. Water Spenders- c.Water Collectors
- *Drought Avoidance- drought escape*
- *Desiccation Tolerance- Ability to function while dehydrated condition*

  Some plants protoplasm can tolerate severe drought without killing-protoplasmic tolerance (True Xerophytes, Lichens **)**

Resurrection plants

Some plants protoplasm dry out during drought and becomes fresh immediately after watering (Selaginellasp)

Ephemerals

These are short lived plants and they complete their life cycle within a short favourable period during rainy season. They pass dry periods in the form of seeds. They are called as drought escaping plants.

Succulent plants

These plants accumulate large quantities of water and use it slowly during dry period. Thus, they pass dry periods or drought without facing it. Such plants develop several morphological adaptations for reducing transpiration such as thick cuticle, reduced leaf area, sunken stomata etc.

Non succulent plants

These plants are in fact the real drought enduring (tolerant) plants. They tolerate drought without adapting any mechanism to ensure continuous supply of water. They develop many morphological adaptations which are collectively called xeromorphy. They develop, in general, greyish colour, reflecting surfaces, smaller leaves, extensive root system, leaf fall during dry season, sunken stomata and thick cuticle etc. They develop an elaborated conducting system. The stomata remain closed mostly in dry periods.

Compatible osmolytes

Compatible solutes are organic compounds that are osmotically active in the cell but do not destabilize the membrane or interfere with enzyme function at high concentrations, as ions *do. Plant cells can tolerate high concentrations of these compounds without detrimental effects on metabolism. Common compatiblesolutes include amino acids such as proline, sugar alcohols such as sorbitol, and quaternary ammonium compoundssuch as glycine betaine*

Two types of osmolytes

- ***Organic solutes and inorganic ions****, play a key role in osmotic adjustment.*
- ***Organic solutes****, known as compatible solutes, include amino acids, Proline,Glycine betaine, glycerol, polyols, sugar alocohol, sorbitol, mannitol, sugars-sucrose,trehalose, and other low molecular weight metabolites, serve a function in cells to lower or balance the osmotic potential of intracellular and extracellular ions in resistance to osmotic stresses.*
- ***Inorganic ions*** *for osmotic adjustment are mainly* Na^+, K^+, Ca^{2+}, *and* Cl^-
- *Inorganic ions make great contribution in osmotic adjustment by ion transport processes with related ion antiporters and ion channels*

Osmotic adjustment or Osmo regulation

Accumulation of Organic compounds in Cytoplasm

↓

Increases the Osmotic Pressure of leaf

↓

Lowers the leaf water Potential

↓

Absorb the water from soil

↓

Maintains the water balance in Plant is ***Osmotic Adjustment***

Methods to overcome / Alleviation drought

- *Selection of drought tolerant species*
- *Adjusting the tome of sowing in such a way that the crop completes its lifecycle before the onset of drought*
- *Seed hardening with KCl,* KH_2PO_4, $CaCl_2$ *or Thiourea*
- *Thinning of poorly established plants*
- *Mulching to minimize the evaporative loss*
- *Foliar spray of antitranspirants such as Kaolin, PMA, Waxes and Silicone oils*
- *Foliar spray of KCl*
- *Foliar spray of growth retardants such as CCC and MC*

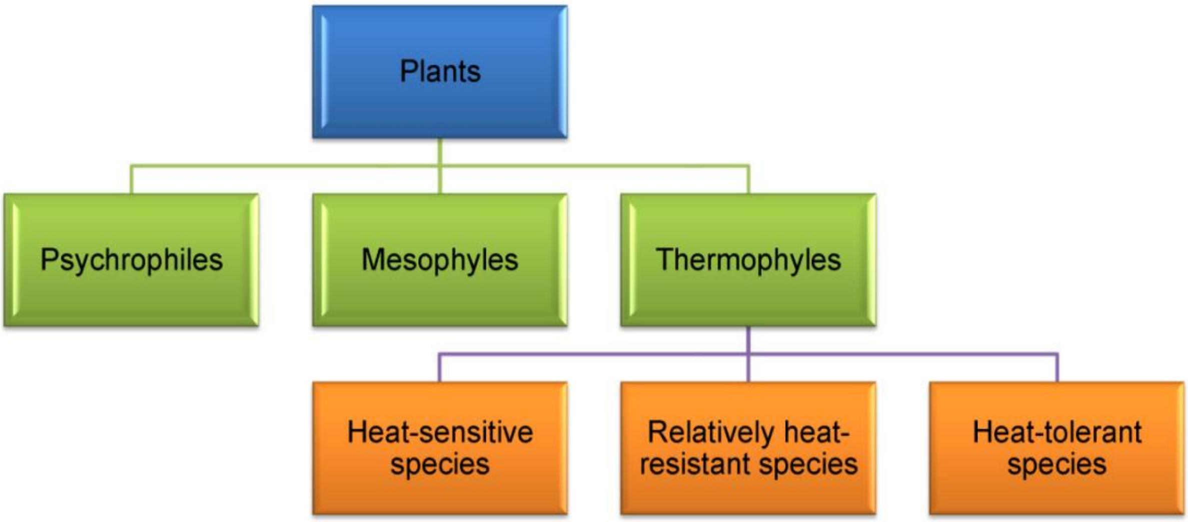
Lecture 31: High and low temperature stress – physiological changes - membrane properties – adaptation

Temperature stress includes both high temperature stress and low temperature stress.

High temperature stress

The effect of high temperature is heat Injury. Heat Injury occurs when plant temperature is higher than that of environment (exceeds 35°C).

Classification of plants on the basis of their heat tolerance



Psychrophiles*: which grow optimally at low temperature ranges between 0 and 10 °C*

Mesophyles*: which favor moderate temperature and grow well between 10 and 30 °C*

Thermophyles*: which grow well between 30 and 65 °C or even higher*

General effects of High temperature

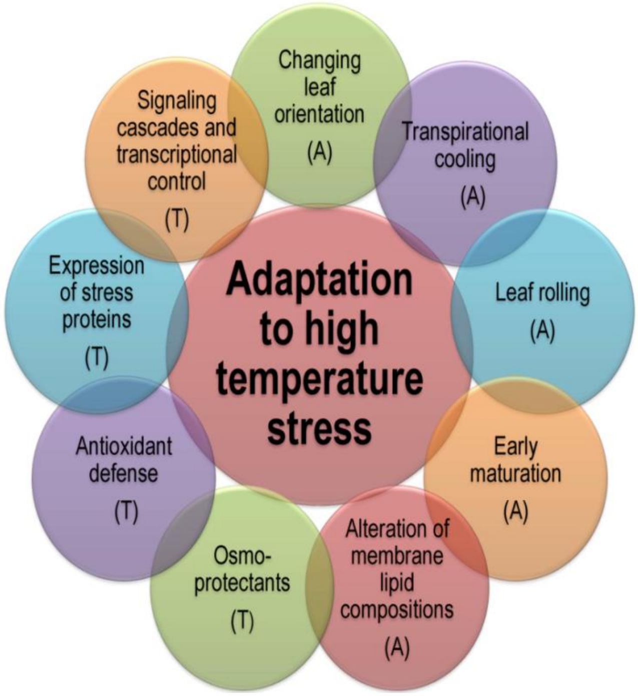
1. *Seedling growth and vigour*
2. *Water and nutrient uptake*
3. *Solute transport*
4. *Photosynthetic activity is more sensitive than respiration to higher temps*
5. *Fertilization and maturation*
6. *at warmer temps, lipids are too fluid and can lead to ion leakage*
7. *Warmer temps also tend to denature/inactivate proteins*

Resistance mechanisms

- *Plants that are adapted to warmer temps tend to have higher concentration of saturated lipids in the membranes*
- *Reflecting infrared radiation (cuticle, trichomes reflect more)*
- *Convection cooling by cooler air around the leaf*
- *Evaporative cooling by transpiration (evaporation of water absorbs heat)*
- *Acute heat stress induces the synthesis of heat shock proteins (HSPs)*

- *HSPs preserve protein structure and assembly at higher temperatures*

Different adaptation mechanisms of plants to high temperature A: Avoidance, T: Tolerance.



Mechanism of Tolerance (Adaptation)-Heat tolerance

1. *Thick bark covering – Insulate covering*
2. *Reflective leaf hairs, Leaf rolling and orientation changes*
3. *Waxy leaf and Dimorphic leaf (White brittlebush)*
4. *High levels of Bound water & high protoplasmic viscosity*
5. *Accumulation of Polyamines*
6. *Synthesis of HSP's and LEA proteins*

Late embryogenesis abundant (LEA) Protein-Cellular Stress Tolerance

- *Late embryogenesis abundant (LEA) proteins are mainly low molecular weight (10-30 kDa) proteins,*
- *Protecting higher plants from damage caused by environmental stresses, especially drought (dehydration), desiccation and high temperature.*
- *Classified into six groups (families) according to their amino acid sequence and corresponding mRNA homology, which are basically localized in cytoplasm and nuclear region.*

HEAT SHOCK PROTEINS (HSP)

- *Master Players for Heat Stress Tolerance*
- *HSPs can be grouped into five different families: HSP100,HSP90, HSP70, HSP60 and HSP 20.*
- *Protect the enzymes and membranes against high temperature*

ALLEVIATING HIGH TEMPERATURE STRESS

1. ***Shade**: It may used for high cash crops (Ornamentals), typically a cloth or lathe house. Shading decreases leaf temperature, not air temperatures.*
2. ***Green house**: It should be whitewash, provide fans, evaporative coolers (Where humidity allows)*
3. ***Overhead Irrigation**: As water evaporates heat is absorbed. Cools plant body, but encourages disease.*
4. ***GA_3 and proline** application exhibit positive effects on stress alleviation through the stimulation of α – amylase expression*
5. ***Zeatin Riboside** is the most effective in slowing leaf senescence and alleviating heat induced lipid peroxidation of cell membranes.*
6. *The inhibitory effect of high temperature on seed germination can be overcome by exogenous application **of ethylene.***
7. *Application of **Glycine Betaine** under heat stress appreciably reduced the leakage of all these ions, particularly Ca^{2+}, K^+ and NO_3.*
8. *Exogenous application of **salicylic acid** enhanced the thermo tolerance ability of both roots and hypocotyls in intact seedlings*

Low temperature stress causes chilling injury and freezing injury.

1. Chilling injury

The tropical origin plants are injured when the temperature drops to some point close to 0°C. The injury which occurs due to low temperature but above zero degree centigrade is called chilling injury.

2. Freezing injury

Freezing injury occurs when the temperature is 0°C or below.

Effect of freezing and chilling injury plants

- *The lipid molecules in cell membrane get solidified i.e. changed from liquid state to solid state. Hence, the semi-permeable nature of the membrane is changed and the membrane becomes leaky.*
- *Inactivation of mitochondria*
- *Streaming of protoplasm is stopped*
- *Accumulation of respiratory metabolites which become highly toxic*
- *Ice formation inside the cell occurs.*

Prevention of cold injury

- *Some plants change the pattern of growth.*
- *The growth is completely arrested during this period.*
- *In cell membrane, unsaturated fatty acid content is increased.*
- *Intracellular ice formation is reduced.*

- *The quantity of free enzymes, sugars and proteins increases.*

Chilling sensitive crops

- *Maize, Rice, Bean, Tomato, Cucumber & Sweet potato*
- *Chilling injury temperature = 10 to 15ºC*

Chilling tolerant crops

- *Spring wheat, peas, potato and cabbage*

Mechanism of chilling tolerance

1. ***Sensitive or resistant*** *depends upon ratio between* **Saturated FA and Unsaturated FA**
   - *Sensitive plant – Higher proportion of SFA*
   - *Resistant plant - Higher proportion of USFA*
   - *SFA – Quick solidification – Disrupting the membrane*
   - *USFA – Allows the membrane remain fluid*

2. ***Cold Acclimation Proteins (CAP)****-Protects the Thylakoid membrane from chilling*
3. ***Ice Recrystalization****-Freeze without damage – slow ice crystal formation – Small round ice – less damage in plants*
4. ***Antifreeze compounds****-Lowers the freezing point of Cytoplasm. Ex: Proline*
5. ***Physical barriers*** *– Pectin – Insoluble in nature-Prevents ice formation – Xylem discontinuity – No Connection to the rest of the plant*

Alleviation of low temperature stress

- *Water spray*
- *Degree of shoot and root pruning*
- *Foliar spray of 0.15 % Ammonium molybdate*
- *Pre-soaking treatment with GA_3 and Proline – Seed germination*
- *Paclobutrazol – Increased activity of Scavenging enzymes*
- *Uniconzole (50 ppm) – Reduce the electrolyte leakage*
- *Use of Cryoprotectants-New frost resistant proteins are synthesized*

Cold acclimation (cold hardening)

- ✓ *Increased freezing tolerance after prior prolonged exposure to low, nonfreezing temperatures*
- ✓ *ABA has a role in induction of freezing tolerance*
- ✓ *Plants develop freezing tolerance at non-acclimating temperatures when treated with exogenous ABA*

Lecture 32: Salt stress - physiological changes - adaptation – compartmentalization – alleviation

Salt stress occurs due to excess salt accumulation in the soil. As a result, water potential of soil solution decreases and therefore exosmosis occurs. This leads to physiological drought causing wilting of plants.

Salts responsible for salinity

Accumulation of salts in the soil from irrigation water is major problem

Na^+, Ca^{2+}, Mg^{2+} , K^+ , Cl^- , SO_4^{2-} & HCO_3^- *- These ions are Usually responsible for soil salinity*

Na^+, Cl^- *and* HCO_3^- *-- Potentially toxic to the plants*

High concentration of Na^+ *- Sodicity*

High concentration of Total Salts – Salinity

Classification of Saline soil: *1. Saline soil 2. Alkaline soil*

1. Saline soil

In saline soils, the electrical conductivity is greater than 4 dS/m, exchangeable sodium percentage is less than 15% and pH is less than 8.5. These soils are dominated by Cl^- *and* SO^{2-}_4 *ions.*

2. Alkaline soil

Alkaline soils are also termed as sodic soils wherein, the electrical conductivity is less than 4 dS/m, exchangeable sodium percentage is greater than 15% and pH of the soil is greater than 8.5. These soils are dominated by CO^-_3 *and* HCO^-_3 *ions.*

Classification of Plants

Plants are classified into two types based on the tolerance to salt stress. They are halophytes and glycophytes.

1. Halophytes

Halophytes are the plants that grow under high salt concentrations. They are again divided into two types based on extreme of tolerance. Ex: ***Atriplex***

Euhalophytes: can tolerate extreme salt stress

Oligohalophytes: can tolerate moderate salt stress

2. Glycophytes

Glycophytes are the plants that cannot grow under high salt concentration.

Effect of Salt stress on plant growth and yield

1. Seed germinaon

Salt stress delays seed germination due to the reduced activity of the enzyme, α-amylase

2. Seedling growth

The early seedling growth is more sensitive. There is a significant reduction in root emergence, root growth and root length.

3. Vegetative growth

When plants attain vegetative stage, salt injury is more severe only at high temperature and low humidity. Because under these conditions, the transpiration rate will be very high as a result uptake of salt is also high.

4. Reproductive stage

Salinity affects panicle initiation, spikelet formation, fertilization and pollen grain germination.

5. Photosynthesis

Salinity drastically declines photosynthetic process. Thylakoid are damaged by high concentration of salt and chlorophyll b *content is drastically reduced.*

Mechanism of salt tolerance

1. *Some plants are able to maintain high water potential by reducing the transpiration rate.*
2. *Salts are accumulated in stem and older leaves in which metabolic processes take place in a slower rate.*
3. *Na^+ (sodium ion) toxicity is avoided by accumulating high amount of K^+ ions.*
4. *Accumulation of toxic ions in the vacuole but not in the cytoplasm.*
5. *Accumulation of proline and abscissic acid which are associated with tolerance of the plants to salt.*

6. *Avoidance -It is the process of keeping the salt ions away from the parts of the plant where they are harmful*

   - *Salt Exclusion-The ability to exclude salts occurs through filtration at the surface of the root Root membranes prevent salt from entering while allowing the water to pass through*

   - ***Salt excretion / extrusion / secretion-Salt excreters - accumulate salts in special cells***
   - *Secreting excess salt by foliar glands*
   - *Salt glands or Vesiculated hairs-Salt Bush-Salt bladders*
   - *Salt cedar – Secretes through cuticle*
   - *Crystallization of salts takes place in salt glands*
   - *Crystallized salt is unharmful*
   - *Succulence – Salt dilution*

Salt Tolerate Mechanism

1. *Compartmentalization of salts - Check to reach photosynthetic area*
   - *Cytotoxic Ions –* Na^+ *and* Cl^-
   - *Compartmentalised into Vacuoles*
   - *Used as Osmotic solutes or Osmoprotectants*
   - *Osmotic adjustment*
   - *Increase the vacuolar volume*
   - *Cellular development*
2. *Accumulation of osmolytes in leaf – Maintain turgor*

Relative salt tolerant crops

Tolerant crops: Cotton, sugar cane, barley

Semi tolerant crops: Rice, maize, wheat, oats, sunflower, soybean

Sensitive crops: Cow pea, beans, groundnut and grams

Alleviation of salt stress

1. *Application gypsum to convert the highly injurious carbonates to less injurious sulphate*
2. *Leaching excess salts from upper to lower layers*
3. *Increased Seed rate*
4. *Older seedlings - more tolerant than younger one*
5. *Auger – hole planting Technique*
6. *Foliar application should be followed*
7. *Sesbania - legume tolerant to alkali*

Lecture 33: Flooding and UV radiation stresses – physiological changes – adaptation

Soil flooding creates composite and complex stress in plants known as either submergence or waterlogging stress depending on the depth of the water table. In nature, these stresses are important factors dictating the species composition of the ecosystem.

Flooding also known as waterlogging or submergence is one of the major abiotic stresses experienced frequently by plants.Flooding is also compound stress composed of interacting changes inside plant cells induced by the flood water surrounding the plant.

Flood types

Flash flood: *Short duration over a few weeks (2 weeks) and not very deep. Water level can reach 50 cm*

Deep flooding: *Lasts for a long time (several months).*

Key words for flooding stress

- *Normoxia - Aerobic respiration*
- *Hypoxia - Too small amounts of oxygen - ATP production via oxidative phosphorylation*
- *Anoxia – absence of oxygen - ATP produced by Fermentative metabolism*

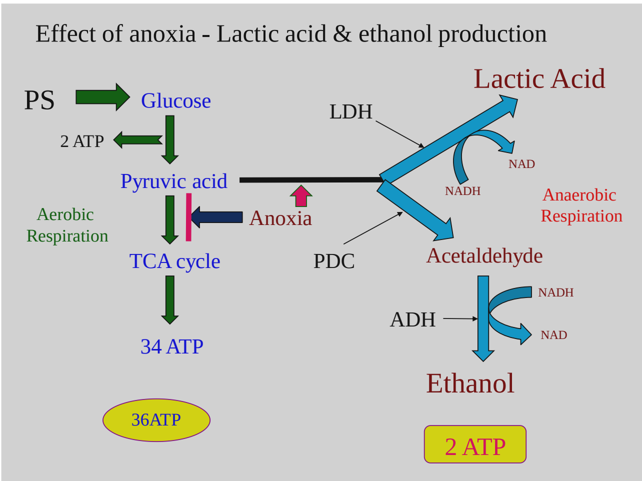
Physiological effects of Flooding

- *Poor aeration-Anaerobic respiration - Fermentation*
- *Degradation of mitochondria*
- *e^- transport & Oxidative phosphorylation, TCA cycle affected*
- *Reduction of ATP -Reduction of water absorption*
- *Production of alcohol (toxic), ethylene & Lactic acid*
- *Cytoplasmic Acidosis – Cell death*
- *Protein synthesis affected*
- *Block of cell division, Elongation & Ion transport*

Plant level effect by Waterlogging

1. *Leaf rolling & curling*
2. *Wilting*
3. *Abscission*
4. *Epinasty*
5. *Lenticels formation*
6. *Nutrient deficiency & Toxicity*

Effect of anoxia on Lactic acid & ethanol production



Lack of O_2 – Anoxic (complete deficit) & Hypoxic (Partly deficitUltraviolet radiation stress

- *Denitrification (NO formation) & Iron toxicity*
- *SO_4 reduced to H_2S + Acetic acid & butyric acid (Anaerobes metabolites)-Unpleasant odour in waterlogged soil*

- *O_2 deficit – ABA production in root – transport to leaf -Stomatal closure occur*

Adaptive mechanisms to Floods

1. *Thick root hypoderm (reduce oxygen depletion)*
2. *Aerenchyma (continuous intercellular spaces for O_2 move)*

   Formation of Aerenchyma -Mediated by Hormone – Ethylene-Signals by Ethylene & Calcium-They cause cell death & cell separation-Enhanced levels of Xylanase&Glucanase -Cell wall degradation occurs-thus aerenchyma formation
3. *Lenticels (oxygen exchange)*
4. *Adventitious aerial roots from stem (Cucurbits)*
5. *Pneumatophores (superficial roots - negative geotropism)*
6. *Increased internode elongation by ethylene*
7. *Suberized & Lignified cells (Not allowing escape of O_2)*
8. *Roots get O_2 through enzymatic process – PR*
9. *Production of Anaerobic Response Proteins (ANP's)*
10. *Increased glycolytic flow (Pasteur effect)*

Flooding sensitive crops

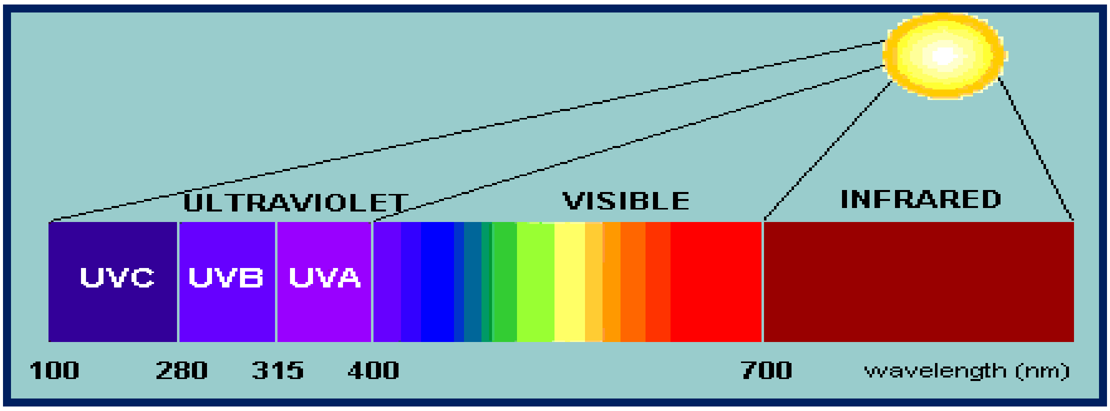
Soybean, Tomato, Peas &Chillies

Flooding tolerant crops

Rice, wheat, sorghum & Maize

ULTRAVIOLET (UV) RADIATION Stress

Plants use sunlight for photosynthesis and, as a consequence, are exposed to the ultraviolet (UV) radiation that is present in sunlight. Ultraviolet (UV) radiation is an integral part of the sunlight that reaches the surface of the Earth. The UV region of the spectrum is by convention divided into three parts: UVA (320–400 nm), UVB (280–320 nm) and UVC (less than 280 nm). Of these, only UVA and longer-wavelength UVB have biological importance because the stratospheric ozone layer very effectively absorbs UV radiation that has wavelengths below 290 nm. UVB represents a small fraction of total solar radiation, yet *exposure to UVB at ambient or enhanced levels is known to elicit a variety of responses and causes stress in all living organisms, including higher plants*



Types	***Wave length (nm)***	***Effects***
UV-A	***320-400 nm***	***Least dangerous form***
UV-B	***280-320 nm***	***Most common; Moderately dangerous form***
UV-C	***100-280 nm***	***Most dangerous*** ***(unable to reach earth surface)***

UV radiation and plant response

1. *UV radiation slows down the growth of plants*
2. *Damage the process of photosynthesis*
3. *Prevent maturation and ripening process*
4. *Accelerate genetic mutation*

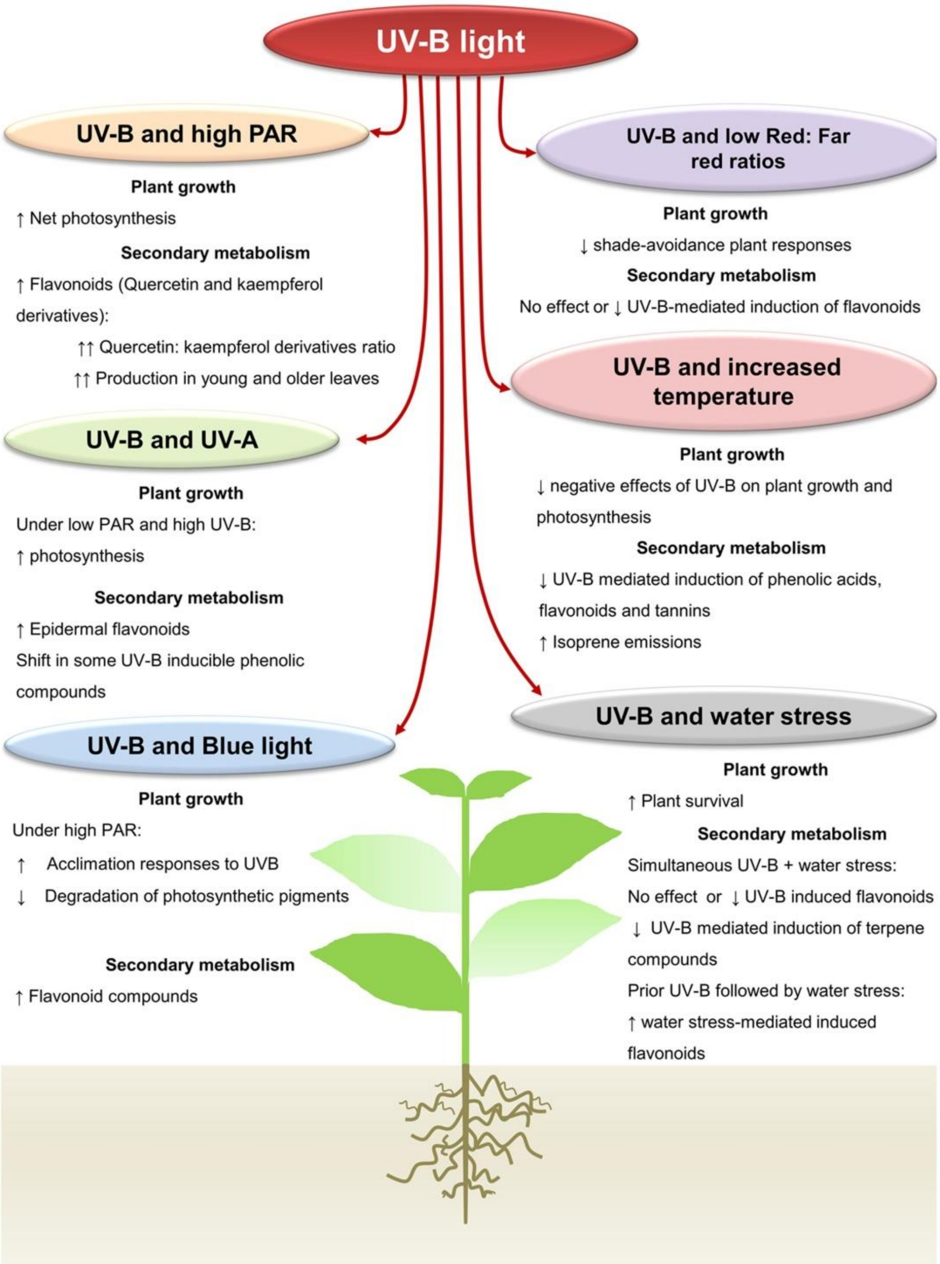
Direct effects of UV-B on plants

- ✓ *Altered gene activity*
- ✓ *Non-specific damage to DNA*
- ✓ *Alterations in plant hormones or nucleic acids*
- ✓ *changes in leaf secondary metabolites*
- ✓ *alterations in leaf anatomy & morphology*
- ✓ *reductions in photosynthesis*
- ✓ *changes in biomass allocation and growth*
- ✓ *Peroxidation of lipids in membranes*
- ✓ *Photooxidation of Hormones*

- ✓ *Formation of ROS*
- ✓ *Dedradation of PSI and PSII-D1 and D2 proteins*
- ✓ *Damaging of thylakoid membranes*
- ✓ *Altered flowering and reduced fertility*

Indirect effects of UV-B on plants

- ✓ *Affect Nutrient mobilization*
- ✓ *Susceptibility of plants to insects and pathogens*
- ✓ *Susceptibility to abiotic stress*



Interactive effects of UV-B light with other abiotic factors on plant growth and production of plant secondary metabolites

Adaptations of Plants to UV radiation

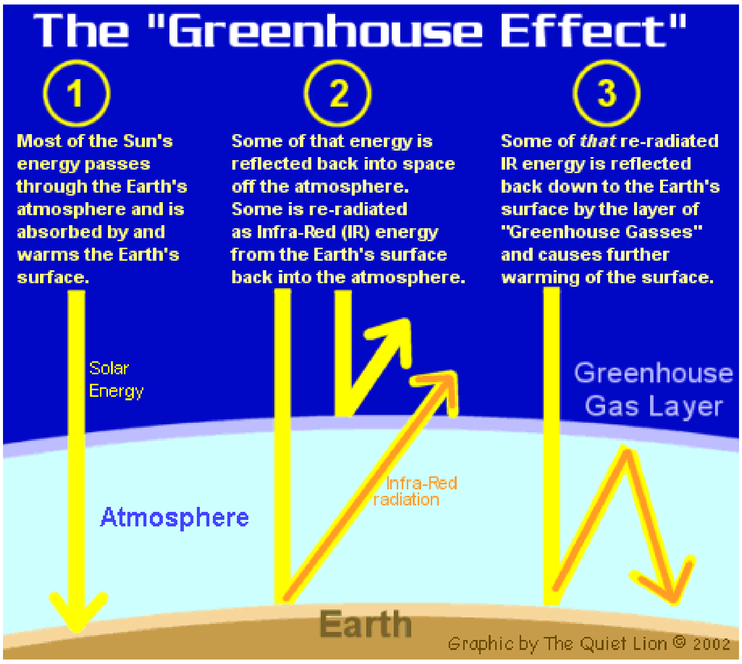
1. *Enhanced UVB radiation significantly decreases plant height and leaf area and increases leaf thickness, Increased Leaf thickness suggests the possibility of a lower penetration of UVB radiation in the deeper mesophyll layer.*
2. *Low dose of UVB radiation can induce alterations in antioxidant status such as regulation of glutathione pathways phenyl propanoids pathways, flavonoids etc..,*
3. *UVB stimulates plants to accumulate specific flavonoids, glycosides which are produced in the vacuoles of epidermal and sub epidermal cells protecting plants from UVB radiation.*
4. *Early light induced proteins and a range of transcription factors, transporters and proteases area activated under light stress, which have a key role in protecting plants against UVB radiation.*

Lecture 34 Global warming - Physiological effects on Crop Productivity

Global Warming is the increase of Earth's average surface temperature due to effect of greenhouse gases, such as carbon dioxide emissions from burning fossil fuels or from deforestation, which trap heat that would otherwise escape from Earth. This is a type of greenhouse effect.

The Sun powers Earth's climate, radiating energy at very short wavelengths, predominately in the visible or near-visible (e.g., ultraviolet) part of the spectrum. Roughly one-third of the solar energy that reaches the top of Earth's atmosphere is reflected directly back to space. The remaining two-thirds are absorbed by the surface and, to a lesser extent, by the atmosphere. To balance the absorbed incoming energy, the Earth must, on average, radiate *the same amount of energy back to space. Because the Earth is much colder than the Sun, it radiates at much longer wavelengths, primarily in the infrared part of the spectrum. Much of this thermal radiation emitted by the land and ocean is absorbed by the atmosphere, including clouds, and reradiated back to Earth. This is called the* ***greenhouse effect.***

The glass walls in a greenhouse reduce airflow and increase the temperature of the air inside. Similarly, but through a different physical process, the Earth's greenhouse effect warms the surface of the planet. ***Without the natural greenhouse effect, the average temperature at Earth's surface would be below the freezing point of water. Thus, Earth's natural greenhouse effect makes life as we know it possible.*** *However, human activities, primarily the burning of fossil fuels and clearing of forests, have greatly intensified the natural greenhouse effect, causing global warming.*



A ***greenhouse gas*** *(GHG) is a gas in an atmosphere that absorbs and emitsradiation within the thermal infrared range. This process is the fundamental cause of the greenhouse effect. The primary greenhouse gases in the Earth's atmosphere are water vapor, carbon dioxide, methane, nitrous oxide, and ozone. When ranked by their direct contribution to the greenhouse effect, the most important are:*

Compound	Formula	Contribution (%)

Water vapor	H_2O	36–72%
Carbon dioxide	CO_2	9–26%
Methane	CH_4	4–9%
Ozone	O_3	3–7%

Other greenhouse gases include nitrous oxide (N_2O), sulfur hexafluoride, hydro fluorocarbons (HFC), perfluorocarbons and chlorofluorocarbons (CFC).

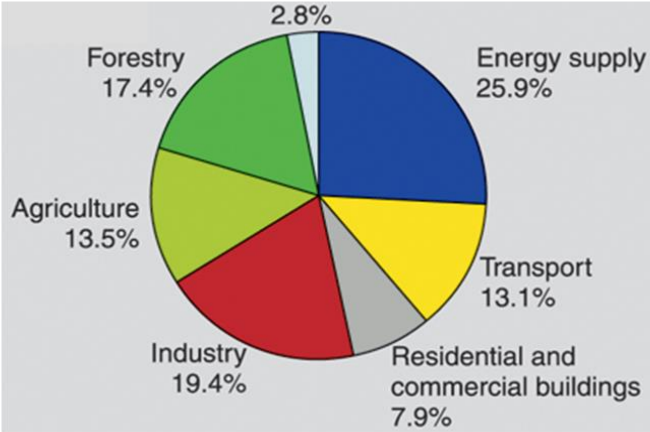
Why CO_2 and water vapour are the major reasons for global warming?

There are two reasons: First, although the concentrations of these gases are not nearly as large as that of oxygen and nitrogen (the main constituents of the atmosphere), neither oxygen or nitrogen is greenhouse gases. This is because neither has more than two atoms per molecule (i.e. their molecular forms are O_2 and N_2, respectively), and so they lack the *internal vibrational modes* that molecules with *more* than two atoms have. **Both water and CO_2, for example, have these "internal vibrational modes", and these vibrational modes can absorb and reradiate infrared radiation, which causes the greenhouse effect.**

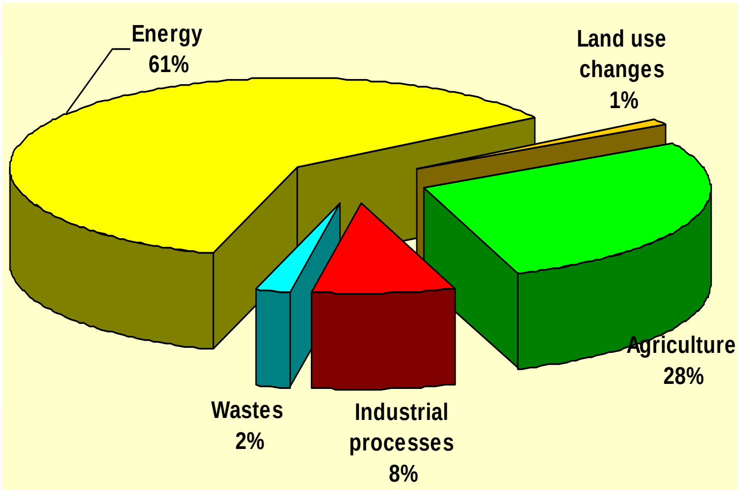
Secondly, CO_2 **tends to remain in the atmosphere for a very long time** (time scales in the hundreds of years). Water vapor, on the other hand, can easily condense or evaporate, depending on local conditions. Water vapor levels therefore tend to adjust quickly to the prevailing conditions, such that the energy flows from the Sun and re-radiation from the Earth achieve a balance. **CO_2 tends to remain fairly constant and therefore behave as a *controlling* factor, rather than a *reacting* factor. More CO_2 means that the balance occurs at higher temperatures and water vapor levels.**

The increased amounts of carbon dioxide (CO_2) and other greenhouse gases (GHGs) are the primary causes of the human-induced component of warming. They are released by the burning of fossil fuels, land clearing and agriculture, etc. and lead to an increase in the greenhouse effect. ***The first speculation that a*** *greenhouse effect* ***might occur was by the*** *Swedish chemistSvante Arrhenius* ***in 1897, although it did not become a topic of popular debate until some 90 years later.***

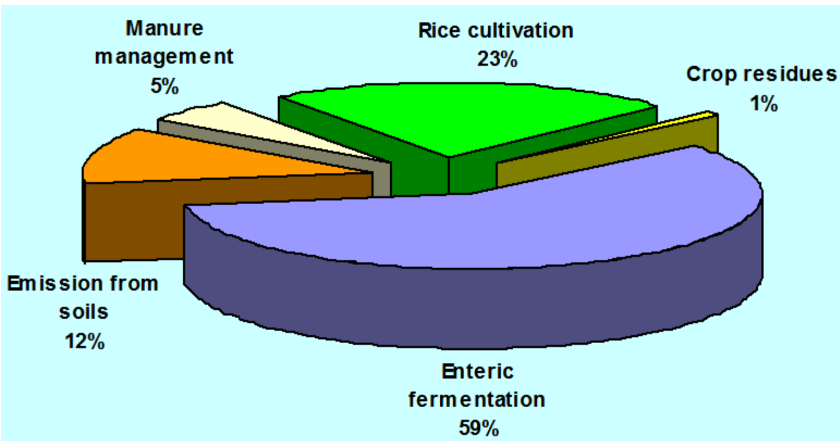
Sources of Greenhouse Gas emissions in world to climate change



Sources of greenhouse gas emissions in India



Contribution of agricultural sector on climate change



Top-10 annual energy-related CO_2 emitters for the year 2009

Country	*% of global total annual emissions*	*Tonnes of GHG per capita*
People's Rep. of China	23.6	5.13

United States	*17.9*	*16.9*
India	*5.5*	*1.37*
Russian Federation	*5.3*	*10.8*
Japan	*3.8*	*8.6*
Germany	*2.6*	*9.2*
Islamic Rep. of Iran	*1.8*	*7.3*
Canada	*1.8*	*15.4*
Korea	*1.8*	*10.6*
United Kingdom	*1.6*	*7.5*

Top-10 cumulative energy-related CO_2 emitters between 1850–2008

Country	***% of world total***	***Metric tonnes CO_2 per person***
United States	*28.5*	*1,132.7*
China	*9.36*	*85.4*
Russian Federation	*7.95*	*677.2*
Germany	*6.78*	*998.9*
United Kingdom	*5.73*	*1,127.8*
Japan	*3.88*	*367*
France	*2.73*	*514.9*
India	*2.52*	*26.7*

Canada	*2.17*	*789.2*
Ukraine	*2.13*	*556.4*

Impact of global warming:

1. ***Rising Seas****--- inundation of fresh water marshlands (the everglades), low-lying cities, and islands with seawater.*
2. ***Changes in rainfall patterns*** *--- Occurrence of droughts and fires in some areas, and flooding in other areas.*
3. ***Increased likelihood of extreme events****--- such as flooding, hurricanes, warmer summers and mild winters etc.*
4. ***Melting of the ice caps*** *--- loss of habitat near the poles. Polar bears are now thought to be greatly endangered by the shortening of their feeding season due to dwindling ice packs.*
5. ***Melting glaciers*** *- significant melting of old glaciers is already observed.*
6. *Widespread loss of animal populations due to widespread habitat loss.*
7. ***Spread of disease*** *--- migration of diseases such as malaria to new, now warmer, regions.*
8. ***Bleaching of Coral Reefs due to warming seas and acidification due to carbonic acid formation*** *--- One third of coral reefs now appear to have been severely damaged by warming seas.*
9. ***Loss of Plankton due to warming seas*** *--- The enormous (900 mile long) Aleution island ecosystems of orcas (killer whales), sea lions, sea otters, sea urchins, kelp beds, and fish populations, appears to have collapsed due to loss of plankton, leading to loss of sea lions, leading orcas to eat too many sea otters, leading to urchin explosions, leading to loss of kelp beds and their associated fish populations.*

Some positive effects of global warming:

1. Increase in the crop yield in mid latitude countries.

2. Increase in rainfall in higher latitudes

3. Reduction in death rate in tropic regions

4. In Arctic there will be more running and standing water, thinner and reduced ice cover.

Projected impacts of climate change on Indian agriculture

The negative impact of global warming will be more severe on developing countries than industrial countries. Most developing countries have less capacity to adapt than do their wealthier neighbors. Most are in warmer parts of the globe, where temperatures are already close to or beyond thresholds at which further warming will reduce rather than increase agricultural output.And agriculture is a larger share of developing economies than of industrial countries.

Climate change can affect agriculture in a variety of ways.

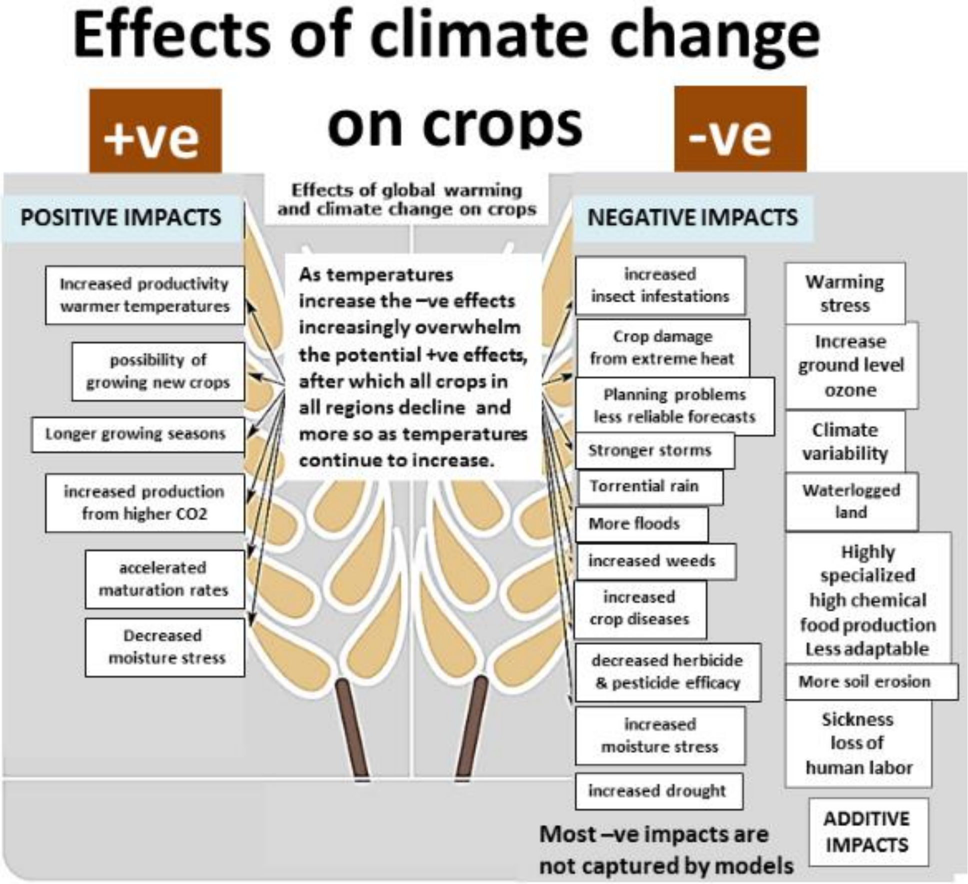
1. Beyond a certain range of temperatures, warming tends to reduce yields because crops speed through their development, producing less grain in the process.

2. Higher temperatures also interfere with the ability of plants to get and use moisture. Evaporation from the soil accelerates when temperatures rise and plants increase transpiration—that is, lose more moisture from their leaves. The combined effect is called "evapotranspiration."

3. But a key culprit in climate change—carbon emissions—can also help agriculture by enhancing photosynthesis in many important, so-called C3, crops (such as wheat, rice, and soybeans)but this phenomenon does not much help for C4 crops (such as sugarcane and maize), which account for about one-fourth of all crops by value.

- *Cereal productivity to decrease by 10-40% by 2100.*
- *Greater loss expected in rabi. Every 1°C increase in temperature reduces wheat production by 4-5 million tons. Loss only 1-2 million tons if farmers could plant in time.*
- *Reduced frequency of frost damage: less damage to potato, peas, mustard*
- *Increased droughts and floods are likely to increase production variability*
- *Increase in temperature, speed up the development and hence reduction in days between sowing and harvesting and hence biomass reduces, so yield reduces.*
- *Increase in carbon dioxide concentration has both positive and negative effect.*
    - *Increase in carbon dioxide concentration increases photosynthesis and hence yield increases. But this advantage is more for C_3 plants than C_4 plants.*

- *Increase in carbon dioxide concentration leads to reduced uptake of nitrogen and reduced uptake of zinc.*



Role of Agriculture in mitigating climate change

There are several adaptation measures that the agricultural sector can undertake to reduce the negative impact of climate change. These include:

i. *Changing planting dates*
ii. *Planting different varieties or crop species*
iii. *Development and promotion of alternative crops*
iv. *Developing new drought and heat-resistant varieties*
v. *Improve crop residue and weed management*
vi. *More use of water harvesting techniques*
vii. *Better pest and disease management*
viii. *Practicing soil conservation techniques*

Who studies global warming, and who believes in it?

Most of the scientific community, represented especially by the Intergovernmental Panel on Climate Change (IPCC - www.ipcc.ch), now believes that the global warming effect is real.

Who are the IPCC?

In 1998, the Intergovernmental Panel on Climate Change (IPCC) was established by the World Meteorological Organization (WMO) and the United Nations Environment Programme (UNEP), in recognition of the threat that global warming presents to the world.

The IPCC is open to all members of the UNEP and WMO and consists of several thousand of the most authoritative scientists in the world on climate change. The role of the IPCC is to assess the scientific, technical and socio-economic information relevant for the understanding of the risk of human-induced climate change.

The IPCC has completed two assessment reports. Results of the ***first assessment (1990--1994):*** *confirmed scientific basis for global warming but concluded that ``nothing to be said for certain yet''.* ***The second assessment (1995),*** *concluded that `` ...the balance suggests a discernable human influence on global climate'', and concluded that, as predicted by climate models, global temperature will likely rise by about 1-3.5 Celsius by the year 2100.* ***The next report, in 2000****, suggested, that the climate might warm by as much as 10 degrees Fahrenheit over the next 100 years, which would bring us, back to a climate not seen since the age of the dinosaurs. The most recent report, in 2001, concluded that* ***"There is new and stronger evidence that most of the warming observed over the last 50 years is attributable to human activities".***

A ***carbon footprint*** *is historically defined as "the total sets of greenhouse gas emissions caused by an organization, event, product or person. Greenhouse gases (GHGs) can be emitted through transport, land clearance, and the production and consumption of food, fuels, manufactured goods, materials, wood, roads, buildings, and services. For simplicity of reporting, it is often expressed in terms of the amount of carbon dioxide, or its equivalent of other GHGs, emitted.*

The most common way to reduce the carbon footprint of humans is to *Reduce, Reuse, Recycle, Refuse.*

1. This can also be done by using reusable items such as thermoses.

2. Another easy option is to drive less. By walking or biking to the destination rather than driving, not only is a person going to save money on gas, but they will be burning less fuel and releasing fewer emissions into the atmosphere. However, if walking is not an option, one can look into carpooling or mass transportation options in their area.

3. Yet another option for reducing the carbon footprint of humans is to use less air conditioning and heating in the home.

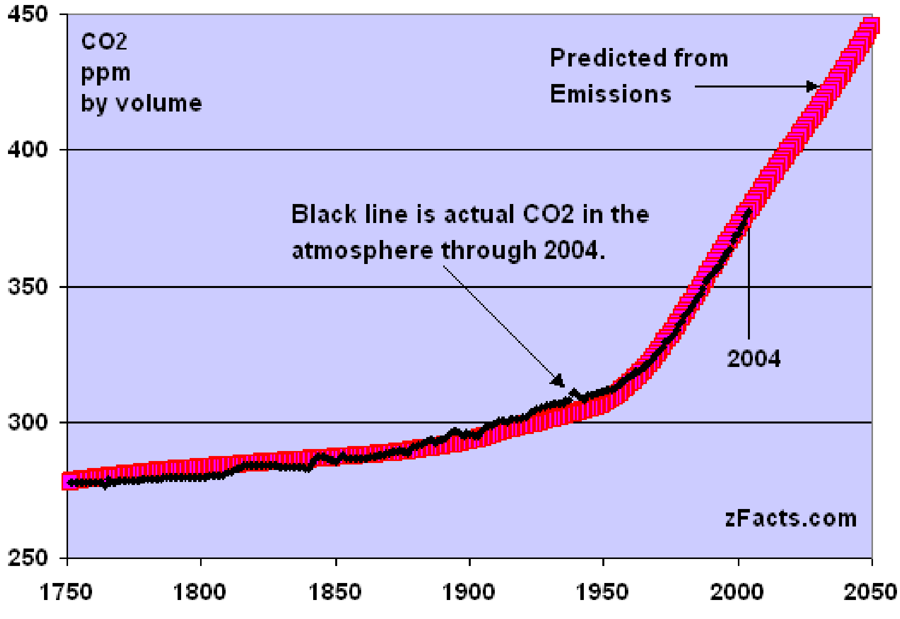
4. Choice of diet is a major influence on a person's carbon footprint. Animal sources of protein (especially red meat), rice (typically produced in high methane-emitting paddies), foods transported long distance and/or via fuel-inefficient transport (e.g., highly perishable *produce flown long distance) and heavily processed and packaged foods are among the major contributors to a high carbon diet.*

Carbon emissions trading:

This one of the steps taken by Kyoto protocol, to reduce carbon emissions. Under Carbon trading, a country having more emissions of carbon is able to purchase the right to emit more and the country having less emission trades the right to emit carbon to other countries. More carbon emitting countries, by this way try to keep the limit of carbon emission specified to them.

Carbon Sequestration - physiological effects on crop productivity

Atmospheric levels of CO_2 have risen from 280 parts per million (ppm) to 375 ppm. This rise in level is primarily due to use of fossil fuels for energy. Predictions of energy use in the next century suggest a continued increase in carbon emissions and rising concentrations of CO_2 in the atmosphere.



The ways to manage the carbon emissions are

1. *To use energy more efficiently to reduce our need for a major energy and carbon source—fossil fuel combustion.*

2. *To increase our use of low-carbon and carbon-free fuels and technologies (nuclear power and renewable sources such as solar energy, wind power, and biomass fuels).*
3. *The third and newest way to manage carbon is through* ***carbon sequestration****.*

*The term "**carbon sequestration**" refers to the sequence of processes whereby CO_2 emitted from large-scale CO_2 emission sources is separated, recovered, and stored under the ground or at sea. More specifically, the exhaust gas (mainly from combustion) from large-scale CO_2 emission sources such as power plants and steel mills first undergoes a process of separation and recovery exclusively for CO_2 utilizing chemical reactions or the properties of CO_2. This is followed by transportation to the storage site by pipeline or tanker (the latter requiring liquefaction). The sequestration is completed with the forced injection of the transported CO_2 into the ground or in the sea.*

TYPES

i. *Bio Sequestration*
ii. *Geo Sequestration*
iii. *Ocean Sequestration*
iv. *Terrestrial Sequestration*

I. *Biosequestration* ***or carbon sequestration through*** *biological processes* ***affects the global*** *carbon cycle****.***

1. Peat production: *These are a very important carbon store. By creating new bogs, or enhancingexisting ones, carbon can be sequestered.*

2. Reforestation:*is the replanting of trees on marginal crop and pasture lands to incorporate carbon*

from atmospheric CO_2 into biomass.

3. Wetland restoration:*Wetland soil is an important carbon sink; 14.5% of the world's soil carbon is found in wetlands, while only 6% of the world's land is composed of wetlands.*

4. Agriculture*: Globally, soils are estimated to contain approximately 1,500 gigatons of organic* *carbon to 1 m depth, more than the amount in vegetation and the atmosphere.*

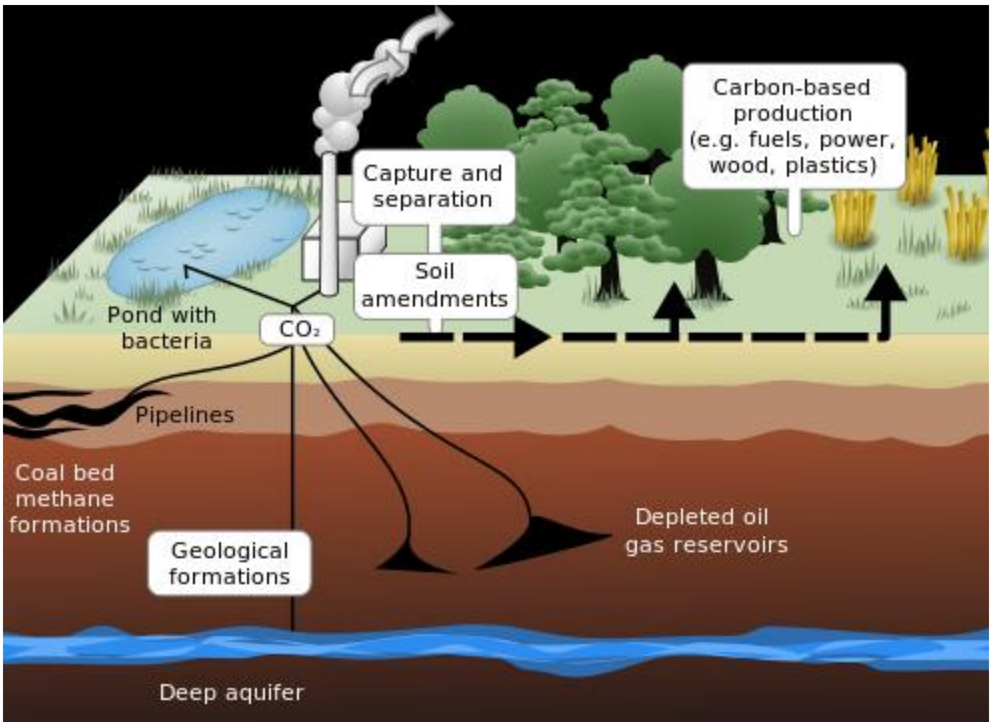
II. Ocean Sequestration:

1. *Plankton photosynthesis creates 45 Gt organic carbon per year. Most carbon gets recycled to atmosphere, but some is drawn down into Deep Ocean. Iron is the limiting factor for phytoplankton growth in 20% of the world's oceans (HNLC zones). Fertilization with iron could enhance growth, fix more carbon.*

2. Injecting CO_2 to ocean depths greater than 1000 meters.

III. Geo-sequestration: *This method involves injecting carbon dioxide, generally in supercritical form, directly into underground geological formations. Oil fields, gas fields, saline formations, unmineable coal seams, and saline-filled basalt formations have been suggested as storage sites.*

IV. Terrestrial sequestration: *involves the capture and storage ofcarbon dioxide by plants and the storage of carbon in soil. During photosynthesis, carbon from atmospheric carbon dioxide is transformed into components necessary for plants to live and grow. As part of this process, the carbon present in the atmosphere as carbon dioxide becomes part of the plant: a leaf, stem, root, etc. Long-lived plants like trees might keep the carbon sequestered for a long period of time. Once the tree dies, or as limbs, leaves, seeds, or blossoms drop from the tree, the plant material decomposes and the carbon is released.*



Sequestration methods		Description	Storage capacity (worldwide)
Underground sequestration	Crude oil recovery	Injection of CO_2 into oil fields on the occasion of tertiary recovery of crude oil, to induce recovery	73.3 - 238.8 billion t-CO_2
	Coal seam methane recovery	Adsorption of CO_2 in unexploitable deep-stratum coal seams, with simultaneous recovery of methane	146.7 billion t-CO_2
	Depleted oil and gas wells	Use of the storage capacity of oil and gas fields that had held reserves of oil and natural gas; proven storage capacity	Oil wells: 366.7 billion t-CO_2 Gas wells: 1,466.7 billion t-CO_2
	Aquifers	Dissolution of CO_2 in underground salt water subject to virtually no fluctuation	At least 3,666.7 billion t-CO_2
Sea-bottom sequestration	Marine dissolution	Injection of CO_2 into the sea to dissolve and diffuse it; termed "gas dissolution" in the case of injection in a gas state and "liquid dissolution" in that of injection in a liquid state	3,666.7 billion t-CO_2
	Deep-sea injection	Formation of CO_2 pools in sea-bottom depressions; expected isolation period of at least 2000 years	
Biological sequestration		Sequestration by flora (plants, sea weed, vegetable plankton, etc.) through photosynthesis	Flora on land: 4.4 billion t-CO_2 per year

<u>Issues and tasks</u>

The aforementioned issues and tasks related to carbon sequestration may be summarized as follows.

(1) Environmental impact of CO_2 storage: *Research must be conducted on the behavior of CO_2 in sequestration to determine the impact, if any, on underground and marine organisms and on ground water.*

(2) Definition of international rules: *Although the technical possibilities of carbon sequestration have been recognized, international rules that would encourage its practical* *application have not yet been determined. Such rules must be determined upon discussion in forums of international negotiation.*

(3) Establishment of monitoring methods: *Programs must be executed to establish technology for monitoring for leakage and other abnormalities over wide areas, and monitoring methods for long-term micro-leaks and leakage caused by earthquakes.*

(4) Cost reduction: *Carbon sequestration is recognized as being cost-competitive with certain other measures of emission reduction, but its cost competitiveness is by no means fullysufficient. Efforts must continue to be made to reduce its cost.*

(5) Improvement of the energy efficiency of recovery: *Separation and recovery require a large input of energy. The efficiency of this energy use must be improved for more efficient use of resources and reduction of cost.*

(6) Selection of sequestration systems adapted to the circumstances: *There is a need for research aimed at the construction of optimal total systems with the right combination of technology for the circumstances, including the exhaust gas properties, emission source, and storage site.*

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