Fundamentals of Crop Physiology Credit hour: 2(1+1)

Chapter-1

Introduction to crop physiology and its importance in Agriculture

Genetic potential of a plant and its interaction with environmental factors decides its growth and development by influencing or modifying certain internal processes. Plant physiology studies about these internal processes and their functional aspects.

Plant physiology is a study of **Vital phenomena** in plant. It is the science concerned with Processes and functions, the responses of plants to environment and the growth and development that results from the responses. It helps to understand various biological processes of the plants like Photosynthesis, respiration, transpiration, translocation, nutrient uptake, plant growth regulation through hormones and such other processes which have profound impact on crop yield.

1. Processes :

Processes means natural event/ sequence of events. Examples of processes that occur in living plants are

- ♦ Photosynthesis
- ♦ Respiration
- ♦ Ion absorption
- ♦ Translocation
- ♦ Transpiration
- ♦ Stomatal opening and closing
- ♦ Assimilation
- ♦ Flowering
- ♦ Seed formation and
- ♦ Seed germination

To described and explain the plant processes is the main task or the first task of plant physiology.

2. Function :

Function means natural activity of a cell or tissue, or organ or a chemical substance. So, the second task of plant physiology is to describe and explain the function of an organ, tissue, cell and cell organelle in plants and the function of each chemical constituent, whether it may be an ion, molecule or a macro molecule.

Both processes and functions are dependent on the external factors and are modified by the external factors such as light and temperature. Since these two factors are modified by the external factors, the third task of plant physiology is to describe and explain how processes and functions respond to change in the environment.

Essentially the overall goal of plant physiology is to evolve a detailed and comprehensive knowledge of all the natural phenomena that occur in living plants and thus to understand the nature of plant growth, development and productivity. Many aspects of practical agriculture can benefit from more intensive research in plant physiology.

Crop: it is a group of plants grown as a community in a specific locality and, for a specific purpose.

Crop Physiology :

Crop physiology is the study of the ways in which plant physiological processes are integrated to cause whole plant responses in communities. The subject matter of crop physiology includes the ways in which the knowledge of plant physiology is applied for better management of crops.

A brief history of Crop Physiology:

W.L. Balls (1915): Crop physiology, with the aim of understanding the dynamics of yield development in crops, really began with the work of W.L. Balls. Along with Holton he analysed the effects of plant spacing and sowing date on the development and yield of Egyptian Cotton plants within crop stands, not in isolated plants. It was from his work the term 'crop physiology' came into existence. From then onwards, various scientists have started applying the advances in physiological knowledge for better crop management.

1924- In England- a rapid development of the methods of growth and yield analysis by different investigators (V.H. Blackman, F.G. Gregory, G.E. Briggs etc.) was started. With the development of various methods of growth analysis, they started explaining 'the physiology of crop yield'

1947: The concept of LAI (Leaf area index) was developed by D.J. Watson. This index has provided a more meaningful way of analyzing growth in crops, and stimulated renewed interest in crop physiology.

1950's: Studies on photosynthetic rate of the leaf and the loss of photosynthates by respiration was studied by the development of 'Infra Red Gas Analysis (IRGA)'method. This method has facilitated the estimation of short term rates of Photosynthesis and respiration by crops in the field.

1953: Monsi and Saeki explained about the manner of light interception by the crop canopy with their concept of light interception coefficient.

1963: Hesketh and Moss showed that photosynthesis by leaves of Maize, Sugarcane and related tropical grasses could reach much higher rates, with less marked light saturation, than leaves of other plants. (This was the starting point for research to find other photosynthetic CO_2 fixation path ways like C4, and CAM Mechanisms). The differences in pathway are associated with differences in photosynthetic rate, in response to light intensity, temperature and oxygen level, in photorespiration, in leaf anatomy and chloroplast morphology, in rate of translocation, and in the efficiency of water use, which can have profound effects on the physiology of yield determination.

Later on, several research works were carried out to understand the processes like translocation of food materials, their partitioning towards economic yield, storage mechanisms, physiology of flowering, effect of stressful environmental factors on crop growth and development, role of plant growth regulators in increasing the crop productivity etc. All these areas have enriched the knowledge of physiological processes and their role in deciding the crop yield.

Importance of crop physiology in agriculture:

Many aspects of Agriculture and Horticulture can be benefitted from more intensive research in plant physiology to provide practical solutions in agriculture and horticulture. Understanding the physiological aspects of seed germination, seedling growth, crop establishment, vegetative development, flowering, fruit and seed setting and crop maturity, plant hormone interaction, nutrientphysiology, stress (biotic/abiotic) physiology etc., provides a reasonable scientific base for effective monitoring and beneficial manipulation of these phenomenon's. Since in agriculture we are interested in economic yield which is the output of these phenomenons and well beingness of plants, Plant Physiology provides a platform for getting better yield of crops. Studying these phenomenon with a view to develop better crop management practices forms the subject matter of crop physiology. The importance of physiology in agriculture and horticulture can be seen from the following examples;

1. Seed Physiology

   Seed is the most important input in agriculture. Germination of seed and proper establishment of seedling depends upon various internal and external factors. Knowledge of Seed physiology helps in understanding of different physiological and morphological changes that occur during germination. Any deviation in these processes causes Seed dormancy. The dormant condition of the seed bars immediate use of harvested seed for next crop which is important in intensive agriculture. By understanding the causes and effects of this problem, Crop physiologists have come up with different methods of breaking the seed dormancy. Example: When ever Paddy is used as a seed material in the very next season it is recommended to treat the seed either with HNO_3 or with GA.

2. Optimum seedling growth and plant population

   By knowing the process of radicle and plumule emergence and their function we can achieve best plant health, which is the outcome of best plant physiology. By knowing the different inputs requirement of plants (water, nutrients, sunlight) we can easily manage the plant population to get highest yield. Input interaction of plants within their body is the matter of plant physiology.

3. Growth measurement of crops

   The first prerequisite for higher yields in crops is high total dry matter production per unit area. High dry matter production is a function of optimum leaf area (Optimum leaf area Index) and Net Assimilation rate. (CGR = LAI X NAR).

   Example: Pruning operation in horticultural crops like Mango is done based on this principle of proper canopy management for better photosynthesis.

4. Harvest index

   The difference between total amount of dry matter produced and the photosynthates used in respiration is the net product of photosynthesis. Economic yield depends on how the dry matter is distributed among different organs of the plant. Partition of total dry matter amongst the major plant organs is of interest to the farmers as they are more interested in its partition towards economic yield. Example: excessive vegetative growth period in Ground nut produces less number of Pods as the reproductive period gets constricted. Thus, groundnut varieties with relatively extended period of reproductive growth are desirable.

5. Mode of action of different weedicides

   The use of herbicides to kill unwanted plants is widespread in modern agriculture. Majority of Herbicides -about half of the commercially important compounds—act by interrupting photosynthetic electron flow (Ex. Paraquat, diuron) or electron flow of respiration. In Photosynthesis when the electron transport is blocked, it virtually stops light reaction of photosynthesis. When light reaction is stopped the dark reaction does not happen and thus CO_2 is not fixed as carbohydrate. Therefore, the weed is killed by starvation.

6. Nutriophysiology

   Nutriophysiology is yet another important area to under stand crop physiology. For the healthy growth of a crop around 17 essential elements are required. Knowledge of nutriophysiology has helped in identification of essential nutrients, ion uptake mechanisms, their deficiency symptoms and corrective measures. It also helps to check the toxicity symptoms of various nutrients. The use of fertilizers and their intake by plants can be totally understand by studying plant physiology.

7. Photoperiodism

   Response of plant to the relative length of day and night is called as photoperiodism. This concept was used to choose photo insensitive varieties. The semi dwarf Rice varieties that have revolutionized Indian agriculture, are lodging resistant, fertilizer responsive, high yielding and photo insensitive. Photo insensitivity has allowed rice cultivation in nontraditional areas like Punjab. Even in traditional areas rice-wheat rotation has become possible only due to these varieties.

8. Plant growth regulators

   Plants can regulate their growth through internal growth mechanisms involving the action of extremely low concentrations of chemical substances called Plant growth substances, phytohormones or Plant growth regulators. The regulation of flowering, seed formation and fruit setting has been controlled through the application of different hormones at the appropriate time of plant height and age.

9. Indian agriculture being predominantly rainfed in nature, so development of drought resistant varieties is very important. Root zone depth, density of roots, plant water potential, relative water content, water use efficiency, xerophytic characters of leaves

etc. are some of the characters helped to bred drought tolerant varieties and to develop efficient irrigation management practices (sprinkler and drip irrigation).

10. Among Several physiological approaches, transpiration efficiency or water use efficiency is the most dependable trait, which is "the amount of dry matter produced per unit amount of water transpired". The importance of water use efficiency (WUE) in influencing grain yield under water limited conditions can be explained by the following model give by passioura.

    Grain Yield = T x TE x HI

    Where T = Total transpiration by the crop canopy

    TE = Transpiration Efficiency or WUE

    HI = Harvest Index (Economic Fraction of Dry matter)

    This relationship provides an analytical tool to select the genotype with high levels of T and TE.

11. Post-harvest Physiology

    Post harvest losses of agriculture and horticulture are causing a great distress to farming community. Moisture and temperature are the two important factors causing physiological changes that reduce the post harvest quality of grains. Control of moisture content and maintenance of low temperatures have proved effective in storage of grains. Being perishable in nature the magnitude of post harvest loss is comparatively higher in horticultural crops. Example: In recent years a method called 'modified atmospheric storage' was developed for prolonged post harvest life of fruits and vegetables. Shelf life of cut flowers can be increased by application of kinetin (cytokinin). This will reduce the burst of ethylene and thus reduces the rate of senescence.

Thus, physiological understanding of crop plants provides the fundamental scientific base about various aspects of metabolism, growth and development. This is immensely important for crop improvement or technology improvement in agriculture or horticulture.

Chapter-2
Plant cell: an Overview

The cell (from Latin cella, meaning "small room") is the basic structural, functional, and biological unit of all known living organisms. A cell is the smallest unit of life. Cells are often called the "building blocks of life". The study of cells is called cell biology. The cell was discovered by Robert Hooke in 1665, who named the biological units for their resemblance to cells inhabited by Christian monks in a monastery. Cell theory, first developed in 1839 by Matthias Jakob Schleiden and Theodor Schwann, states that

1) All organisms are made up of one or more cells and the products of those cells.
2) All cells carry out life activities (require energy, grow, have a limited size).
3) New cells arise only from other living cells by the process of cell division.

Plant cells are the basic unit of life in organisms of the kingdom Plantae. They are eukaryotic cells, which have a true nucleus along with specialized structures called organelles that carry out different functions. Animals, fungi, and protists also have eukaryotic cells, while bacteria and archaea have simpler prokaryotic cells. Plant cells are differentiated from the cells of other organisms by their cell walls, Pastids (chloroplasts and chromoplasts), cell to cell communication by plasmodesmata and central vacuole.

The brief description of the plant cell and various organelles and their functions are as follows:

Cell wall :

Cell wall is a non-living component of the cell and is secreted and maintained by the living portion of the cell, called protoplasm. A typical cell wall is composed of three different regions 1. Middle Lamella 2. Primary cell wall (1-3 µm thick and elastic) 3. Secondary cell wall (5-10 µm thick and rigid)

Functions of cell wall :

1. It protects the inner contents of the cell. 2. It gives definite shape to the cell. 3. It provides mechanical support to the tissues and act as a skeletal framework of plants. 4. It helps in transport of substances between two cells. 5. The cell wall is hydrophilic in nature and it imbibes water and helps in the movement of water and solutes towards protoplasm. It also acts as a permeable structure during absorption of minerals and solutes.

Protoplasm :

It is the living, colloidal and semi fluid substance. It is also called as cytoplasm. Cell devoid of cellwall is called protoplast. Protoplast is enclosed by a membrane called as cell membrane or plasma membrane.

Cell membrane :

All cells are enclosed by a thin, membrane called plasma membrane or plasmalemma. The plasma membrane and sub cellular membrane are collectively called biological membrane. Cell membrane consists of proteins, lipids and other substances.

1. Proteins:- The proteins present in the membranes can be categorized into two types

a. Intrinsic proteins or integral proteins: - Which are embedded or buried in the lipid layer. These proteins associate with hydrophobic interactions to the tails or fatty acid chains of the lipid layer. In addition to the hydrophobic associations, integral proteins also posses hydrophilic aminoacid residues which are exposed at the surface of the membrane. These proteins cannot be removed easily.

b. Extrinsic proteins or peripheral proteins: - They are attached to the membrane surface by weak ionic interactions. These proteins are not much involved in the architecture of membrane. Peripheral proteins are bound to hydrophilic proteins of the integral proteins protruding from the lipid layer.

2. Lipids: - The cell membrane consists of phospholipids and glycolipids. The fatty acid chains in phospholipids and glycolipids usually contain 16-20 even numbered carbon atoms. Fatty acids may be saturated or unsaturated.

3. Other substances like polysaccharide, salicylic acid etc. are found attached to the proteins or lipids on the membrane.

Functions of cell membrane:

1. The cell membrane surrounds the protoplasm of the cell, thus separating the intracellular components from the extracellular environment.

2. It anchors the cytoskeleton to provide shape to the cell, and in attachment to the extracellular matrix.

3. The plasma membrane is differentially permeable and able to regulate the transport across the membrane.

4. The cell membranes maintain the cell potential.

Cell nucleus :

It is oval or spherical in shape and is generally larger in active cells than in resting cells. A nucleus consists of three main parts viz. nuclear envelope, nucleolus and chromatin. The nucleus is separated from the cytoplasm by a double membrane called the nuclear envelope. The space between the outer and inner membrane is known as nuclear pores which provide direct connection between nucleus and cytoplasm. Nucleolus is a spherical, colloidal body found in the nucleus and is the place where almost all DNA replication and RNA synthesis occur. Chromatin is the basic unit of chromosome and contains genes which play important role in the inheritance of characters to offspring from parents.

Functions of cell nucleus:

1. It regulates growth and reproduction of cells.

2. The nuclear envelope allows the nucleus to control its contents, and separate them from the rest of the cytoplasm where necessary.

3. The DNA replication, transcription and post transcriptional modification occur in the nucleus.

Chloroplast :

Chloroplasts are organelles found in plant cells and other eukaryotic organisms that perform photosynthesis because of the presence of green pigment, chlorphyll. They are flattened discs usually 2-10 micrometers in diameter and 1 micrometer thick. The chloroplast is surrounded by double layered membrane. The space between these two layers is called intermembrane space. Stroma is the aqueous fluid found inside the chloroplast. The stroma contains the machinery required for carbon fixation, circular DNA, 70 S ribosomes (thatswhy called as semiautonomous organelle) etc. within the stroma the stacks of thylakoids are arranged as stacks called grana. A thylakoid has a flattened disc shape and has a lumen or thylakoid space. The light reactions occur on the thylakoid membrane.

Functions of chloroplast:

1. The important processes of photosynthesis i.e, light and dark reactions occur within the chloroplast.

2. The granum is the site of NADP reduction forming $NADPH+H^+$ and photophosphorylation i.e., formation of ATP in presence of light. Thus, light reaction of photosynthesis takes place in the granum region.

3. The stroma is the main site for the dark reaction of photosynthesis.

4. The chloroplast has its own genetic system and is self replicating. Thus, associated with cytoplasmic inheritance.

Mitochondria :

Mitochondria are rod shaped cytoplasmic organelles, which are main sites of cellular respiration. Hence, they are referred to as power house of the cell. Each mitochondrion is enclosed by two concentric unit membranes comprising of an outer membrane and an inner membrane. The space between the two membranes is called perimitochondrial space. The inner membrane has a series of infoldings known as cristae. The inner space enclosed by cristae is filled by a relatively dense material known as matrix. The matrix is generally homogeneous but may rarely show finely filamentous or fibrous structures. The matrix contains several copies of round or circular DNA molecules and 70 S ribosomes (thatswhy it is also called as semiautonomous organelle).

Functions of mitochondria:

1. ATP, the readily available form of energy is produced in mitochondria.

2. Krebs cycle takes place in the matrix of mitochondria.

3. The enzymes of electron transport chain are found in the inner membrane or cristae of mitochondria.

4. Heme synthesis occurs in mitochondria.

5. Controls the cytoplasmic Ca^{2+} concentration

Ribosomes :

Chemically, ribosomes are ribonucloprotein complexes. This is a membrane less Ribosomes are of two types. Ribosomes of prokaryotes have sedimentation coefficient of 70 S and consist of two sub units of unequal sizes 50S and 30 S subunits. Ribosomes of eukaryotes have 80 S sedimentation coefficient (40S & 60 S). The two or more ribosomes become connected by a single m RNA and then may be called polyribosome. The major function of the smaller subunit of ribosome is to provide proper site for binding of mRNA and its translation. The larger subunit of ribosome supports translation and translocation processes coupled with polypeptide synthesis.

Functions of Ribosomes: 1. They provide the platform for protein synthesis 2. They have the machinery for protein synthesis.

Golgi complex :

Golgi bodies is an assemblage of flat lying cisternae one above the other in close parallel array. Each golgi complex has 3 to 12 interconnected cisternae which are composed of lipoproteins.

Functions of Golgi complex: 1. It helps in Packaging of proteins for exporting them. 2. It plays a role in sorting of proteins for incorporation into organelles. 3. It is involved in the formation of the cell wall of plant cells.

Endoplasmic reticulum :

Endoplasmic reticulum arises from the outer membrane of the nucleus forming an intermediate meshed network. It is of two types. The granular or rough endoplasmic reticulum in which the outer surface of endoplasmic reticulum is studded with ribosome and agranular or smooth endoplasmic reticulum in which the ribosomes are not attached.

Functions of Endoplasmic reticulum: 1. Rough endoplasmic reticulum is associated with the synthesis of proteins. 2. Smooth endoplasmic reticulum is associated with synthesis of lipids and glycogen. 3. It acts as an inter-cellular transport system for various substances. 4. It contains many enzymes which perform various synthetic and metabolic activities.

Vacuole :

It is a membrane bound organelle found in plant cell and occupies most of the area in the plant cell. A vacuole is surrounded by a single layer membrane called tonoplast. It is an enclosed compartment filled with water containing inorganic and organic molecules including enzymes in solution. It maintains the cell's turgor, controls movement of molecules between the cytosol and sap, stores useful material and digests waste proteins and organelles.

Functions of vacuole:

1. Isolating materials that might be harmful or a threat to the cell.
2. Stores waste products.
3. Maintains internal hydrostatic pressure or turgor within the cell.
4. Maintains an acidic internal pH.
5. Exports unwanted substances from the cell.
6. Allows plants to support structures such as leaves and flowers due to the pressure of the central vacuole.
7. Most plants stores chemicals in the vacuole that react with chemicals in the cytosol.
8. In seeds, stored proteins needed for germination are kept in protein bodies which are modified vacuole.

Microbodies :

Microbodies are ubiquitous organelles found in the majority of eukaryotic plant cells. They are mostly spherical and have a diameter ranging from 0.2um to 1.5um. Two types of microbodies, peroxisomes and glyoxysomes, have been characterized. These organelles differ in their distribution and enzyme composition, although both have the capacity to transform non-carbohydrate material into carbohydrate.

Peroxisomes :

Peroxisomes are found in leaves of higher plants. It is a small organelle present in the cytoplasm of many cells, which contains the reducing enzyme catalase and usually some oxidases.

Functions of Peroxisomes: Peroxisomes act in parallel with chloroplast in higher plants and are believed to undertake photorespiration.

Glyoxysomes :

A glyoxysome is a specialized form of peroxisome (a type of microbody) found in some plant cells, notably the cells of germinating seeds. Glyoxysomes are temporary as they occur during transient periods in the life cycle of a plant such as in certain beans and nuts which store fats in their seeds as energy reserves. Glyoxysomes appear in the first few days after seed germination in endosperm cells and associate closely with lipid bodies. They disappear after the storage fats are broken down and converted into carbohydrate.

Functions of Glyoxysomes: Glyoxysomes are involved in the formation of sugars by the breakdown of fatty acids in germinating seeds.

Cytoskeleton :

The cytoskeleton is scaffolding contained within the cytoplasm and is made up of protein. The cytoskeleton is present in all cells. The cytoskeleton provides the cell with structure and shape.

There are three main kinds of cytoskeleton filaments:

1. Microfilament: - They are composed of actin subunits.
2. Intermediary filaments: - They function in the maintenance of cell shape by bearing tension. They also participate in the cell-cell and cell matrix junctions.
3. Microtubules: - They are like hollow cylinders mostly comprising of 13 protofilaments which in turn are alpha and beta tubulin. They are commonly organized by the centrosome.

Functions of cytoskeleton: 1. Provides mechanical support 2. Anchors organelles 3. Helps to move substances intracellular.

Plasmodesmata :

Plasmodesmata (singular: plasmodesma) are microscopic channels which traverse the cell walls of plant cells and some algal cells, enabling transport and communication between them. Specialized cell-to-cell communication pathways known as plasmodesmata, pores in the primary cell wall through which the plasmalemma and endoplasmic reticulum of adjacent cells are continuous. Unlike animal cells, almost every plant cell is surrounded by a polysaccharide cell wall. Neighbouring plant cells are therefore separated by a pair of cell walls and the intervening middle lamella, forming an extracellular domain known as the apoplast. Although cell walls are permeable to small soluble proteins and other solutes, plasmodesmata enable direct, regulated, symplastic transport of substances between cells. There are two forms of plasmodesmata: primary plasmodesmata, which are formed during cell division, and secondary plasmodesmata, which can form between mature cells.

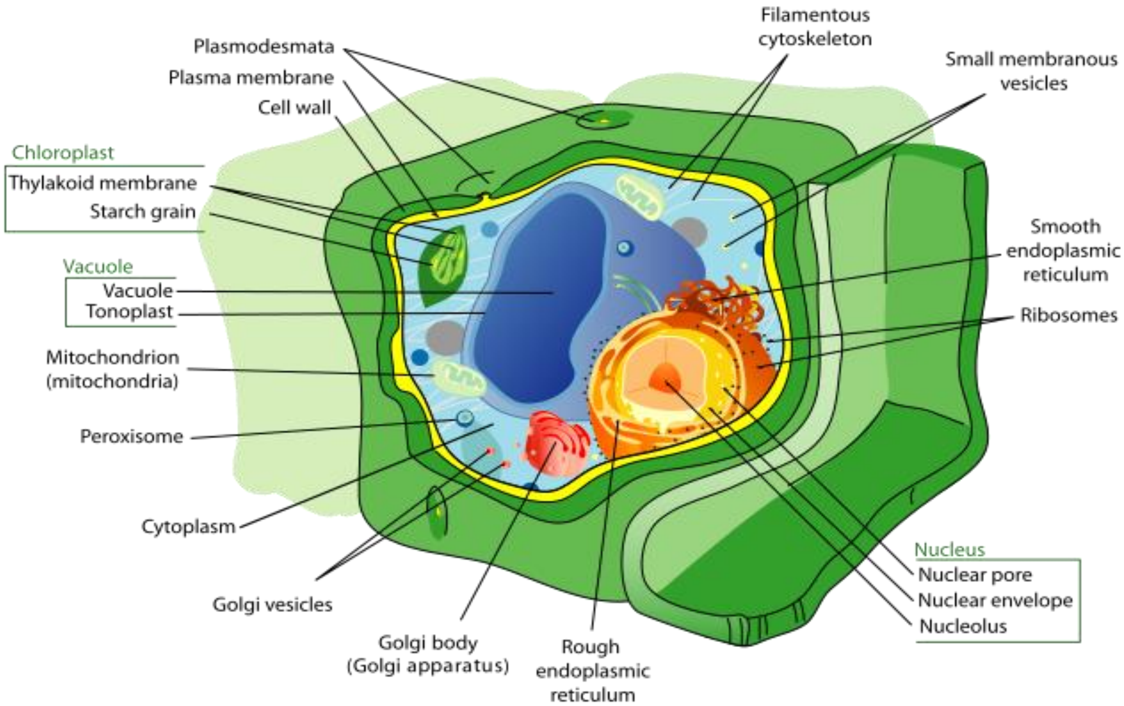


Fig. Diagramatic representation of typical plant cell

Chapter-3

Diffusion and osmosis; Absorption of water, transpiration and Stomatal Physiology

Diffusion and Osmosis :

Diffusion -the process by which molecules spread from areas of high concentration, to areas of low concentration. Diffusion occurs when the spontaneous net movement of particles or molecules spreads them from an area of high concentration to an area of low concentration through a membrane. It is simply the statistical outcome of random motion. As time progresses, the differential gradient of concentrations between high and low will drop (become increasingly shallow) until the concentrations are equalized. Molecules will always move down the concentration gradient, toward areas of lesser concentration. Think of food coloring that spreads out in a glass of water, or air freshener sprayed in a room. Diffusion increases entropy (randomness), decreasing Gibbs free energy, and therefore is a clear example of thermodynamics.

Equilibrium - When the molecules are even throughout a space.

Concentration gradient - a difference between concentrations in a space.

Osmosis - Osmosis is the process of diffusion **of water** across a semipermeable membrane. Water will move in the direction where there is a high concentration of solute (and hence a lower concentration of water). Water molecules are free to pass across the cell membrane in both directions, either in or out, and thus osmosis regulates hydration, the influx of nutrients and the outflow of wastes, among other processes.

*A simple rule to remember is: salt sucks.

Salt is a solute, when it is concentrated inside or outside the cell, it will draw the water in its direction. This is also why we get thirsty after eating something salty.

Type of Solutions :

1. Isotonic Solutions

If the concentration of solute (salt) is equal on both sides of membrane, the water will move back in forth, but it won't have any result on the overall amount of water on either side. "ISO" means the same.

2. Hypotonic Solutions

The word "HYPO" means less, in this case there are less solute (salt) molecules outside the cell, since salt sucks, water will move into the cell.

The cell will gain water and grow larger. In plant cells, the central vacuoles will fill, and the plant becomes stiff and rigid, the cell wall keeps the plant from bursting. In animal cells, the cell may be in danger of bursting, organelles called contractile vacuoles will pump water out of the cell to prevent this.

3. Hypertonic Solutions

The word "HYPER" means more, in this case there are more solute (salt) molecules outside the cell, which causes the water to be sucked in that direction. In plant cells, the central vacuole loses water and the cells shrink, causing wilting. In animal cells, the cells also shrink. In both cases, the cell may die.

Therefore it is dangerous to drink sea water - its a myth that drinking sea water will cause you to go insane, but people marooned at sea will speed up dehydration (and death) by drinking sea water. This is also why "salting fields" was a common tactic during war, it would kill the crops in the field, thus causing food shortages.

Both Diffusion and Osmosis are types of **passive transport**, that is, no energy is required for the molecules to move into or out of the cell. Sometimes, large molecules cannot cross the plasma membrane, and are "helped" across by carrier proteins - this process is called **facilitated diffusion**.

Water potential and its components :

Water potential or chemical potential of water is a quantitative expression of the free energy associated with water. Water potential is symbolized by the Greek letter ψ (psi) and is defined relative to the water potential of pure water, which is zero. Hence the value of psi is always negative. The units of water potential are mega Pascal (MPa). It is a relative quantity and depends on concentration, pressure and gravity at the same temperature.

Water potential as the sum of component potentials which may be written as

$\Psi = \Psi s + \Psi m + \Psi p + \Psi g$

Where, Ψs = Solute osmotic potential (symbol π)

Ψm = Matric potential (symbol T)

Ψp = Pressure potential (symbol P)

Ψg = Gravitational potential (symbol G)

Osmotic potential :

The osmotic potential, Ψs (or π) is the component produced by the solute dissolved in the cell sap, chiefly vacuolar sap.

Matric Potential :

The matric potential Ψm (or T) refers to water held in micro capillaries or bound on surfaces of the cell walls and other cell components.

Pressure potential :

The pressure potential Ψp (or P) is the turgor pressure produced by diffusion of water into protoplasts enclosed in walls which resist expansion. In the xylem of transpiring plants Ψp is usually negative and in guttating plants it is positive as a result of root pressure.

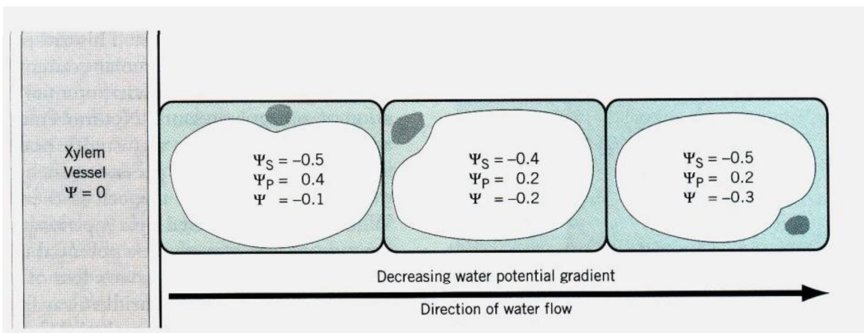
Gravitational Potential :

The effect of gravity, Ψg (or G) is a term of negligible importance within root or a leaf but becomes important in comparing potentials in leaves at different heights on trees and in soils.

Upward movement of water in a tree trunk must overcome a gravitational force of 0.01 Mpa/m and gravity causes drainage of water downward in soil. The volume of matric water is very small as compared to the volume of vacuolar water in parenchyma, therefore potential water constitutes a small fraction of the total water, matric potential can control the cell water potential. Thus, for herbaceous plants and annual field crops of a short vertical height (less than 10 m) the values of the matric potential and gravitational potential are small and are commonly omitted. Thus

$$\Psi = \Psi s + \Psi p \text{ or } P\_\pi$$

Water always moves from less negative water potential to more negative water potential.(Figure)



Reference: Hopkins WG & Huner NPA. 2004.
Introduction to Plant Physiology. John Wiley & Sons

Importance of water potential :

Water potential is a diagnostic tool that enables the plant scientist to assign a precise value to the water status in plant cells and tissues. The lower the water potential in a plant cell or tissue, the greater is its ability to absorb water. Conversely, the higher the water potential, the greater is the ability of the tissue to supply water to other more desiccated cells and tissues.

Thus, water potential is used to measure water deficit and water stress in plant cells and tissues. As a general rule, leaves of most plants rooted in well watered soils are likely to have water potentials between about -2 to -8 bars. With decreasing soil moisture supply, leaf water potential will become more negative than -8 bars and leaf growth rates will decline. Most plant tissues will cease growth completely (i.e., will not enlarge) when water potential drops to about -15 bars.

Uptake of water :

The way in which water is entered into the root hair and the precise mechanism of water absorption can be explained by two different approaches:

(a)Active uptake: Water is absorbed because of activities in the root itself and does not concern with any process in shoot.

(b)Passive uptake of water: The governing force of water absorption originates in the cells of transpiring shoots rather than in root itself.

Although the absorption of water by roots is believed to be a passive, pressure driven process, it is nonetheless dependent on respiration. Respiratory inhibitors (such as cyanide), anaerobic conditions (waterlogged condition) decrease in the hydraulic conductance of most roots. These are some supporting points for active absorption of water. However, the exact role of respiration and active uptake is not clear.

Except for few exceptions, it is now believed that uptake of water is a Passive process. Tension or negative pressure originating at the actively transpiring leaf surface creates a pulling force for water movement in xylem (Cohesion-tension theory of Dixon and Jolly).

The movement of water inside the plant is driven by a reduction in free energy, and water may move by diffusion, by bulk flow or by a combination of these fundamental transport mechanisms. Water diffuses because molecules are in a constant thermal agitation, which tends to even out concentration differences. Water moves by bulk flow in response to a pressure difference, whenever there is a suitable path way for bulk movement of water. Thus, water potential difference (i.e., solute potential and pressure potential) across the cells starting from root hairs to xylem plays an important role in uptake and transport of water.

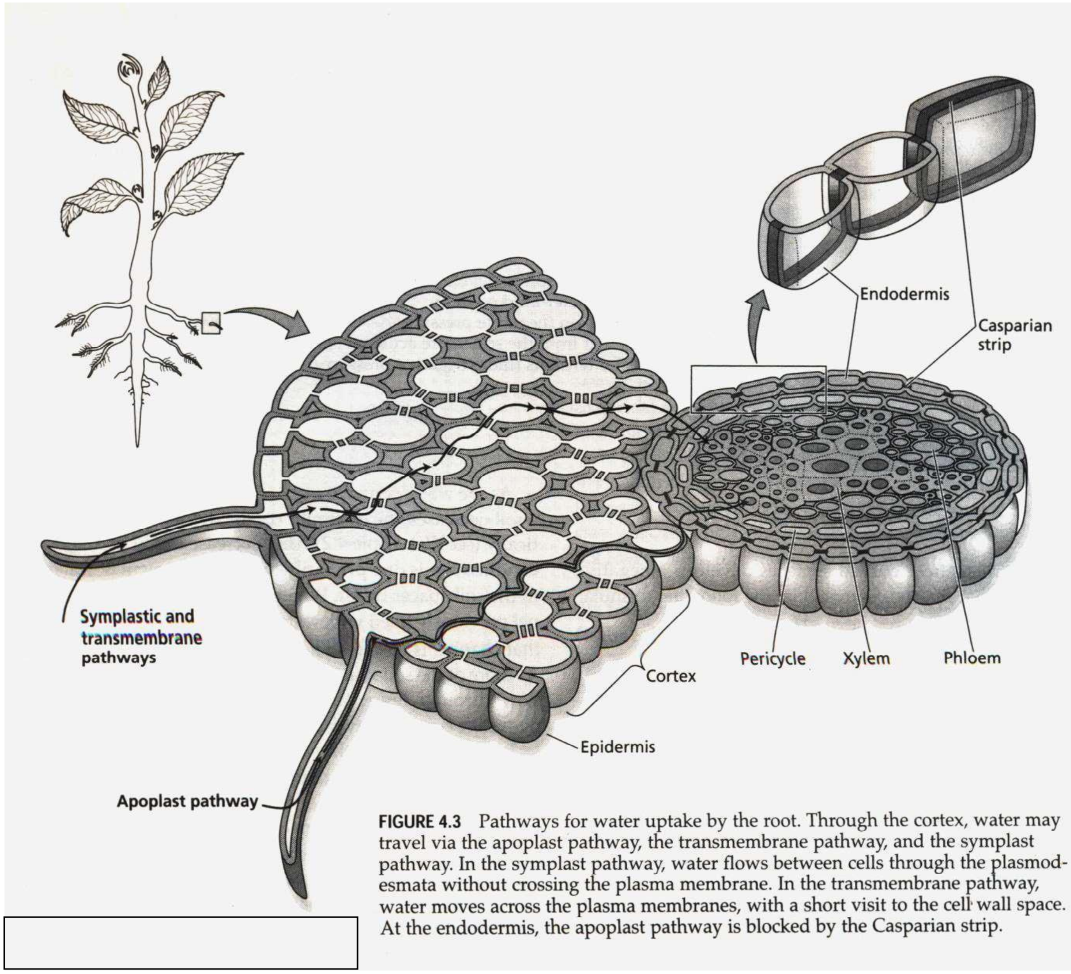


FIGURE 4.3 Pathways for water uptake by the root. Through the cortex, water may travel via the apoplast pathway, the transmembrane pathway, and the symplast pathway. In the symplast pathway, water flows between cells through the plasmodesmata without crossing the plasma membrane. In the transmembrane pathway, water moves across the plasma membranes, with a short visit to the cell wall space. At the endodermis, the apoplast pathway is blocked by the Casparian strip.

Reference: Hopkins WG & Huner NPA. 2004. Introduction to Plant Physiology. John Wiley & sons.

Water uptake and movement (transpiration stream) :

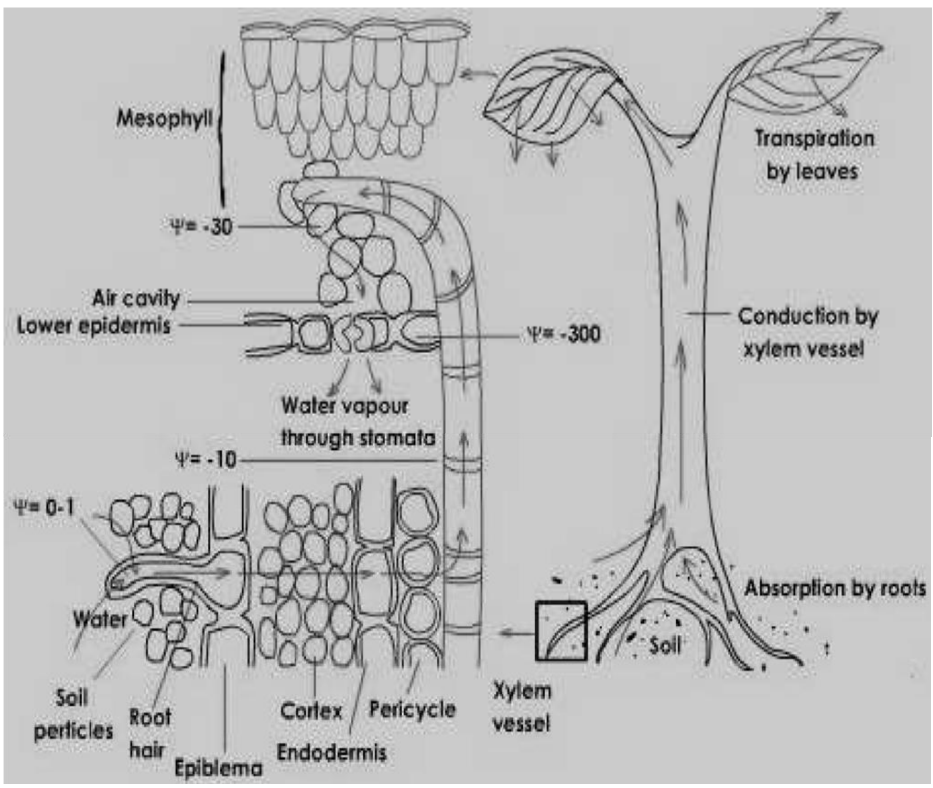
Air (atmosphere) usually has extremely low water potential, compared with plants or soils, so water gradient develops from the soil to the air. Since water moves from a high water to a low water, water will flow from roots to leaves.

Water lost by transpiration must be replenished by absorption of an equivalent amount of water from the soil through the root system. This establishes an integrated flow of water from the soil through the plant, and into the atmosphere, referred to as the soil-plant-atmosphere continuum (SPAC) (transpiration stream).

Soil is a very complex medium, consisting of solid phase comprised of inorganic rock particles and organic material, a soil solution containing dissolvent solutes and a gas phase generally in equilibrium with the atmosphere. Soil structure affects the porosity of a soil and ultimately, its water retention and aeration. When a soil is freshly watered, such by rain or irrigation, the water will percolate down through the pore space until it has displaced most of the air. The soil is then saturated with water. Water will drain freely from the large spore space due to gravity.

The water that remains after free drainage is held in the capillary pores. At this point, the water in the soil is said to be at field capacity. Consequently, soil water at or below field capacity will be under tension and its water potential will be negative, as the water content of the soil decreases, either by the evaporation from the soil surface or because it is taken up by the roots the air water interface will retreat into capillary spaces between the soil particle.

As water is removed from the soil by a root, tensions in the soil will draw more bulk water toward the root. The effectiveness of the roots as absorbing organs is related to the extent of the root system. Plant can only absorb water from the soil when the water potential of the plant sap (cell sap) is lower than water potential of the soil.



Movement of water towards more negative water potential

METHODS OF MEASURING WATER STATUS IN PLANTS :

There are two general ways to describe the water status or internal water balance of plant and plant tissue:

A. The first one is based on the energy associated with water in the plant tissue. Water potential is considered by most plant physiologists to be the most useful and significant way to describe the water status of plant tissues. In terms of water potential, water deficit exists in a tissue whenever its water potential is less i.e., more negative than zero mega Pascal (Mpa). The water potential is measured by (1) liquid immersion method (dye method) (2) vapor equilibration method (Thermocouple Psychrometer) and (3) pressure chamber method.

1. Liquid immersion method or dye method or Chardakov's Falling Drop Method:

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

After the tissue is incubated for 15 to 30 minutes, it is removed from each tube. The actual time of incubation can be just long enough for osmosis to proceed and change the concentration of each solution in the test series; the attainment of equilibrium is not necessary. After the tissue is removed, a small drop of the respective control series solutions is introduced below the surface of its corresponding test solution. If the drop rises in the test solution, it means that the drop is lighter and that the tissue incubation solution is more concentrated; an indication that water from the solution entered the tissue. Conversely, if the drop falls, it means that the test solution is lighter-an indication that water has left the tissue and diluted the solution. In this latter instance, the water potential of the solution initially is more negative than that of the tissue. Accordingly, if the density of the drop from the methylene blue solution is the same as that of the test solution, the drop will diffuse into the solution uniformly. At this point (called the null point), the water potential of the tissue and solution is equal.

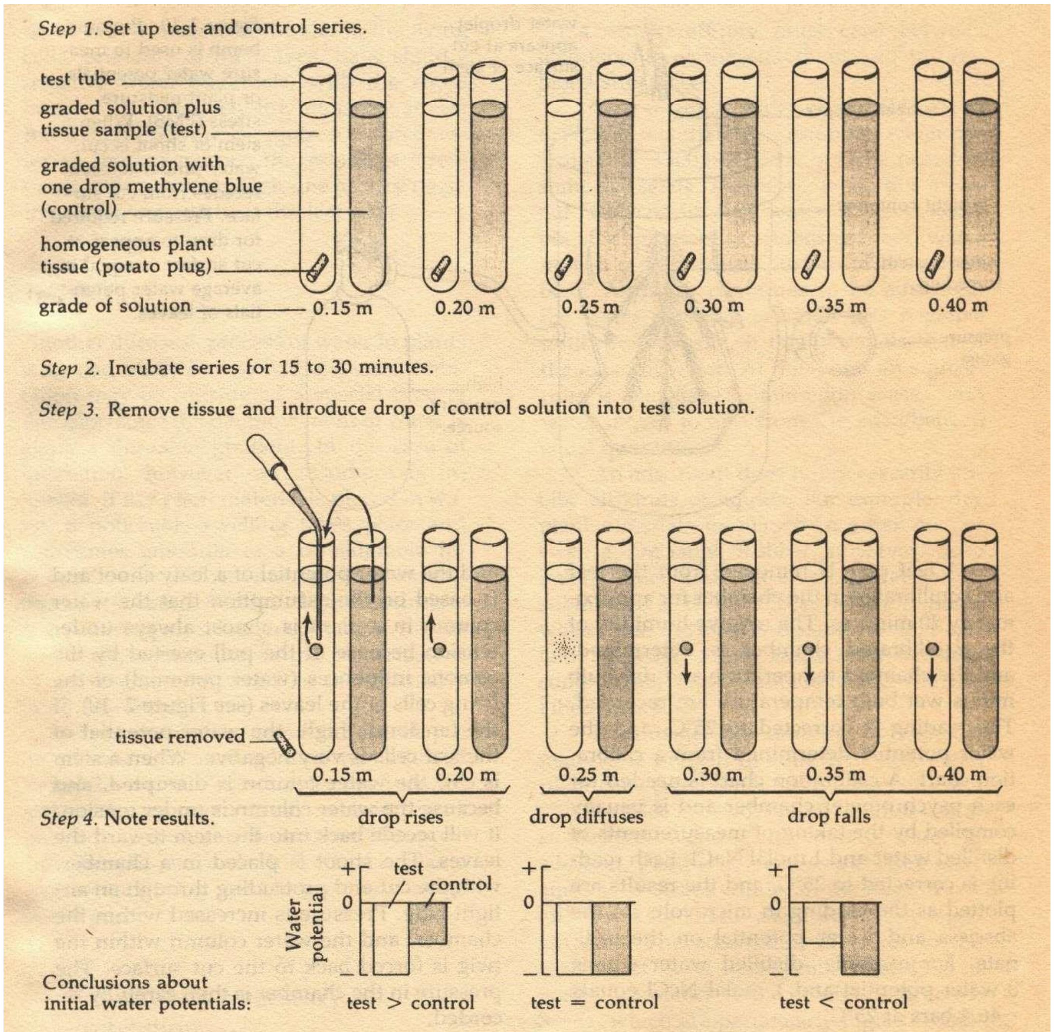
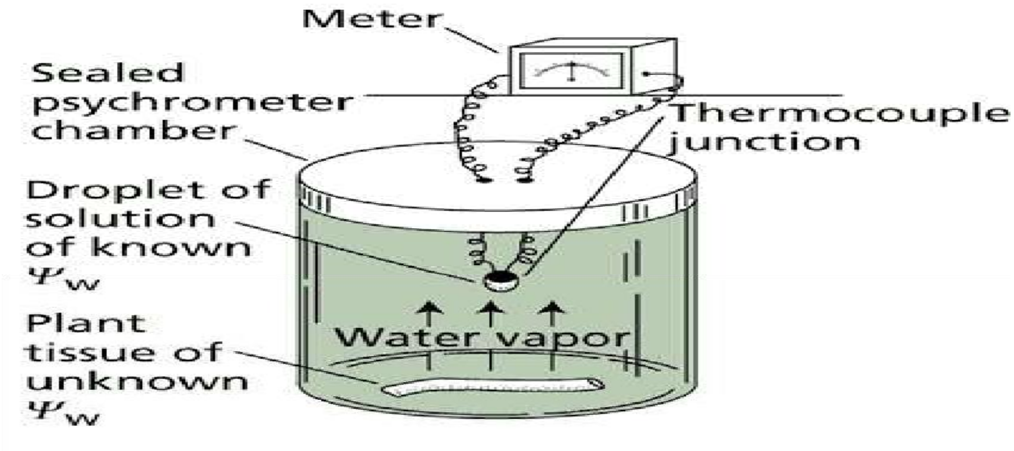


Figure 2–9. Chardakov's, or falling drop, method. Drop rising in test solution indicates test solution became more dense than its counterpart. Water diffused out of test solution into tissue, which initially had a more negative ψ_w. Reverse is true when drop descends in test solution. Diffusion of drop in test solution indicates no change, hence water potentials were equal initially.

2 Vapour equilibration (Thermocouple Psychrometer) Method

Psychrometry (the prefix "psychro-" comes from the Greek word psychein, "to cool") is based on the fact that the vapor pressure of water is lowered as its water potential is reduced. Psychrometers measure the water vapor pressure of a solution or plant sample, on the basis of the principle that evaporation of water from a surface; cools the surface.



Investigators make a measurement by placing a piece of tissue sealed inside a small chamber that contains a temperature sensor (in this case, a thermocouple) in contact with a small droplet of a standard solution of known solute concentration (known Ψ_s and thus known Ψ_w). If the tissue has a lower water potential than that of the droplet, water evaporates from the droplet, diffuses through the air, and is absorbed by the tissue. This slight evaporation of water cools the drop. The larger the difference in water potential between the tissue and the droplet, the higher the rate of water transfer and hence the cooler the droplet. If the standard solution has a lower water potential than that of the sample to be measured, water will diffuse from the tissue to the droplet, causing warming of the droplet. Measuring the change in temperature of the droplet for several solutions of known Ψ_w makes it possible to calculate the water potential of a solution for which the net movement of water between the droplet and the tissue would be zero signifying that the droplet and the tissue have the same water potential.

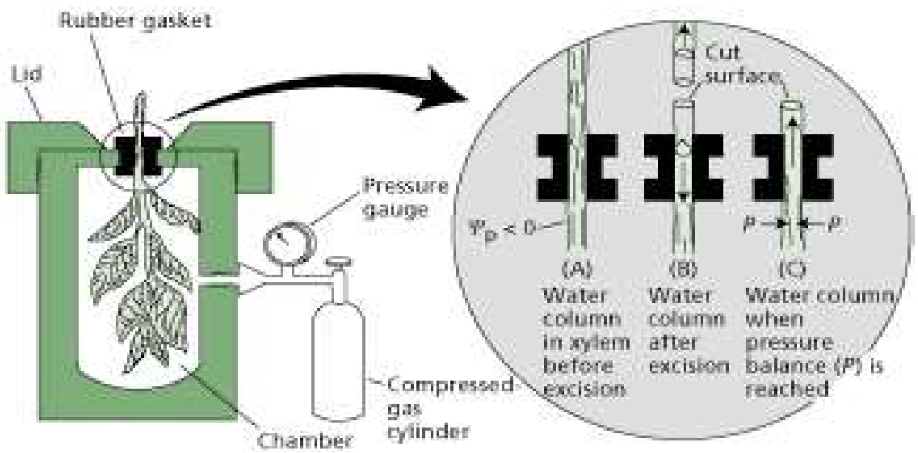
Psychrometers can be used to measure the water potentials of both excised and intact plant tissue. Moreover, the method can be used to measure the Ψ_s of solutions. This can be particularly useful with plant tissues.

A major difficulty with this approach is the extreme sensitivity of the measurement to temperature fluctuations. For example, a change in temperature of 0.01°C corresponds to a change in water potential of about 0.1 MPa. Thus, psychrometers must be operated under constant temperature conditions. For this reason, the method is used primarily in laboratory settings.

3. Pressure chamber method :

A relatively quick method for estimating the water potential of large pieces of tissues, such as leaves and small shoots, is by use of the pressure chamber. This method was popularized by P. Scholander and coworkers.The pressure bomb is a device that is used to determine the plant moisture stress and the water potential of a leafy shoot and is based on the assumption that the water column in a plant is almost always under tension because of the pull exerted by the osmotic influences (water potential) of the living cells of the leaves. If the tension is high, the water potential of the leaf cells is very negative. When a stem is cut, the water column (in xylem) is disrupted and because the water column is under tension, it will recede back into the stem toward the leaves. The shoot is placed in a chamber, with the cut end protruding through an airtight hole. Pressure is increased within the chamber and the water column within the twig are forced back to the cut surface. The pressure in the chamber is then carefully recorded.

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



B. The second way to describe water status is to measure the quantity of water in a tissue i.e. its water content and to express it in relation to a selected reference.

Three of these methods are

1. fresh weight method
2. dry weight method and
3. relative water content (RWC) method.

Stomatal Physiology and transpiration :

Stomata :

Stomata was discovered by Pfeffer & name 'stomata' was given by Malphigii. Stomata cover 1-2% of leaf area. It is minute pore present in soft aerial parts of the plant. Algae, fungi and submerged plants do not possess stomata.

Charecteristics of stomata

(a) Stomata are minute pores of eliptical shape, consists of two specialized epidermal cell called guard cells.

(b) The guard cells are kidney shape in dicotyledon and dumbell shape in monocotyledon.

(c) The wall of the guard cell surrounding the pore is thicken and inelastic due to rest of the walls are thin, elastic and semi-permeable.

(d) Each guard cell has a cytoplasmic lining, central vacuole. Its cytoplasm contains single nucleus and number of chloroplast. The chloroplast of guard cell is capable of very poor photosynthesis, because the absence of RUBISCO enzyme.

(e) Guard cells are surrounded by modified epidermal cells, known as subsidiary cells or accessory cells, which supports in the movement of guard cells.

(f) The Size and shape of stoma and guard cell vary from plant to plant. When fully open, the stomatal pore measures 3-12 µ in width and 10-40 µ in length.

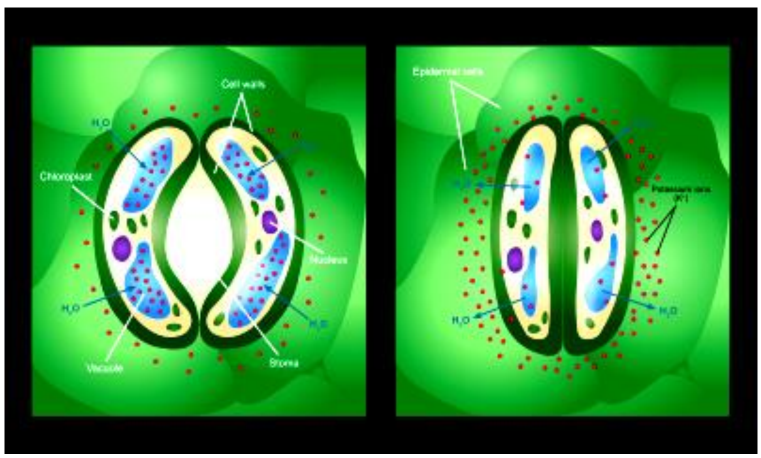


Fig. Opening and closing of stomata

(g) In many gymnosperms and xerophytic plants (plants growing in desert), the stomata are present embedded deeply in the leaves, so that they are not exposed to sunlight directly. Such deeply embedded stomata are called sunken stomata. This is an adaptation to check excessive transpiration in these plants.

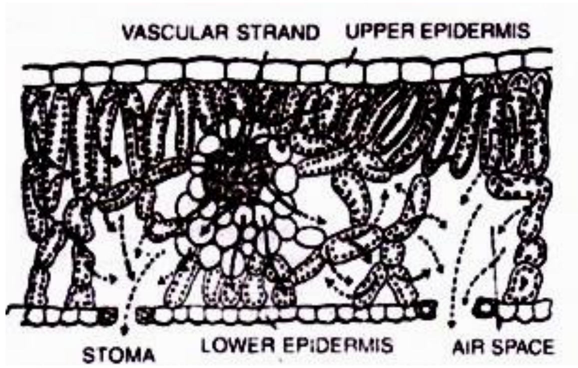


Fig. Vertical section of leaf blade showing the passage of water vapours during transpiration

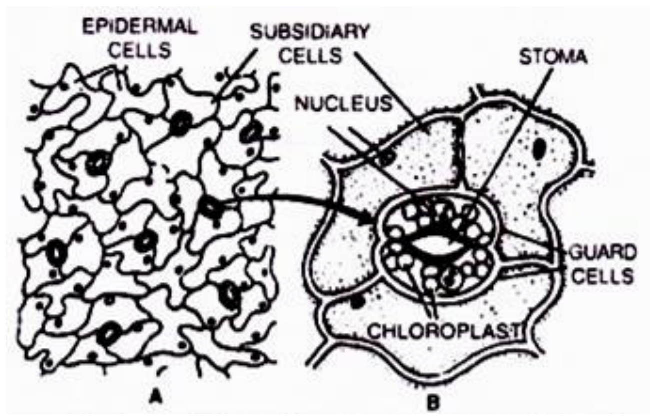


Fig. A) Portion of Lower Epidemis of Leaf Magnified to show stomata, B) A stoma magnified

Number of Stomata (Stomatal Frequency) :

The number of stomata in a definite area of leaf varies from plant to plant. Xerophytes possess larger number of stomata than mesophytes. Number of stomata/sq cm. is 1000-60,000 in different plant species. The number of stomta per unit area of leaf is called Stomatal Frequency.

Stomata frequency of trees and shrubs is higher than herbs. Stomata occupy nearly one to two percent of total leaf area when fully open. In isobilateral leaves (in monocots). approximately the same number of stomata are found on upper surface (adaxial) and lower (abaxial) surface. But in dorsiventral leaves (indicots) the number of stomata on the upper surface is much less in comparison to those found on the lower surface.

Distribution and Types of Stomata :

A. Depending upon the distribution and arrangement of stomata in the leaves five categories of stomatal distribution have been recognized in plants (shown in fig).

1. Apple or mulberry (hypostomatic) type:

   Stomata are found distributed only on the lower surface of leaves, e.g., apple, peach, mulberry, walnut, etc.

2. Potato type:

   Stomata are found distributed more on the lower surface and less on its upper surface, e.g., potato, cabbage, bean, tomato, pea, etc.

3. Oat (amphistomatic) type:

   Stomata are found distributed equally upon the two surfaces, e.g. maize, oats, grasses, etc.

4. Water lily (epistomatic) type:

   Stomata are found distributed only on the upper surface of leaf, e.g., water lily, Nymphaea and many aquatic plants.

5. Potamogeton (astomatic) type:

   Stomata are altogether absent or if present they are vestigeal. e.g., Potamogeton and submerged aquatics.

B. Types of Stomata based on Movement

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

1. Alfalfa Type: The stomata remain open throughout the day and closed all night, eg., peas, bean, mustard etc.
2. Potato Type: The stomata will open throughout the day and night except for few hours in the evening, eg., Allium, cabbage, pumpkin, etc.
3. Barley Type: The stomata open only for a few hours in a day, eg., Barley and other cereals.

C. Types of Stomata based on Behavior

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

1. Photo-active movements: Light directly or indirectly controls stomatal movements. Such stomata remain open during day time and closed in nights (dark).
2. Skoto-active movements: Stomata remain closed during day time and open during night. Such cases are found in succulent plants and other CAM Plants.
3. Hydro-active movements: In some cases, stomata open due to excessive loss of water from the epidermal cells and close due to turgid conditions of epidermal cells. This is usually found during mid-day.
4. Autonomous movements: In certain cases, stomata open and close at a rate of 10-15 minutes showing diurnal or rhythmic pulsation.

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

Theories of stomatal movement :

Factors affecting stomata opening and closing:

i) There is an endogenous rhythm (a biological clock). Stomata open during the day and close during the night. (Though certain succulents, which are native to hot, dry conditions have a reversed rhythm to enable them to economize on water loss.) However, stomata continue to open and close on an approximately 24-hour clock (circadian = about a day) even when switched to continuous light. The phase of this opening and closure can be shifted (made to occur at other times of the day) by control of the end of the dark period.

ii) The water balance of a plant affects stomatal aperture. Wilting plants close their stomata. The plant growth regulator abscisic acid (ABA) seems to act as a mediator under these conditions. Water stress in the roots can transmit its influence to stomata in leaves by the signal of ABA.

iii) Low concentrations of CO_2 cause stomata to open. If CO_2 free air is blown across stomata in darkness, their stomata open. High CO_2 causes stomata to close.

iv) Light causes stomata to open. The minimum light level for opening of stomata in most plants is 1/1000 to 1/30 of full sunlight, just enough to cause some net photosynthesis. Blue light (430-460nm) is nearly 10 times as effective as red light (630-680nm). The wavelengths that are effective in the red part of the spectrum are the same as those that are effective in photosynthesis that is absorbed by chlorophyll. However, the blue light effect is quite independent of photosynthesis. Photosynthesis will change intercellular CO_2 concentrations and may have its effect through the mechanism written in point iii) above.

Mechanism of stomatal opening and closing :

The pigment zeaxanthin (a carotenoid) detects blue-light wavelengths of daylight, and activate proton pumps in the guard cell membranes, which proceed to extrude protons from the cytoplasm of the cell; this creates a "proton motive force" (an electrochemical gradient across the membrane) which opens voltage operated channels in the membrane, allowing K^+ ions to flow passively into the cell, from the surrounding tissues. Chloride ions also enter the cell, with their movement coupled to the re-entry of some of the extruded protons (Cl/H symport) to act as a counter-ion to the potassium. Water passively follows these ions into the guard cells, and as their turgidity increases, the stomatal pore opens, in the morning. As the day progresses the osmotic role of that of sucrose, which can be generated by several means, including starch hydrolysis and photosynthesis, supplements potassium. At the end of the day (by which time the potassium accumulation has dissipated) it seems it is the fall in the concentration of sucrose that initiates the loss of water and reduced turgor pressure, which causes closure of the stomatal pore.

ABA also seems to trigger a loss of K^+ ions from guard cells. Some scientists suggest that in some species, ABA alters turgor pressure without changing solute potential or water potential. There is evidence of a role for increased cytoplasmic calcium (Ca^{2+}) in closure, possibly by effects on opening/closing of ion channels at the plasma membrane.

Break down of starch to phosphoenol pyruvate (PEP) is stimulated by blue light. This PEP then combines with CO_2 to form oxaloacetic acid, which is converted to malic acid. It is H^+ ions from the malic acid, which leave the cell in the mechanism outlined above. Thus, the intake of K ions is matched by formation of anions from malic acid in the guard cells. This causes an increase in osmotically active substances in exchange for the breakdown of starch in guard cells.

Transpiration :

Transpiration is the process of evaporation from plants (i.e. it is the loss of water from the plant in the form of water vapor). The greatest loss (more than 90%) of water vapor from plants occurs through leaves and is driven by differences in vapor pressure between the internal leaf spaces (intercellular space) and ambient air. A small amount of water vapor may be lost through small openings (lenticels) in the bark of young twigs and branches. The cuticle serves to restrict evaporation of water directly from the outer surfaces of leaf epidermal cells and protects both the epidermal and underlying mesophyll cells from potentially lethal desiccation. The integrity of the epidermis and the overlying cuticle is occasionally interrupted by small pores called stomata. Each pore is surrounded by a pair of guard cells. The guard cells function as hydraulically operated valves that control the size of the pore. The interior of leaf is mainly comprised of photosynthetic mesophyll cells. Stomata, when open, provide a route for the exchange of gasses (oxygen, carbon dioxide and water vapor) between the internal air space and the bulk atmosphere surrounding the leaf.

Diffusion of water through the stomatal pores known as stomatal transpiration cover 90 to 95 % of water loss from leaves. The remaining 5 to 10% is counted for by cuticular transpiration (although the cuticle is composed of waxes and other hydrophobic substances and generally impermeable to water, small quantities of water vapor can pass through).

Significance of transpiration :

Transpiration is advantageous because:

1. It creates suction force and helps in the ascent of sap.
2. It helps in the absorption of water and minerals by roots.
3. It helps in evaporating excess amount of water from moist soil.
4. It plays a role in translocation of food from one part of the plant to the other.

5. It brings opening and closure of stomata which indirectly influences the gaseous exchange for the processes of photosynthesis and respiration.
6. It helps in dissipating the excess energy absorbed from the sun, which will otherwise raise the leaf temperature.
7. It maintains suitable temperature of leaves by imparting a cooling effect.

According to Curtis (1926) transpiration is regarded as an unavoidable (necessary) evil. It is unavoidable because leaf structure (stomata) which is favorable for uptake of CO_2 and O_2 necessary for photosynthesis and respiration is also favorable for the loss of water through transpiration. One of the advantages of transpiration is that it reduces the temperature of the leaf and if it does reduce the temperature then it must be advantageous to plants because we understand the importance of temperature in a cell and how it affects enzymes. Some claim that transpiration helps in translocation of mineral salts to the upper parts of the body. But studies show that only 1-2% of transpiration is sufficient to translocate the mineral salts.

Transpiration is an 'evil' because often it causes injury by dehydration due to heavy transpiration loss when the atmospheric conditions are aggressive such as high light intensity, hot winds, depleted soil moisture and poor water retentive capacity of soil.

Evapotranspiration: The total amount of water lost from the field by both evaporation and plant transpiration.

A. Environmental factors affecting ET:

1. Solar radiation,
2. Temperature,
3. Relative humidity,
4. Wind

B. Plant factors affecting ET:

1. Stomatal closure
2. Stomatal number and size
3. Leaf amount
4. Leaf rolling or folding
5. Root depth and proliferation

Knowing how environment and the plant influence evapotranspiration helps to explain the daily pattern of evapotranspiration in the field.

Soil Water Availability :

Available water: water that can be extracted from the soil by plant roots. It is the difference between the amount of water in the soil at field capacity and the amount of water in the soil at permanent wilting percentage.

Field capacity: water held in the soil against the gravitational force.

Permanent wilting percentage: the percentage of soil moisture at which a plant will wilt and not recover in an atmosphere of 100% relative humidity.

Water use efficiency:

The water use efficiency (WUE) of field crops is defined as 'Amount of dry matter produced per unit amount of water transpired'

$$WUE = \frac{\text{Dry matter production (DM)}}{\text{Evapotranspiration}}$$

This is expressed as g DM kg^{-1} water. WUE measurements can be made on plants in containers, on individual plants, and on crop communities. They can be used for economic yield as well as total dry matter calculations.

A related term, water requirement is the reciprocal of WUE. Water requirements is usually expressed in weights of equal magnitude, such as g water $(g\ DM)^{-1}$.

Water use efficiency in C3, C4 and CAM plants :

Field data for WUE, when regrouped into C3 and C4 species, illustrate a two fold increase for C4 species when calculated for either grasses or dicots. Water use Efficiency (g DM kg^{-1} water) for C4 and C3 species.

Species	Grasses	Dicots
C3	1.49	1.59
C4	3.14	3.44

Large differences in WUE occur when species are categorized by CO_2 fixation pathway. It is now accepted that the WUE of C4 species is generally higher than C3 species. The higher WUE of C4 species is a result of higher photosynthetic rate under high light intensity and temperature and lower transpiration rates under low light.

The WUE values for both C3 and C4 species are low compared with CAM plants. One CAM species, pineapple (*Ananas comosis*), has shown a WUE of 20g DM kg^{-1} water. Use of crop species with CAM is limited because the CO_2 fixation and overall productivity of CAM plants is low (CAM is only a survival mechanism but not a productive mechanism).

Chapter-4

Mineral nutrition of Plants: Functions and deficiency symptoms of nutrients, nutrient uptake mechanisms

Mineral nutrients are elements acquired primarily in the form of inorganic ions from the soil. Although mineral nutrients continually cycle through all organisms, they enter the biosphere predominantly through the root systems of plants, so in a sense plants act as the "miners" of Earth's crust. The large surface area of roots and their ability to absorb inorganic ions at low concentrations from the soil solution make mineral absorption by plants a very effective process. After being absorbed by the roots, the mineral elements are translocated to the various parts of the plant, where they are utilized in numerous biological functions. Other organisms, such as mycorrhizal fungi and nitrogen-fixing bacteria, often participate with mots in the acquisition of nutrients.

The study of how plants obtain and use mineral nutrients is called mineral nutrition.

Mineral nutrition

• The chemical compounds required by an organism are termed as nutrients

• Nutrition may be defined as the supply and absorption of chemical compounds needed for plant growth and metabolism

• For plant growth and metabolism, 17 elements are essential. They are C, H, O, N, P, K, Ca, S, Mg, Fe, Mn, Zn, B, Cu, Mo, Cl and Ni.

These essential elements are classified into two groups

1. Major elements (macro nutrients)

2. Minor elements (Micro nutrients) (Trace elements)

Major elements :

The essential elements which are required by the plants in comparatively large amounts are called as major elements or macro nutrients. According to another definition minerals found in >1000 ppm concentration are macronutrients. They are C, H, O, N, P, K, Ca, S, Mg.

Minor 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. According to another definition minerals found in <100 ppm concentration are micronutrients. They are Fe, Zn, Mn, B, Cu and Mo. Si is now transferred from list of beneficial elements to essential elements.

Beneficial elements : (Na, Si and Co)

Sodium has beneficial effect, and, in some case, it is essential. There are some plant species, particularly the Chenopodiaceae plants and species adapted to saline conditions that take up this element in relatively high amounts. Na is also required for turnips, sugar beets and celery. The same is true for Si, which is an essential nutrient for rice. Cobalt is an essential element for the growth of the Bluegreen algae, but it has not been shown to be essential for other algae or for higher plants. It is also required by certain legumes to fix atmospheric nitrogen. Here, however the cobalt ion is necessary for the symbiotic bacteria present in the nodules associated with the roots.

Criteria of essentiality :

The term essential mineral element was proposed by Arnon and Stout (1939). According to them an element to be considered essential, three criteria must be met:

1. A given plant must be unable to complete its life cycle in the absence of mineral elements.
2. The function of the element must not be replaceable by another mineral element
3. The elements must be directly involved in plant metabolism. For eg. as a component of an essential plant constituents or it must be required for a distinct metabolic step such as an enzyme reaction.

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

Classification of plant nutrients based on their biochemical role and physiological function :

Essential elements are now classified according to their biochemical role and physiological function. Based on the biochemical behavior and physiological functions, plant nutrients may be divided into four groups.

Nutrient elements	Uptake	Biochemical function
1st group C, H, O, N, S	In the form of CO_2, HCO^-_3, H_2O, O_2, NO_3, NH^+_4, $N_2SO_4^2$, SO_2. The ions from the soil solution, the gases from the atmosphere.	Major constituents of the organic compounds of the plant. Essential elements of atomic groups which are involved in enzymatic processes. Assimilation by oxidation reduction reactions.

2nd Group P, B, Si	In the form of phosphates, boric acid or borate, silicate from the soil solution.	They are important in energy storage reactions or in maintaining structural integrity. Elements in this group are often present in plant tissues as phosphate, Borate and silicate esters in which the elemental group is bound to the hydroxyl group of an organic molecule (i.e. sugar-phosphates) (Esterification*). The phosphate esters are involved in energy transfer reactions.
3rd Group K, Na, Mg, Ca, Mn, Cl	In the form of cations from the soil solution except chlorine	Present in plant tissues as either free ions or ions bound to substances such as the pectic acids present in the plant cell wall. Of particular importance of their roles as enzyme cofactors and in regulation of osmotic potentials.
4th Group Fe, Cu, Zn, Mo	In the form of ions or chelates from the soil solution	Present predominantly in a chelated form Incorporated in prosthetic groups. Enable electron transport by valency change.

(Source: Taiz and Zeiger 2002)

*Esterification: Compounds formed by condensation of an acid and alcohol with elimination of water ADP + Pi = ATP

Mineral deficiencies produce visible symptoms :

When minerals are deficient, the growth of the plant is stunted, or the plant shows other symptoms. The combination of symptoms observed for deficiency of a particular mineral can be traced to the roles that mineral plays in metabolism or physiology.

1. Stunted growth is a symptom for many deficiencies, especially stunted stems with nitrogen deficiency and stunted roots in phosphorus deficiency.

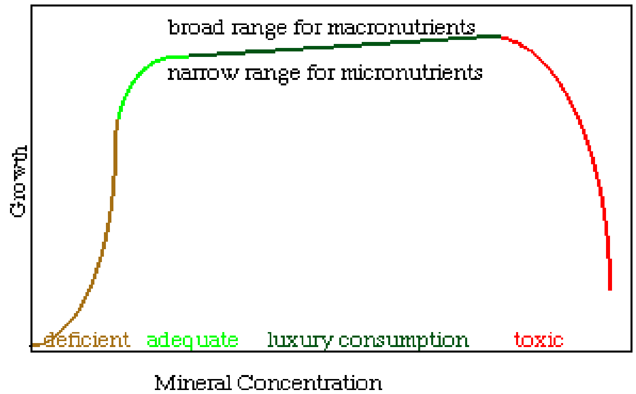
2. Chlorosis decreased chlorophyll synthesis or increased chlorophyll degradation, is observed with magnesium, nitrogen, and iron deficiencies. Magnesium is the central atom for the electron cloud of chlorophyll from which electrons flow through the light reactions.

3. Necrosis, dead spots or zones, is observed when magnesium, potassium or manganese deficiencies are present.

4. Colour changes such as excessive **anthocyanin production** is observed in stems with phosphorus deficiency. They generally pick up an intense purple colour sometimes extending onto the leaves.

Mineral availability shows an interesting dose effect :

The following graph demonstrates how deficiency reduces growth. As the mineral availability is increased, growth increases. As the mineral content continues to be increased there is no further increase in growth, but quality may be continuing to increase. This zone is called the luxury zone. However, continuing to increase the mineral concentration ultimately reaches toxic levels and growth is diminished.



The goal of a plant grower is to keep the plant in the sufficient to luxury zone but never to get as low as deficiency or as high as toxicity for any one of the macronutrients or micronutrients. The trouble with that goal knows how wide the luxury zone is in terms of concentrations. For minerals like boron, the zone is very narrow, and it is easy to achieve toxic levels or to be in deficiency. For minerals like phosphorus, the luxury zone is quite broad and large amounts can be given and the plants will respond nicely in spite of that. As a result, it is difficult to overdose plants on phosphorus.

Specific roles of essential mineral elements :

A. The macronutrients :

1. Nitrogen specific role :

- Nitrogen is important constituent of proteins, nucleic acids, porphyries (chlorophylls & cytochromes) alkaloids, some vitamins, coenzymes etc.
- Thus N plays very important role in metabolism, growth, reproduction and heredity.

Deficiency symptoms

- Plant growth is stunted because protein content cell division and cell enlargement are decreased • N deficiency causes chlorosis of the leave i.e yellowing older leaves are affected first
- In many plants eg. tomato, the stem, petiole and the leaf veins become purple coloured due to the formation of anthocyanin pigments.

2. Phosphorus :

- It is important constituent of nucleic acids, phospholipids, coenzymes NADP, NADPH2 and ATP
- Phospholipids along with proteins may be important constituents of cell membranes
- P plays important role in protein synthesis through nucleic acids and ATP
- Through coenzymes NAD, NADP and ATP, it plays important role in 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 due to the synthesis and accumulation of anthocyanin pigments.

3. Potassium Specific role :

- Although potassium is not a constituent of important organic compound in the cell, it is essential for the process of respiration and photosynthesis
- It acts as an activator of many enzymes involved in carbohydrate metabolism and protein synthesis
- It regulates stomatal movement
- Regulates water balance

Deficiency symptoms

- Mottled chlorosis of leaves occurs
- Neurotic areas develop at the tip and margins of the leaf
- Plants growth remains stunted with shortening of internodes.

4. Calcium :

- It is important constituent of cell wall
- It is essential in the formation of cell membranes
- It helps to stabilize the structure of chromosome
- It may be an activation of may enzymes

Deficiency symptoms

- Calcium deficiency causes 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

5. Magnesium :

- It is very important constituent of chlorophylls
- It acts as activation of many enzymes in nucleic acid synthesis and carbohydrate metabolism

- It plays important role in binding ribosomal particles during protein synthesis.

Deficiency symptoms

- Mg deficiency causes mottled chlorosis with veins green and leaf tissues yellow or white appearing first on older leaves
- Dead neurotic patches appear on the leaves
- In cotton Mg deficiency leads o reddening of leaves and disorder is called as reddening in cotton.

6. Sulphur specific role :

- It is important constituent of some amino acids (cystine, cysteine and methionine) with which other amino acids form the protein
- S helps to stabilize the protein structure
- It is also important constituent of vitamin i.e biotin, thiamine and coenzyme A
- Sulphhydryl groups are necessary for the activity of many enzymes.

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.

B. Micronutrients :

1. Iron specific role :

- Important constituent of iron porphyrin proteins like cytochromes, peroxidanes, catalases, etc.
- It is essential for chlorophyll synthesis
- It is very important constituent of ferredoxin which plays important role in photochemical reaction in photosynthesis and in biological nitrogen fixation.

Deficiency symptoms

Iron deficiency causes chlorosis of young leaves which is usually interveinal.

2. Zinc specific role :

- It is involved in the biosynthesis of growth hormone auxin (indole 3 acetic acid)
- It acts activator of many enzymes like carbonic anhydrase and alcohol dehydrogenase, etc.

Deficiency symptoms

- 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’

- Stalks will be very short.

3. Manganese :

- 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 photosynthesis

Deficiency symptoms

- The young leaves are affected by mottled chlorosis
- Veins remain green
- Small necrotic spots developed on the leaves with yellow strips

4. Copper specific role :

- It is an important constituent of plastocyanin (copper containing protein)
- It is also a constituent of several oxidizing enzymes.

Deficiency symptoms

- Copper deficiency causes necrosis of the tip of the young leaves
- It also causes die-back of citrus and fruit trees
- Also causes reclamation disease or white tip disease of cereals and leguminous plants.

5. Boron specific role :

- Boron facilitates the translocation of sugars by forming sugar borate complex.
- It involves in cell differentiation and development since boron is essential for DNA synthesis
- Also involves in fertilization, hormone metabolism etc.

Deficiency symptoms

- Boron deficiency causes death of shoot tip
- Flower formation is suppressed
- Root growth is stunted
- The other diseases caused by B deficiency is Heart rot of beet, Stem crack of celery, Brown heart of cabbage, Water core of turnip, Internal cork formation in apple, Hen and chicken in grapes.

6. Molybdeneum :

- It is constituent of the enzyme nitrate reductase and thus plays an important role in nitrogen metabolism
- It is essential for flower formation and fruit set.

Deficiency symptoms

- Molybdenum deficiency causes interveinal chlorosis of older leaves

- Flower formation is inhibited
- Causes whiptail disease in cauliflower plants.

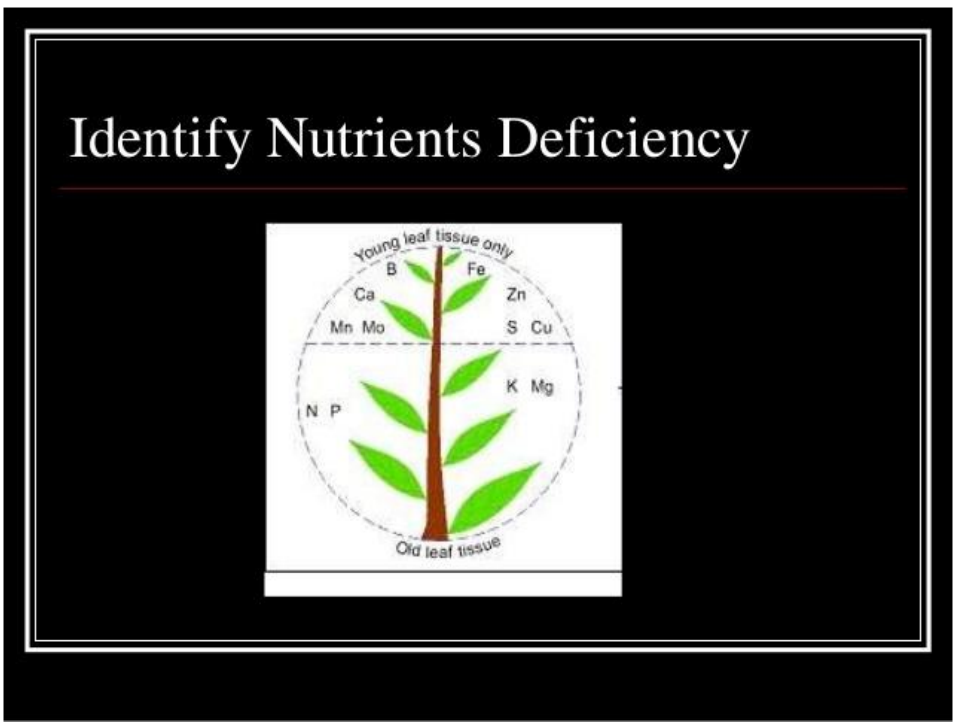
7. **Chlorine :**

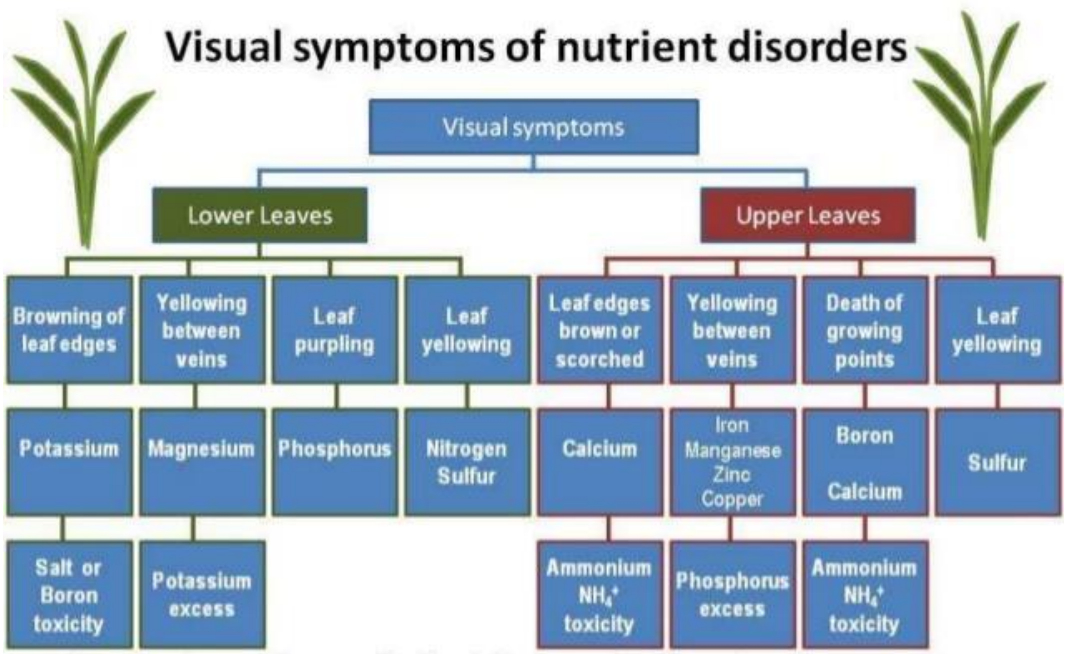
Specific role

- Chlorine has been shown to be involved in the oxygen evolution in photosystem II in photosynthesis (Cl and Mn are important for this reaction)
- It raises the cell osmotic pressure
- Chlorine accelerates the activation of amylase which converts starch into soluble sugars

Deficiency symptoms

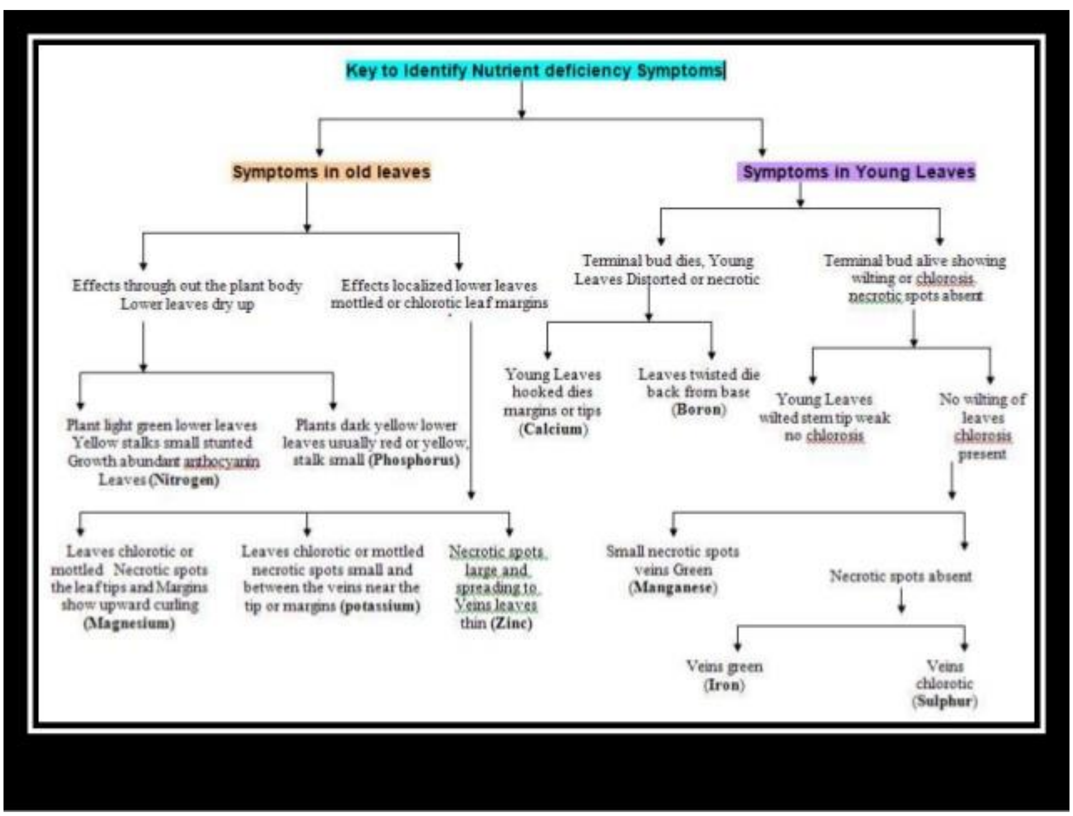
- Chlorosis of younger leaves and an overall wilting of the plant
- In some plant species, like tomato, leaves show chlorotic mottling, bronzing and tissue necrosis





Note: Symptoms can be caused by other factors – e.g., drought can also cause browning of leaf edges

Source: Modified from T.H. Fairhurst, C. Witt, R.J. Buresh, and A. Dobermann (eds). 2007. *Rice: A Practical Guide to Nutrient Management (2nd edition)*. IRRI, IPNI and IPI, Singapore.



DEFICIENCIES OF NUTRIENT ELEMENTS											
Symptoms	Suspected Element										
	N	P	K	Mg	Fe	Cu	Zn	B	Mo	Mn	OF
Yellowing of Younger Leaves					✓					✓	
Yellowing of Middle Leaves									✓		
Yellowing of Older Leaves	✓		✓	✓			✓				
Yellowing Between Veins				✓						✓	
Old Leaves Drop	✓										
Leaves Curl Over				✓							
Leaves Curl Under			✓			✓					✓
Younger Leaf Tips Burn								✓			
Older Leaf Tips Burn	✓						✓				
Young Leaves Wrinkle/Curl			✓				✓	✓	✓		
Necrosis			✓	✓	✓		✓			✓	
Leaf Growth Stunted	✓	✓									
Dark Green/Purple Leaf & Stems		✓									
Pale Green Leaf Color	✓								✓		
Molting							✓				
Spindly	✓										
Soft Stems	✓		✓								
Hard/Brittle Stems		✓	✓								
Growing Tips Die			✓					✓			
Stunted Root Growth		✓									
Wilting						✓					

Physiology of nutrient uptake :

Mineral nutrients are found either as soluble fractions of soil solution or as adsorbed ions on the surface of colloidal particles. Various theories proposed to explain the mechanism of mineral salt absorption can be placed in two broad categories:

I) Passive Absorption

II) Active Absorption

Ion uptake is both active and passive :

After several decades of research on this process of ion uptake it is now believed that the process involves both passive and active uptake mechanisms.

Whether a molecule or ion is transported actively or passively across a membrane (casparian band, plasma membrane or tonoplast) depends on the concentration and charge of the ion or molecule, which in combination represent the electrochemical driving force.

Passive transport across the plasma membrane, occurs along with the electrochemical potential. In this process ions and molecules diffuse from areas of high to low concentrations. It does not require the plant to expend energy.

Active transport, (in contrast, to passive transport) energy is required for ions diffusion against the concentration gradient (electro chemical potential). Thus, active transport requires the cell to expend energy.

Passive transport mechanism:

A) Diffusion: Simple diffusion to membranes occurs with small, non-polar molecules (i.e. O_2, CO_2). In this process ions or molecules move from the place of higher concentration to lower concentration. It needs no energy.

B) Facilitated diffusion: For small polar species (i.e. H_2O, Ions and amino acids) specific proteins in the membrane facilitate the diffusion down the electrochemical gradient. This mechanism is referred to as facilitated diffusion. Eg.

a) Channel proteins: The specific proteins in the membrane form channels (channel proteins), which can open and close, and through which ions or H_2O molecules pass in single file at very rapid rates. A K^+ and $NH4^+$ channel also operates by the same process of facilitated diffusion. In addition, Na^+ can also enter the cell by this process.

b) Transporters or Co-transporters or carriers: Another mechanism involves transporters or co-transporters responsible for the transport of ions and molecules across membranes. Transporter proteins, in contrast to channel proteins, bind only one or a few substrate molecules at a time. After binding a molecule or ion, the transporter undergoes a structural change specific to a specific ion or molecule. As a result, the transport rate across a membrane is slower than that associated with channel proteins.

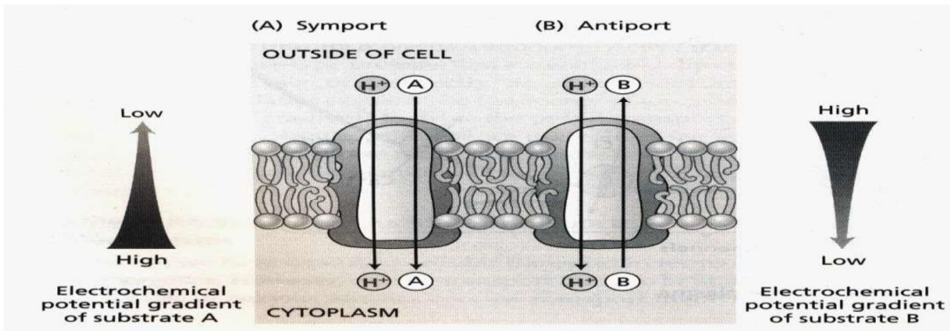
Three types of transporters have been identified:

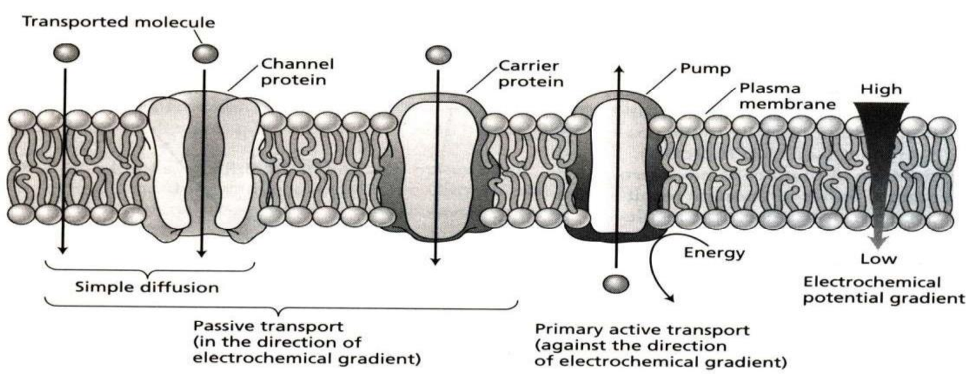
1. Uniporters: transport one molecule (i.e. glucose, amino acids) at a time down a concentration gradient.
2. Antiproters: catalyze movement of one type of ion or molecule against its concentration gradient. This is coupled with the movement of a different ion or molecule in the opposite direction. Examples of antiporter transport are H^+/Na^+ and H^+ /Ca^{+2} transport into the vacuole.
3. Symporters: catalyze movement of one type of ion or molecule against its concentration gradient coupled to movement of a different ion or molecule down its concentration gradient in the same direction. The high H^+ concentration in the apoplast provides the energy for symporter transport of NO_3^- and the other anions.

Therefore, the energy for antiporter and symporter transport originates from the electric potential and/or chemical gradient of a secondary ion or molecule, which is often H^+.

Active transport mechanism: Larger or more-charged molecules have great difficulty in moving across a membrane, requiring active transport mechanisms (i.e., sugars, amino acids, DNA, ATP, ions, phosphate, proteins, etc.). Active transport across a selectively permeable membrane occurs through ATP-powered pumps that transport ions against their concentration gradients. This mechanism utilizes energy released by hydrolysis of ATP. The Na^+-K^+ ATP pump transports K^+ into the cell and Na^+ out of the cell, another example is the Ca^{+2} -ATP pump.

Thus, it can be understood from the above discussion that the ion transport mechanisms operate both actively and passively. For some of the ions the uptake mechanism is active and for some others it is passive.





Reference: Lincoln Taiz and Eduardo Zeiger 2006, Plant Physiology, Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.

Chapter 5

Photosynthesis: Light and Dark reactions, C3, C4 and CAM plants

Photosynthesis :

Photosynthesis is the absorption of light energy and its conversion into chemical energy. During photosynthesis, CO_2 and water transformed into simple carbohydrates and O_2 is liberated into the atmosphere.

♦ The simple CH_2O_3 produced during photosynthesis are converted by additional metabolic process, into lipids, nucleic acids, proteins and other organic molecules.

♦ These organic molecules in turn, are elaborated into leaves, stems, roots, tubers, fruits, seeds and other tissues and organ system.

Thus, the overall reaction of oxygenic photosynthesis can be represented as.

$$6CO_2 + 12\,H_2O \xrightarrow[\text{CHLOROPHYLL}]{\text{LIGHT}} C_6H_{12}O_6 + 6\,H_2O + 6\,O_2\uparrow$$

This equation is frequently represented by the simplified form:

$$CO_2 + 2\,H_2O \longrightarrow (CH_2O) + H_2O + O_2$$

The photosynthetic process is carried out by three steps:

i. The absorption of light and retention of light energy.
ii. The conversion of light energy into chemical potential.
iii. The stabilization and storage of chemical potential.

Based on the three steps, the yield of a crop can be expressed by an equation

$Y = Q \times I \times E \times H$

Q = Quantity of solar radiation received by the leaf or striking the leaf.

I = Fraction of Q utilized by plants.

E = Overall photosynthetic efficiency of the canopy (i.e. efficiency of the conversion of solar energy to chemical energy) in terms of total dry matter produced by the plants.

H = Fraction of dry matter allocated to the harvested parts (Harvest index).

Light phase of photosynthesis :

The absorption of radiant energy of green leaves is due to the presence of several pigments:

1. Chlorophyll a 2. Chlorophyll b 3. Carotenoids

Chlorophyll a and b account for the absorption of red light (600-700nm) and blue light (400 to 500 nm). The carotenoids also absorb in the blue region of the spectrum. In the absence of light chlorophyll 'a' synthesis is impaired. That is why plants grown in dark are usually lack chlorophyll ‘a’. So they are usually yellow in colour and possess elongated growth habit. Their leaf development is strongly reduced. Plants displaying the characters are said to be etiolated plant. So brief exposure of radiant energy of appropriate wavelength is sufficient to induce the formation of chlro ‘a’ in etiolated plants.

Photosynthesis is one of the most thoroughly studied photophysiological reaction. Chlorophyll pigments absorb lights energy. But they chiefly absorb in the blue and red part of the spectrum. Apart from chlorophyll several leaf pigments participate in the absorption of radiant energy used in photosynthesis. But chlorophyll ‘a’ only participate directly in the conversion of light energy into chemical energy. Whereas the other pigments (accessory pigments) transfer their excitation energy to chlorophyll ‘a’. The transfer of excitation energy between the pigments is occur by the process known as inductive resonance.

This can be illustrated by an example. Consider two pigments A and B.

A + radiant energy = A*

A pigment with the absorption of radiant energy, is converted to an excited state. Where this excitation energy is transferred to another pigment B. Then B gets excited. This is called inductive resonance. Inductive resonance normally occurs from accessory pigments to chlorophyll ‘a’ but not from chlorophyll ‘a’ to accessory pigments.

Absorption and action spectrum :

Action Spectra: An action spectrum is the rate of a physiological activity plotted against wavelength of light.

Absorption Spectra: An absorption spectrum is a spectrum of radiant energy whose intensity at each wavelength is a measure of the amount of energy at that wavelength that has passed through a selectively absorbing substance e.

The absorption of radiation by a substance can be quantified with an instrument called a spectrophotometer. This is a device that produces a beam of monochromatic ("single-color") radiation that can be shifted progressively across the spectrum; passes the beam through a solution of the substance and measures the radiation that gets through.

The relationship between the action spectrum for photosynthesis and absorption of light by chlorophylls and other pigments (carotenoids) indicates that the pigments are involved in photosynthesis.

Photochemical reaction :

In a photochemical event, only one above or one molecule is activated for each photon absorbed. Therefore, the number of excited molecules equals the number of photons absorbed. While observing the structure of a pigment molecule, nucleus possess the protons and neutrons. Whereas electrons are seen at various distances away from the nucleus. The electrons have different energies, depending on the distance from the nucleus. Nearer the electron to the nucleus, greater is the pull or attraction of the nucleus on electrons. If a photon of appropriate energy strikes the pigment, the electron in an inner shell is raised to an outer shell and the pigment is said to be in an excited state. The excited molecule will participate in the chemical reaction (chlorophyll 'a') or it may transfer the excitation energy (accessory pigment) to the neighboring pigments molecule by resonance transfer. Otherwise, the excited molecule may return to the ground state by two processes. 1. By emitting the radiant energy (Emission of radiant energy) or 2. By dissipating the heat (Heat dissipation).

Emission of radiant energy :

Chlorophyll molecules are capable of absorbing both red light and blue light. Red light is lesser energetic than blue light. Following the absorption of red light (660 nm), chlorophyll molecule attains the excited level called the first excited level. The lifetime of the excited molecule is quite short, often of the order of 10^{-10} to 10^{-8} s. When the energy is transferred to another pigment, excited chlorophyll returns to the ground state through the loss of energy by the emission of light. The emission of light within this short period of time (10^{-10} to 10^{-8}S) is referred to as fluorescence. The red light at the wavelength of 700 nm has raised the electron to the first excited level, whereas more energetic blue light of shorter wavelength (400 nm) raised the electron to the second excited level. The life time of this excited molecule is long i.e. 10^{-2} to 10^{-1} seconds. Such long lived excited molecules have much greater probability of interacting with neighboring molecules and participating in photochemical reaction or the energy of the long lived molecule is emitted as light. This process is referred to as 'Phosphorescence'. The major difference between fluorescence and phosphorescence is that fluorescence occurs rapidly whereas the light emission by phosphorous is delayed. The excited chlorophyll molecule is involved in the transformation of radiant energy to chemical energy. As the result of the transformation of radiant energy, the chemical potentials such as ATP and NADPH are found besides releasing O_2.

ATP formation :

Regarding ATP formation, it is generated during

1.) The oxidation of glucose to CO_2 and H_2O in mitochondria. This process is known as oxidative phosphorylation

2.) The formation of ATP by the absorption of radiant energy by the chlorophyll pigments is known as photophosphorylation.

$$\text{ADP + inorganic phosphate (pi)} \xrightarrow[\text{Chloroplast}]{\text{Radiant energy}} \text{ATP}$$

NADPH formation :

NADPH is formed by accepting electrons from water molecules and releasing O_2.

$$4H_2O \xrightarrow{\text{Radiant Energy}} O_2 + 4\,(H^+ + e^-) + 2H_2O \quad \text{(Photolysis of water)}$$

$$4H_2O + 2\,NADP \xrightarrow[\text{Chloroplast}]{\text{RE}} 2NADPH + O_2 + 2H^+ + 2H_2O$$

Therefore, the illuminated chloroplasts are capable of generating both ATP and NADPH through the following reaction.

$$2\ ADP + 4\ H_2O + 2Pi + 2NADP \xrightarrow[\text{Chloroplast}]{\text{RE}} 2\ ATP + 2NADPH + 2H_2O + O_2 + 2H$$

Reaction scheme for ATP and NADPH formation :

Two pigment systems are involved:

1. Photo system I (PS I) (Photo system I contains chlorophyll 'a', 'b' and carotenes)
2. Photo system II (PS II) (Photo system II contains chlorophyll 'a', 'b' and xanthophylls)

The two photo systems are connected by several intermediates:

1. Plastoquinone 2. Cytochrome 3. Plastocyanin

Non-cyclic electron transport and phosphorylation :

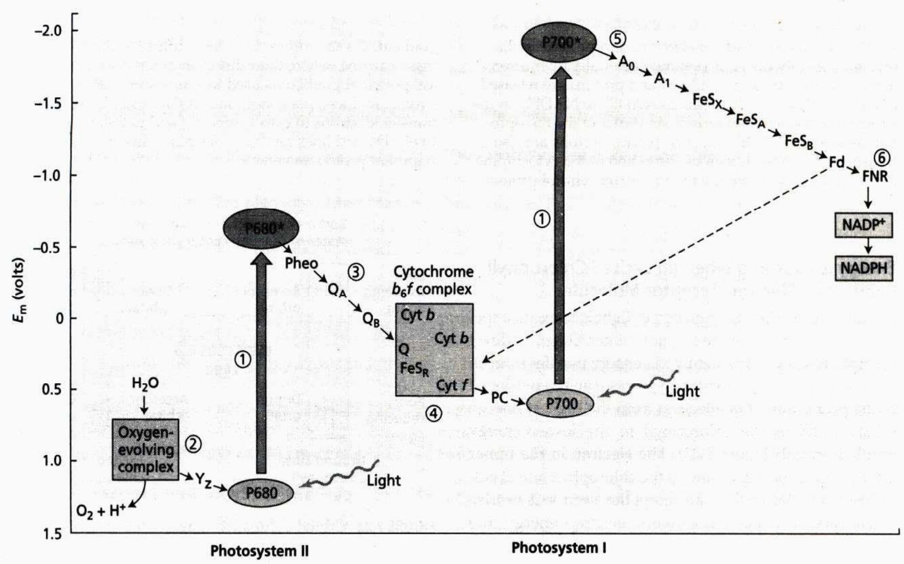


FIGURE Detailed Z scheme for O_2-evolving photosynthetic organisms. The redox carriers are placed at their midpoint redox potentials (at pH 7). (1) The vertical arrows represent photon absorption by the reaction center chlorophylls: P680 for photosystem II (PSII) and P700 for photosystem I (PSI). The excited PSII reaction center chlorophyll, P680*, transfers an electron to pheophytin (Pheo). (2) On the oxidizing side of PSII (to the left of the arrow joining P680 with P680*), P680 oxidized by light is re-reduced by Y_z, that has received electrons from oxidation of water. (3) On the reducing side of PSII (to the right of the arrow joining P680 with P680*), pheophytin transfers electrons to the acceptors Q_A and Q_B, which are plastoquinones. (4) The cytochrome b_6f complex transfers electrons to plastocyanin (PC), a soluble protein, which in turn reduces P700+ (oxidized P700). (5) The acceptor of electrons from P700* (A_0) is thought to be a chlorophyll, and the next acceptor (A_1) is a quinone. A series of membrane-bound iron–sulfur proteins (FeS_X, FeS_A, and FeS_B) transfers electrons to soluble ferredoxin (Fd). (6) The soluble flavoprotein ferredoxin–NADP reductase (FNR) reduces $NADP^+$ to NADPH, which is used in the Calvin cycle to reduce CO_2 (see Chapter 8). The dashed line indicates cyclic electron flow around PSI. (After Blankenship and Prince 1985.)

*

Reference: Lincoln Taiz and Eduardo Zeiger 2006, Plant Physiology, Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.

In 1960, Hill and Bendall proposed this scheme hill and Bendall scheme (Z scheme). This is the light requiring process in which electrons are removed from H_2O resulting in the evolution of O_2 as a byproduct and transfer of these electrons via a number of carriers to produce a strong, negative reducing potential with the subsequent formation of $NADPH_2$, a reducing agent with the potential of -0.34 V. Two ATP molecules can be simultaneously formed from two ADP and two pi, so that energy is stored in the form of this high energy compounds. The $NADPH_2$ and ATP are the 'assimilatory powers' required to reduced CO_2 to CH_2O.

The components of non-cyclic electron transport pathway are organized into three components that span the chloroplast membranes. These components are PS II complex, chlorophyll 'b' complex and PSI complex. Plastoquinone, plastocyanin and ferridoxin are the mobile carriers that shuttle electrons across the complexes. An electron is transferred from H_2O to NADP in almost 20 ms. Two different light reactions each occurring in different photosystem are required to raise the electrons from redox level of H_2O (+ 0.82V) to the redox level of $NADPH_2$ (-0.34V). Photosystem I has a predominance of chlorophyll 'a' with an absorption maximum at 680 nm (bluish green in colour), whereas PS II the closely related chlorophyll 'b' which has its maximum absorption peak at 650 nm (Yellowish green in colour).

Cyclic photophosphorylation :

The cyclic photophosphorylation operates when chloroplasts are illuminated with wave lengths of light greater than 680nm. Under these circumstances only photo system I is activated and electrons are not removed from H_2O. When the flow of electrons from H_2O is stopped, non-cyclic assimilation retarded, oxidized NADP is no longer available as an electron acceptor. Activation of photo system I by wave lengths of light greater than 680 nm causes electron to flow from P700 to chlorophyll molecule and Ferridoxin. Then the electrons instead of passing on to NADP return back to P700 via cyt b6-f complex, plastoquinone and plastocyanin.

Cyclic transport system is likely to result in the synthesis of ATP at two locations. One is between Fe-s protein and cyt-b6 complex and another between cyt-b6 and cytochrome f.

Significance :

Evidence for the operation of cyclic electron transport in C3 plants *in vivo* is limited but it has been demonstrated under physiological conditions *in vivo* in C4 plants where there is an additional ATP requirement in their carbon fixation pathway. It may also play an important role in the synthesis of ATP required for protein synthesis during PS II repair, following photo inhibition.

Pseudo cyclic phosphorylation :

Another source of generation of ATP is that electrons might be transferred from ferridoxin back to oxygen reducing it to water. It is possible that this process might also involve an electron transport chain and produce ATP. Here the electron that is cycled back to reduce molecular oxygen to water is not the same that is released from the water. Hence it is called as pseudo cyclic phosphorylation.

Dark reaction (CO_2 fixation) :

All photosynthetic eukaryotes, from most primitive algae to the most advanced angiosperms, reduce CO_2 to carbohydrates via the basic mechanism, the C3 photosynthetic carbon reduction (PCR) cycle. The PCR cycle is sometimes referred to as Calvin cycle in honour of its discovered, the American biochemist, Melvin Calvin.

In PCR cycle, CO_2 from atmosphere and water are enzymatically combined with a five-carbon acceptor molecule to generate 2 molecules of a three carbon intermediate. These intermediates are reduced to carbohydrate using the photochemically generated ATP and NADPH. The cycle is complete by regeneration of 5 carbon acceptor.

C3 PCR cycle involves three stages:

1. Carboxylation (Carboxylation of CO_2 acceptor, ribulose 1-5 bisphosphate to form two molecules of 3 phosphoglycerate, the first stable intermediate of the PCR cycle)
2. Reduction (Reduction on this 3 PGA to a carbohydrate in the form of glyceraldehyde 3 phosphate)
3. Regeneration (Regeneration of the CO_2 acceptor, ribulose, 1-5 bisphosphate from glyceraldehydes 3-phosphate)

Carboxylation of Ribulose bisphosphate :

CO_2 enters the PCR cycle by reacting with ribulose 1,5-bisphosphate to yield two molecules of 3-phosphoglycerate, a reaction that is catalysed by the chloroplast enzyme ribulose bisphosphate carboxybae / oxygenase (Rubisco). Carboxylation of ribulose 1,5-bisphosphate, catalyzed by Rubisco proceeds in two stages: Carboxylation and hydrolysis.

1. Carboxylation involves the addition of CO_2 to carbon 2 (O_2) ribulose 1,5- bisphosphate to form an unstable enzyme bound intermediate 2 carboxy 3 keto arabinitol 1,5-bisphosphate, which undergoes hydrolysis to yield two molecules of stable product, 3-phosphoglycerate. Rubisco, the enzyme responsible for fixing 200 billion tons of CO_2 annually, is without doubt the world's most abundant enzyme. Rubisco accounts for some 40% of the total soluble protein of most leaves. The concentration of Rubisco active sites within the chloroplast stroma is calculated to be about 4mM or about 500 times greater than the cone of one of its substrates CO_2. The molecular mass of Rubisco is 560 KDa. It is composed of eight large subunits each of which has an active site, and eight small subunits.

The gene for the large submit is encoded by the chloroplast genome and this sub unit is synthesized by the chloroplast ribosomes. Gene for the small subunit is synthesized by cytosolic ribosomes, transported into the chloroplast and assembled there with large sub units for L8 S8 Rubisco molecules.

The Reduction step of the C3 PCR cycle :

In this stage, the 3 phosphoglycerate formed as a result of the carboxylation of ribuloze bisphosphate is first phosphorylate to 1-3 bisphosphoglycerate by the ATP generated in the light reactions and is then reduced to glyceraldehydes -3-phosphate, using the NADPH generated by the light reaction. The chloroplast enzyme NADP: glyceraldehydes 3 phosphate dehydrogenase catalysis this step.

The regeneration of ribulose 1,5- Bisphosphate :

Continued fixation of CO_2 requires that the CO_2 acceptor, ribulose 1,5 bisphosphate, be consultancy regenerated. To avoid depleting the cycle of intermediates, 3 molecules of Ribulose 1-5, bisphosphate are formed by reshuffling the carbon from 5 molecules of triosephosphate. One molecule of glyceraldehydes 3-phosphate is converted to dihydroxyacetone 3 phosphate. Dihydroxyacetone 3 phosphate then undergoes condensation with a molecule of glyceraldehyde 3 phosphate to give fructose 1,6 bisphosphate. This product is hydrolysed to fructose 6- phosphate. This compound reacts with third molecule of glyceraldehydes 3-phosphate to give erythrose 4 phosphate and xylulose 5-phosphate. E4P then combines with a fourth molecule of triose phosphate to yield seven carbon sugar sedoheptulose 7 phosphate. This reacts with fifth molecule of glyceraldehydes 3 phosphate and produces ribose 5-phoshate and xylulose 5 phosphate. The two molecules of xylulose 5-phosphate are epimerized to give ribulose 5- phosphate. Ribose 5phosphate is also isomerized to ribuloses 5 phosphate. Finally, ribulose 5-phosphate is phosphorylated with ATP, thus generating the CO_2 acceptor ribulose 1,5-bisphosphate.

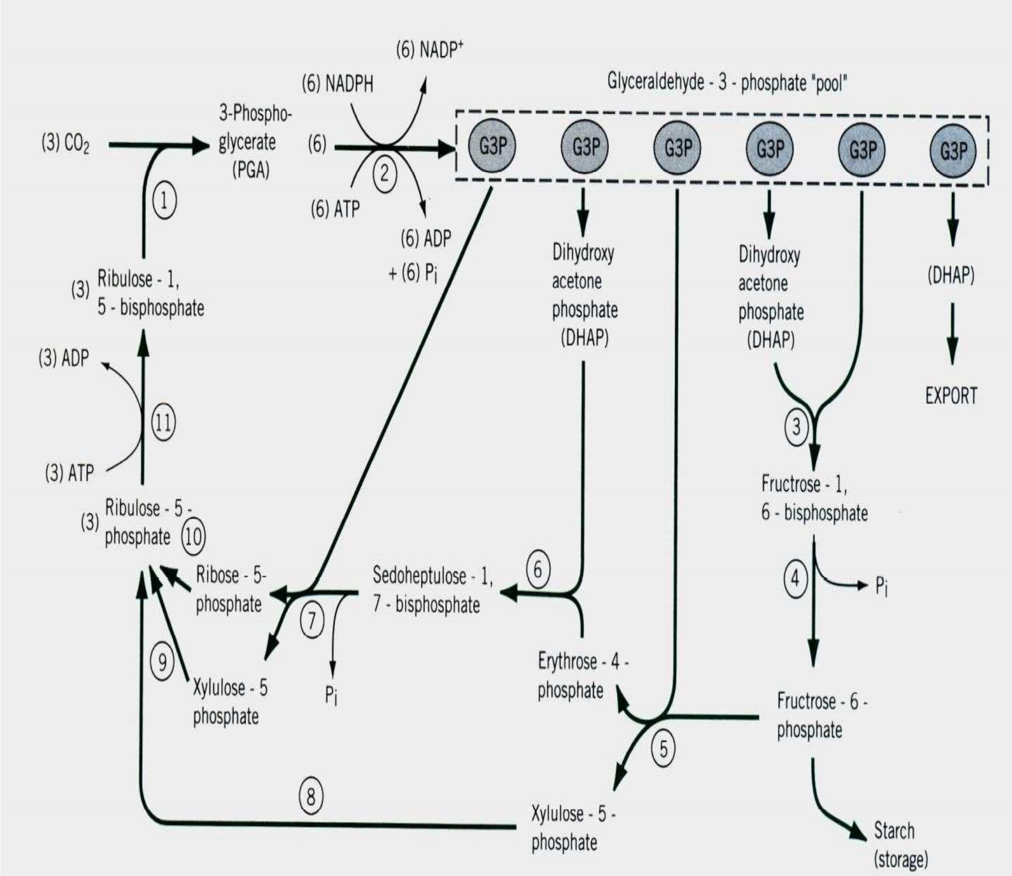


FIGURE 10.3 The photosynthetic carbon reduction (PCR) cycle. Numbers in brackets indicate stoichiometry. Enzymes, indicated by circled numbers are: (1) ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco); (2) 3-phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase; (3) aldolase; (4) fructose-1,6-bisphosphatase; (5) transketolase; (6) aldolase; (7) sedoheptulose-1,7-bisphosphatase; (8, 9) ribulose-5-phosphate epimerase; (10) ribose-5-phosphate isomerase; (11) ribulose-5-phosphate kinase.

References: Hopkins WG & Huner NPA. 2004. Introduction to Plant Physiology. John Wiley & Sons

Chapter-6

Respiration: Glycolysis, TCA cycle and electron transport chain

Plant Respiration :

"Plant respiration is the chemical reaction by which plants cells stay alive." The process of respiration is expressed as:

Glucose + Oxygen → Carbon Dioxide + Water (+ Energy)

Do Plants Breathe?

The answer to this question is not direct. Yes, plants need oxygen for respiration but at the same time they also give out carbon dioxide. Thus, plants have proper system to ensure the availability of oxygen. Unlike animals, plants do not possess any specialized organs for exchange of gases but they have lenticels and stomata (present in stems and leaves respectively) that carry out the function of gaseous exchange.

Plants do not have any specialized organ to respire and exchange gases because each part of the plant takes care of the need of gases themselves. The parts of the plant do not display any great demand for exchange of gases. Added to this, stems, leaves and roots respire at very lower rate as compared to animals. But during the process of photosynthesis, large exchange of gases takes place and each part of the plant is well adapted to fulfil its need of gases. Availability of oxygen is not a problem during photosynthesis because the cells release oxygen within cells. It is important to note that each living cell in a plant is located quite close to the surface of the plant and in case of stems, the living cells are arranged in the form of thin layers beneath and inside the bark and have openings which are referred as lenticels. Thereby, the respiration and translocation take place at every part of the plant.

The complete combustion of glucose produces $\mathbf{H_2O}$ and $\mathbf{CO_2}$ as end products and release energy in the form of heat. In case, this energy is required by the cell, it will utilize accordingly. Following reaction explains the entire process:

$$\mathbf{C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + Energy}$$

During the process of respiration, $\mathbf{O_2}$ is utilized and carbon dioxide, energy and water are released as products. There is also a situation when then the oxygen is not available. For instance, the first cell on this planet must have carried out reaction in the absence of oxygen and even in the current living world we are aware of several living organisms adapted to anaerobic conditions. Some of these organisms are facultative and some are obligate. In any of these cases, all living organisms retain enzymatic machinery to partially oxidize glucose in the absence of oxygen. This process is also called as **Glycolysis** which includes breaking down of glucose to **Pyruvic Acid**.

Respiration in Roots :

The process of respiration in roots is carried out in the following manner:

- Air occurs in several interspaces of soil. The hairs of the roots are in direct contact with them.
- Oxygen of the soil gets diffused via root hairs and reaches all internal cells of the root for respiration.
- Carbon dioxide produced during the diffusion is released in the opposite direction.

Respiration in Stems :

- In the condition of water logging, air gets deficient in soil and in this case, metabolic activity of the roots declines. The stems of herbaceous plants possess stomata and the air gets diffused via it and reaches the cells for respiration.
- The carbon dioxide produced during the process gets diffused in the air via stomata.

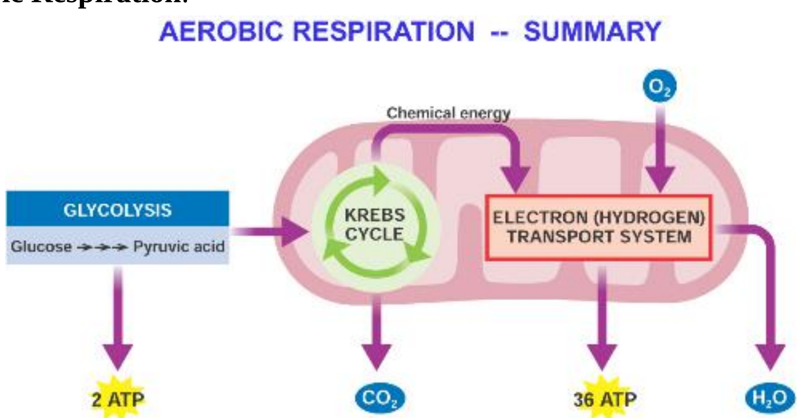
Respiration in Leaves :

- When the stems are woody, this gaseous exchange is carried out by lenticels. Leaves of the plants have tiny pores which are referred as stomata. The exchange of gases takes place by the process of diffusion via stomata. The stomata are present in large number on lower surface of leaves of plant. Each stoma is surrounded and controlled by **Guard Cells** (two kidney shaped cells). Then the stoma, open gaseous exchange takes place between **atmosphere** and **interior of leaves**.

Types of Respiration :

Respiration is of two types :

- **Aerobic Respiration:** In this type of respiration, the food substances are completely oxidized into $\mathbf{H_2O}$ and $\mathbf{CO_2}$ with the release of energy. It requires atmospheric oxygen and all higher organisms respire aerobically. Following figure shows the steps included in **Aerobic Respiration**.



References: https://www.askiitians.com/biology/respiration-in-plants/

- **Anaerobic Respiration:** In this type of respiration, partial oxidation of food takes place and energy is released in the absence of oxygen. This type of respiration occurs in prokaryotic organisms like bacteria and yeast. Ethyl alcohol and carbon dioxide are formed in this process.

Glycolysis :

The term glycolysis is derived from two Greek words, i.e. *Glycos* which means sugar and *lysis* means splitting. The scheme of glycolysis was given by Otto Meyerhof, J. Parnas and Gustav. In case of anaerobic respiration, respiration is carried out via glycolysis which occurs in cytoplasm of the cells. In it, partial oxidation of glucose is carried out resulting in two molecules of pyruvic acid. Glucose and fructose are phosphorylated to give rise to glucose-6-phosphate via enzyme hexokinase. This phosphorylated glucose is isomerized to produce fructose-6-phosphate.

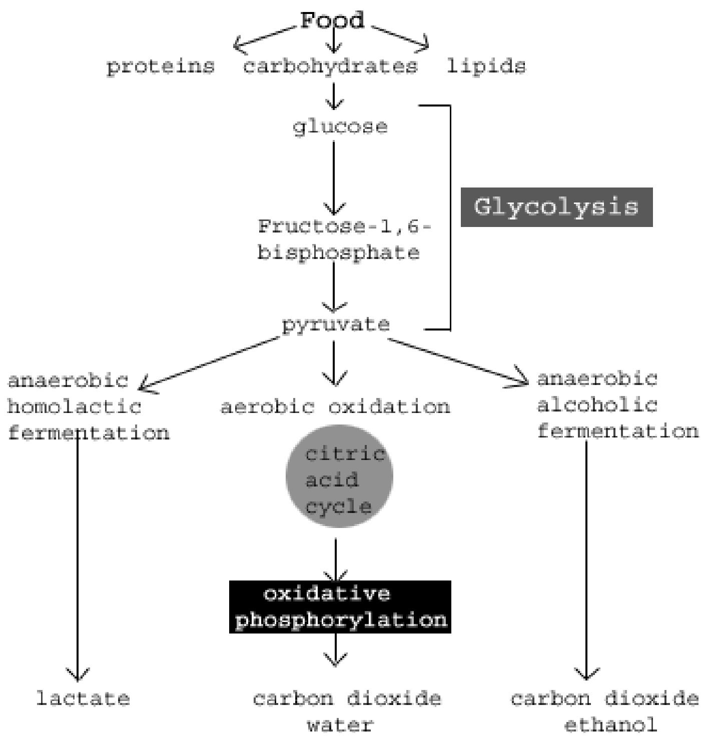
The several steps of Glycolysis are depicted in the figure below. In this process, chain of ten reactions takes place under the control of various enzymes and the outcome is pyruvate. ATP is utilized at two steps:

- During the conversion of glucose into glucose-6-phosphate.
- During the conversion of fructose-6-phosphate in fructose 1 and 6-diphosphate.

The fructose 1, 6 diphosphate is broken into

(i). **Dihydroxyacetone Phosphate** and

(ii). **3-Phosphoglyceraldehyde** (PGAL).



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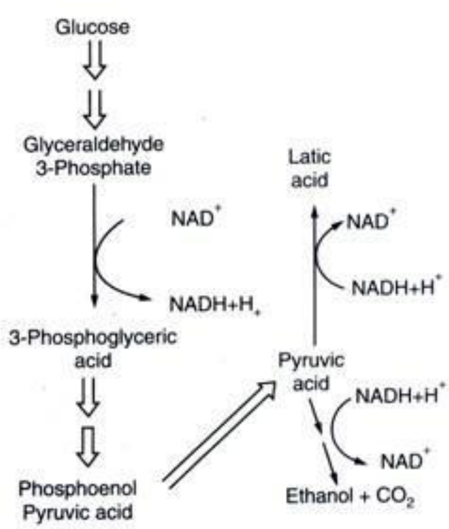
Fermentation



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In this process, the incomplete oxidation of glucose is carried out under anaerobic conditions via set of reactions where pyruvic acid is converted into ethanol and carbon dioxide. These reactions are catalyzed by two enzymes, i.e. achohol dehydrogenase and acid-decarboxylase. Other organisms such as bacteria produce lactic acid from pyruvic acid. The detailed steps are depicted in the figure below. In animal cells as well, during muscle exercise, in case of inadequacy of oxygen for cellular respiration, pyruvic acid is reduced to lactic acid by lactate dehydrogenase. **$NADH^+$ and H^+** are the reducing agent which is oxidized to **NAD^+** in the process.

In both alcohol and lactic acid fermentation, very less energy is released. Both these processes are hazardous because alcohol or acid is produced during the process. Fermentation process is used in our daily life such as in the formation of curd, vinegar, bread and alcoholic drinks.

Aerobic Respiration :

For aerobic respiration to take place in mitochondria, pyruvate is transported into mitochondria from cytoplasm. The most important events in this respiration are:

- The hydrogen atoms, that leaves 3 molecules of **CO_2**.
- Passing on of electrons removed as a part of hydrogen atoms to molecular oxygen with simultaneous synthesis of Adenosine Triphosphate (ATP).

Pyruvate, formed during glycolytic catabolism of carbohydrates in cytosol, enters the matrix of mitochondria and it undergoes oxidative decarboxylation by the complex set of reaction. This entire process is catalyzed by pyruvic dehydrogenase and this reaction requires involvement of several coenzymes such as Coenzyme A and NAD^+.

$$\text{Pyruvic acid} + \text{CoA} + \text{NAD}^+ \xrightarrow[\text{Pyruvate dehydrogenase}]{\text{Mg}^{2+}} \text{Acetyl CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+$$

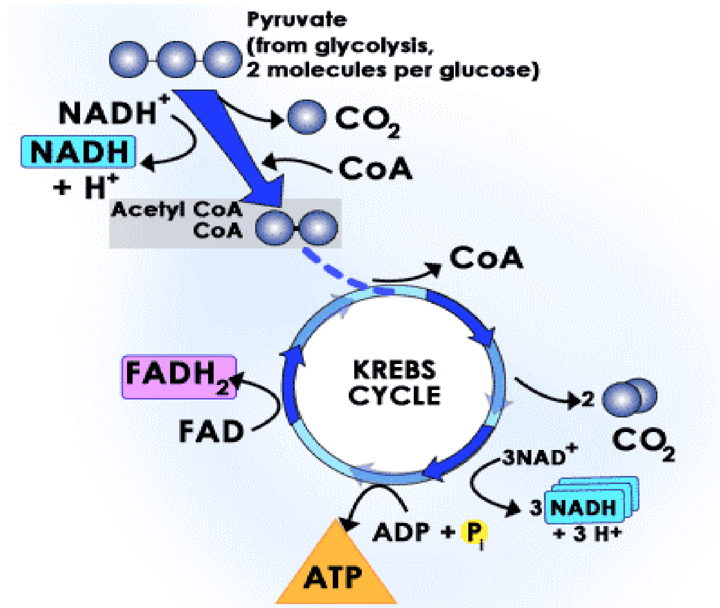
During this entire process, 2 molecules of NADH are produced from the metabolism of 2 molecules of pyruvic acid. The acetyl CoA enters a cyclic pathway called as Kreb's cycle or tricarboxylic acid. The name Krebs Cycle is mentioned after the name of scientist Hans Kreb who first elucidated this cycle.

Tricarboxylic Acid Cycle :

It is the second stage of cellular respiration. It plays an integral role in catabolism of breaking down of organic fuel molecule i.e. glucose, sugar, fatty acid and amino acids. The cycle starts with the condensation of acetyl group with oxaloacetic acid and water to release citric acid. The overall reaction of Krebs cycle is –

$2\ \text{acetyl CoA} + 6NAD^+ + 2FAD + 2ADP + 2P_i \rightarrow 4\ CO_2 + 6\ NADH + 6H^+ + 2\ FADH_2 + 2ATP$

This reaction is catalyzed by citrate synthase enzyme and a molecule of CoA is released. This citrate is then isomerized to isocitrate followed by decarboxylation that results in the formation of α-ketoglutaric acid and succinyl-CoA. Then succinyl-CoA is oxidized to OAA allowing the cycle to continue. During this conversion of succinyl-CoA to succinic acid one molecule of GTP is synthesized. In a coupled reaction GTP is converted to GDP along with the synthesis of ATP from ADP. Added to this, at three places in the entire cycle, NAD^+ is reduced to NADH + H^+ and at one-point FAD^+ is reduced to $FADH_2$. The entire cycle is shown in the figure below:



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Furthermore, the continued oxidation of acetyl CoA in this cycle requires continued replenishment of oxaloacetic acid, i.e. the first member of the cycle. The summary equation of entire process is given below:

$$\text{Pyruvc acid} + 4NAD^+ + FAD^+ + 2H_2O + ADP + Pi \xrightarrow{\text{Mitlochondrial Matrix}} 3CO_2 + 4NADH + 4H^+ + FADH_2 + ATP$$

Electron Transport System (ETS) and Oxidative Phosphorylation :

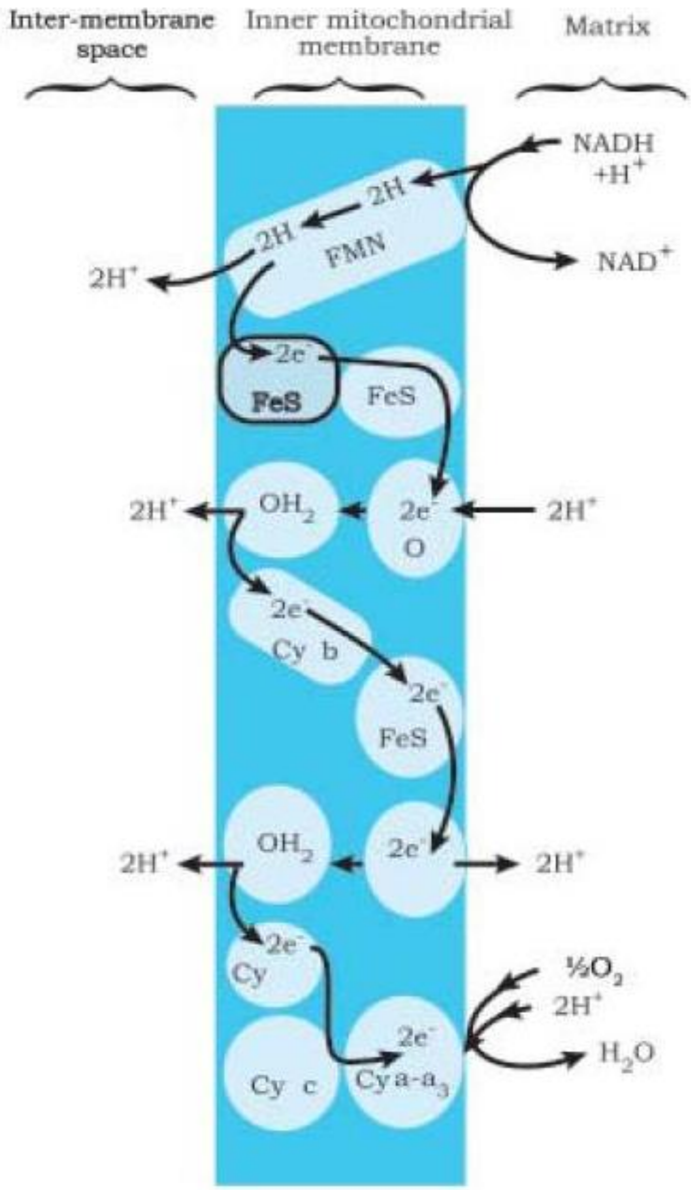


Figure shows the entire Electron Transport System in detail

References: https://www.askiitians.com/biology/respiration-in-plants/

NADH and $FADH_2$ carry electrons to the Electron Transport System. After the completion of Krebs cycle, oxygen enters in pathway as the electron acceptor at the end of electron transport system. "*The metabolic pathway, through which the electron passes from one carrier to another, is called electron transport system and is present in the inner mitochondrial membrane."* The electrons produced from NADH in the matrix of mitochondria during krebs/ citric acid cycle are oxidized by an NADH dehydrogenase (Complex I). The electrons are then transported to ubiquinone present in the inner membrane. This ubiquinone also receives reducing equivalents by $FADH_2$ (Complex II). This $FADH_2$ is generated during the oxidation of succinate in Krebs cycle. This reduced ubiquinone is oxidized with the transfer of electrons to cytochrome *c* with cytochrome bc_1 complex (Complex III). The small protein cytochrome *c* attached to outer surface of inner membrane acts as mobile carrier that transfers the electrons from Complex III to Complex IV. This Complex IV is cytochrome oxidase complex which contains cytochrome *a* and a_3, along with two copper centres.

When the transference of electron takes place from one carrier to another via complex I to IV, they are coupled to Complex V or ATP synthase for the production of ATP from ADP. The number of molecules of ATP synthesis depends on the nature of electron donor. Oxidation of 1 molecule NADH results in 3 molecules of ATP.

It is important to note that presence of oxygen is important for aerobic respiration, but its role is limited in the terminal stage of the process. Presence of oxygen is important because it drives the entire process by eliminating hydrogen from the process or it can be said that oxygen is the final hydrogen acceptor.

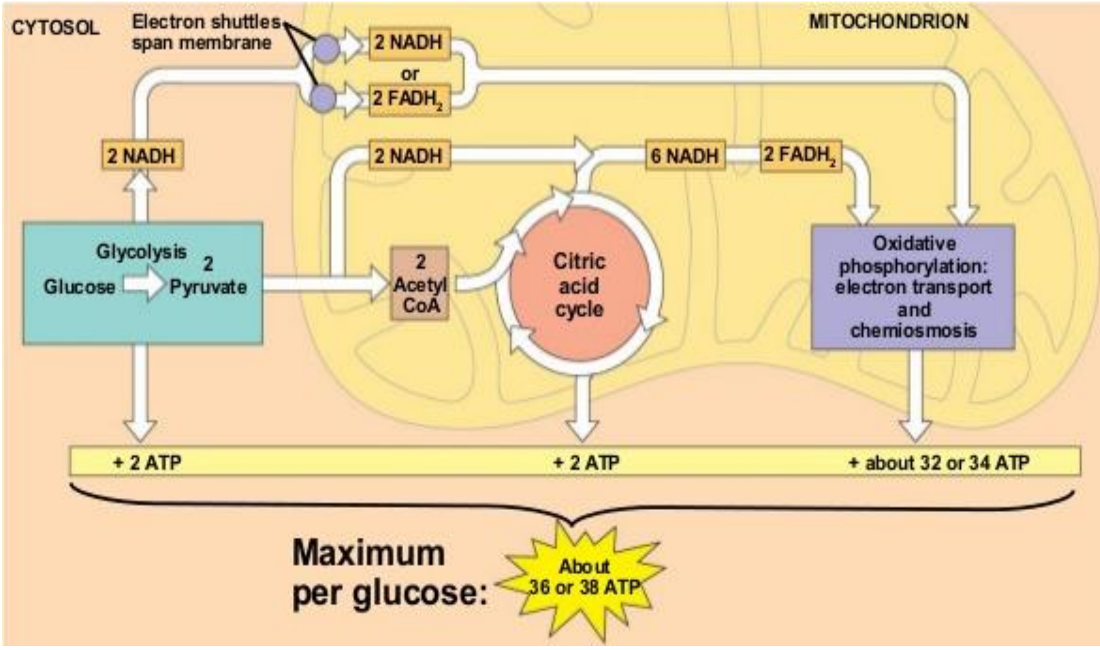
The Respiration Balance Sheet :

Theoretically, we can calculate the net gain of ATP for every molecule of oxidized glucose and this calculation is based on following assumptions –

- There is an orderly and sequential functioning of the pathway; with one substrate forming the next with glycolysis, Krebs cycle and Electron Transport System following one after another.
- The NADH formed during glycolysis is transferred into mitochondria and oxidative phosphorylation takes place.
- None of the intermediates in any process is utilized to synthesize any other compound.
- No alternative substrates except glucose are respired.

All the pathways work simultaneously but none of the above-mentioned assumptions are really valid in living system. Substrate that enters the pathways are extracted as and when required, ATP is utilized as and when required, the rate of enzyme is controlled by several means. On the other hand, doing this exercise is important as it appreciate the efficiency and beauty of the living system in extracting and storing energy. Thus, there can be net gain of 36 ATP molecules from one molecule of glucose in case of aerobic respiration.

Following figures explains the net gain of ATP



References: https://www.askiitians.com/biology/respiration-in-plants/

Amphibolic Pathway :

The term amphibolic is used to explain *"biological pathway that involves both catabolism and anabolism."*

Example of Amphibolic Pathway :

Krebs cycle is an example of Amphibolic Pathway because it includes both catabolism of fatty acids and carbohydrates and synthesis of anabolic precursors for amino acid synthesis. Thus, the pathway with both catabolism and anabolism potential is known as amphibolic pathway.

Respiratory Quotient :

This is another aspect of respiration. *"Respiratory quotient is the ratio of CO_2 produced to O_2 consumed while food is being metabolized."*

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

Where, RQ stands for Respiratory Quotient

RQ depends on the type of respiratory substrate used in respiration. When carbohydrate is used as substrate and is completely oxidized, RQ becomes 1. It implies equal amount of O_2 and CO_2 are consumed and evolved. This reaction is displayed in the figure below –

$$C_6 + H_{12}6O_2 \rightarrow 6CO_2O + \text{Energy}$$

$$RQ = \frac{6CO_2}{6O_2} = 1.0$$

In case, fats are used during the process of respiration, RQ becomes less than 1. Following equation shows the calculation for fatty acid and tripalmitin is used as substrate –

$$\underset{\text{Tripalmitin}}{2(C_{51}\ H_{98}\ O_6)} + 145O_2 \rightarrow 102\ CO_2 + 98H_2O + \text{energy}$$

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

When protein is used as respiratory substrates the ratio comes out to be 0.9.

Factors affecting Respiration in Plants

There are eight environmental factors that has significant impact on respiration in plants –

- Oxygen content of the atmosphere
- Effect of water content
- Effect of temperature
- Effect of availability of light
- Impact of respirable material
- Effect of concentration of carbon dioxide in atmosphere
- Protoplasmic conditions, i.e. younger tissues have greater protoplasm as compared to older tissues.
- Other factors, i.e. fluorides, cyanides, azides, etc.

Chapter-7

Fat Metabolism: Fatty acid synthesis and Breakdown

Fats (lipids) :

Fats are a heterogenous group of molecules. They contain atom of carbon, oxygen and hydrogen. Fats and fat like molecules are generally called as 'lipids'. They are insoluble in water and soluble in organic solvents. Fats serve as reserve food materials primarily in seeds, whereas the fat like materials mainly phospholipids and glycolipids, are constituents of all cell membrane. The cuticular waxes are also lipids but are quite different in their composition from the fats. The complete metabolism of fat leads to oxidation to CO_2 and water and the liberation of energy equivalent to 9 Calories /gram of fat.

Biosynthesis of lipids

The basic lipid unit is phosphatidic acid which is synthesized from glycerol and fatty acid. Glycerol is synthesized from glyceraldehyde-3-phosphate or from glucose. The fatty acids are synthesized from acetyl CoA. Fatty acids are also the precursor of waxes.

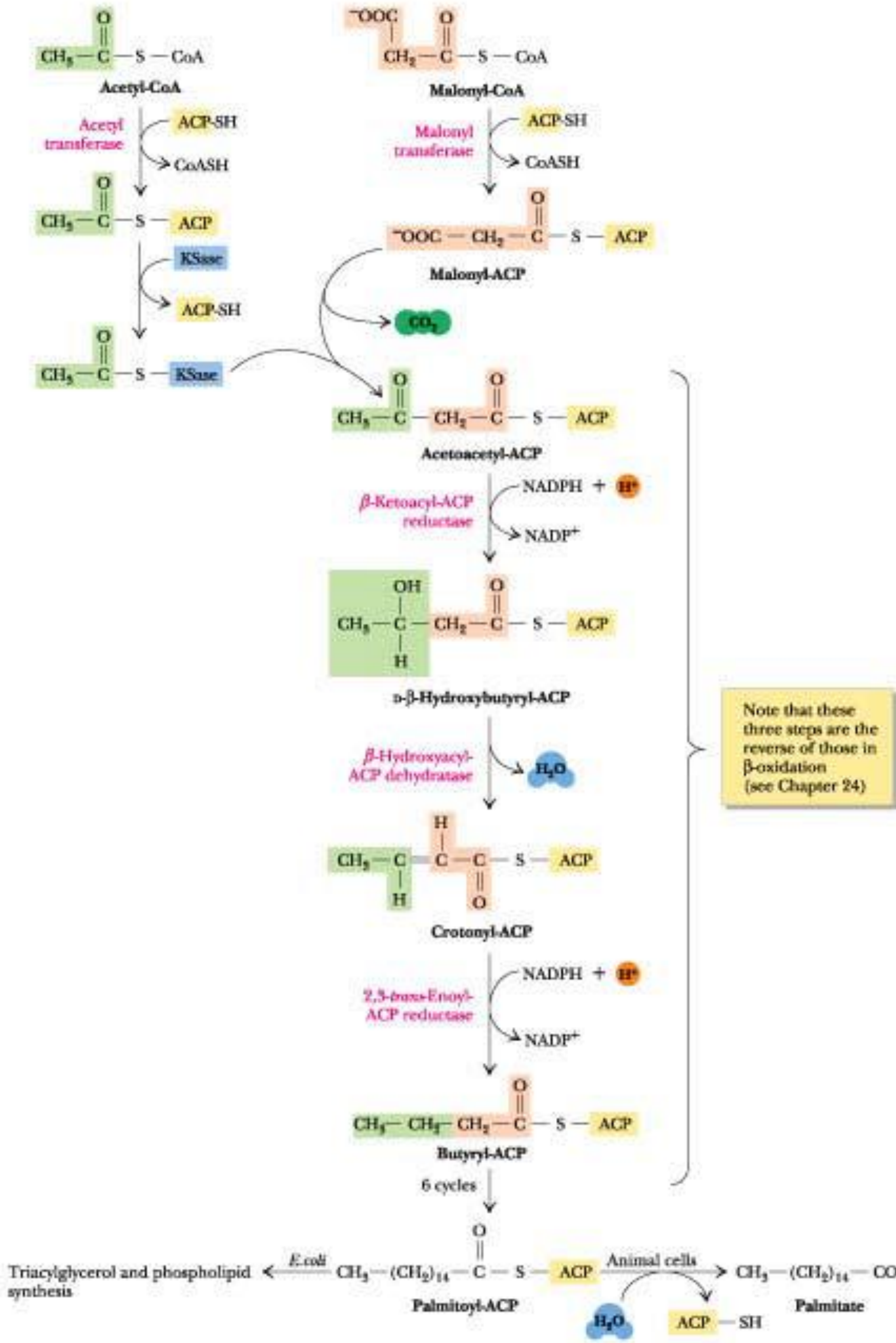
Phosphatidic acid may form triglycerides (neutral fats) or phospholipids by interacting with choline or glycolipids by interacting with sugars. The membrane of chloroplasts and mitochondria contain complex lipid molecules.

Fatty acids consist of even number of C atoms. Fatty acids may be saturated and unsaturated fatty acids Eg. Palmitic acid and Steric acid. Palmitic acid is commonly found in vegetable fats (palm oil) and linolinic acid is in linseed oil.

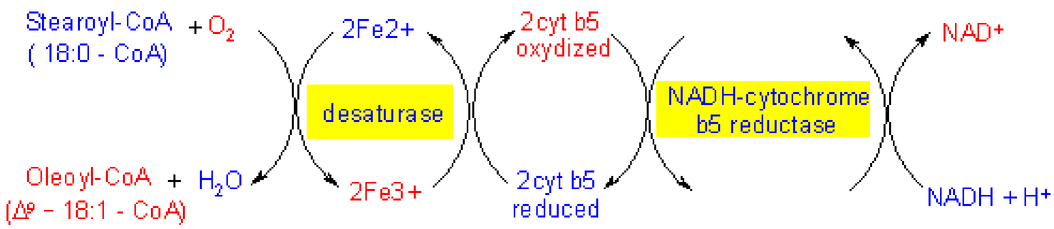
Anabolism of saturated fatty acids: Fatty acid synthesis is characteristic of all living organisms. Multienzyme complexes referred to as type I fatty acid synthases are essential for fatty acid synthesis. The first reaction of the sequence is carboxylation of acetyl CoA to malonyl coenzyme A in the presence of the enzyme acetyl CoA carboxylase.

$$\text{Acetyl CoA} + CO_2 + \text{ATP} \xrightarrow[\text{Acetyl CoA Carboxylase (Biotin)}]{} \text{Malonyl CoA} + \text{ADP} + \text{Pi}$$

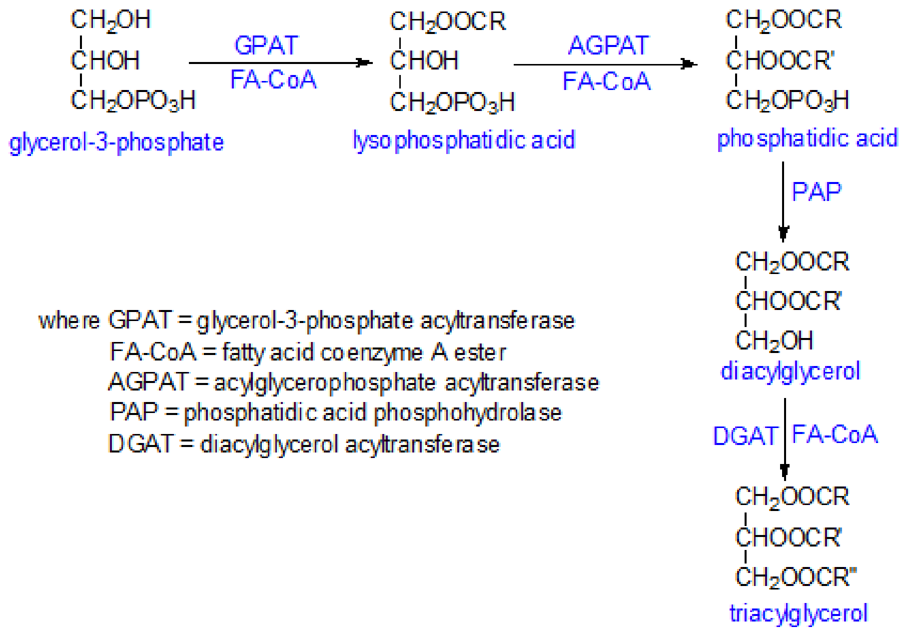
One molecule of acetyl CoA and one molecule of malonyl CoA are converted to their corresponding ACP derivatives in the presence of the enzymes transacylases.



Anabolism of unsaturated fatty acids: The fatty acid synthesized generally is palmitate which is a 16:0 fatty acid. Unsaturation of fatty acids occurs in both the mitochondria and endoplasmic reticulum in presence of the enzyme fatty acyl CoA desaturases.

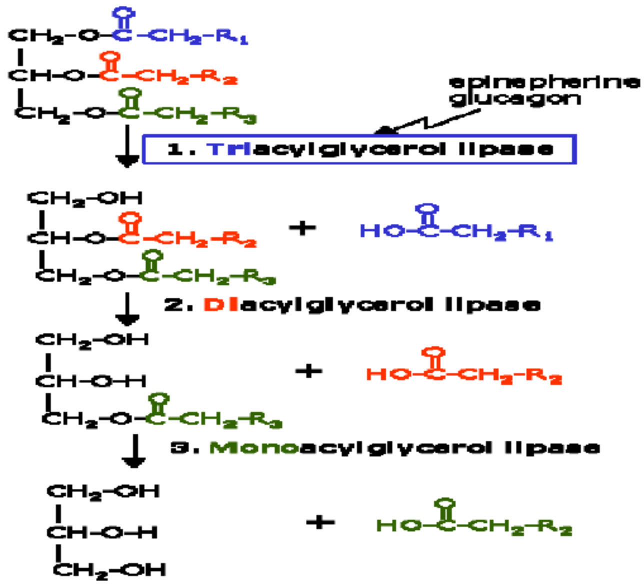


Anabolism of triacyl glycerol: The most important route to triacylglycerol biosynthesis is Kennedy pathway as illustrated below:

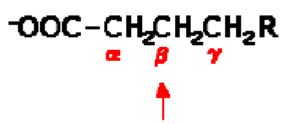


Breakdown of fatty acids

Catabolism of lipids: The first step in lipid catabolism is the hydrolysis of the lipid in the cytoplasm to produce glycerol and fatty acids.



Catabolism of fatty acids: In the mitochondria, fatty acids are broken down by various types of oxidations such as α, β and γ oxidation.



α – oxidation: alpha oxidation occurs in plants where odd number of carbon containing fatty acids are present. By this process, the long chain fatty acid is gradually broken down until it is

reduced to 12 C- atmos. Fatty acids with less than 13C atoms are not affected by this process. One complete α - oxidation results in the elimination of one carbon atom in the form of CO_2 from the COOH group of the fatty acid while α - C atom i.e. C atom no. 2, which is adjacent to – COOH) is oxidized.

α - oxidation takes place as follows:

1. The fatty acid is oxidatively decarboxylated in the presence of fatty acid peroxidase and H_2O_2 to form an aldehyde. In this reaction CO_2 comes from COOH (Carboxylic) group and oxidation takes place at α - C-atom which is converted into the aldehyde group.

β α Fatty acid peroxidase

$RCH_2CH_2CH_2\ COOH \longrightarrow RCH_2CH_2CHO + CO_2 + H_2O$

Fatty acid 2(H_2O_2) Aldehyde

2. The aldehyde is further oxidized in the presence of aldehyde dehydrogenase to form the new fatty acid containing one carbon atom less than in the original fatty acid. NAD is reduced in this reaction.

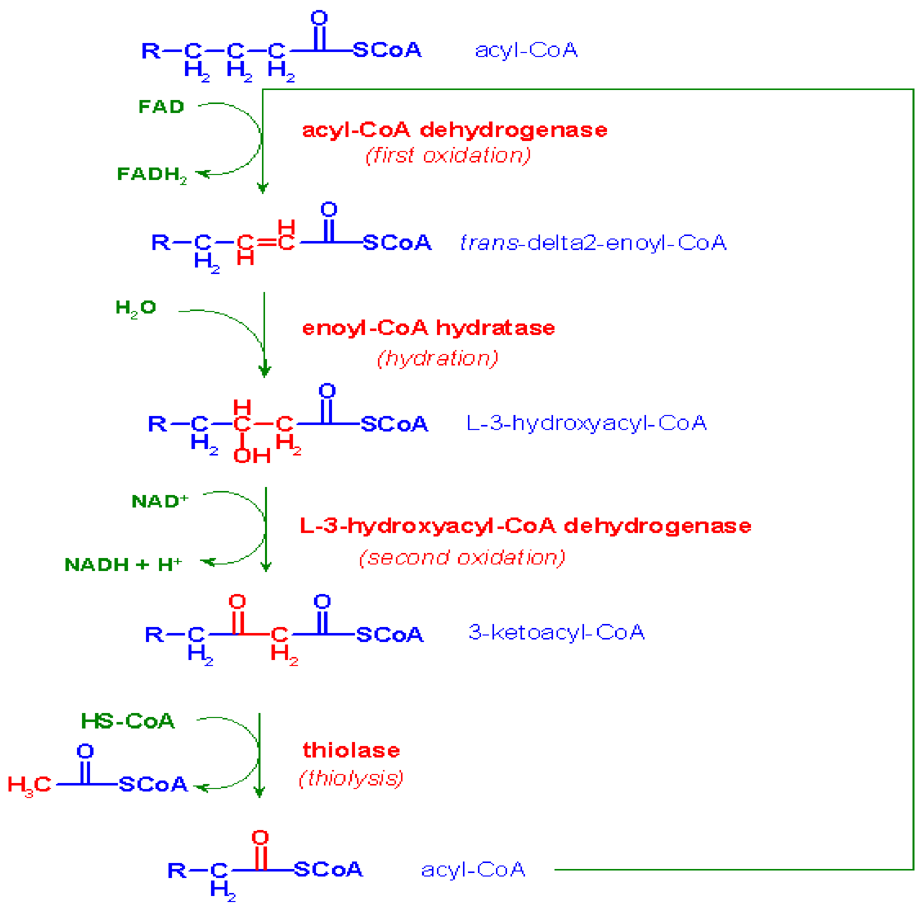
Aldehyde Dehydrogenase α

RCH2CH2CHO+NAD ——————→ RCH2CH2COOH+NADH + H

H_2O New fatty acid

The cuticular waxes are also lipids.

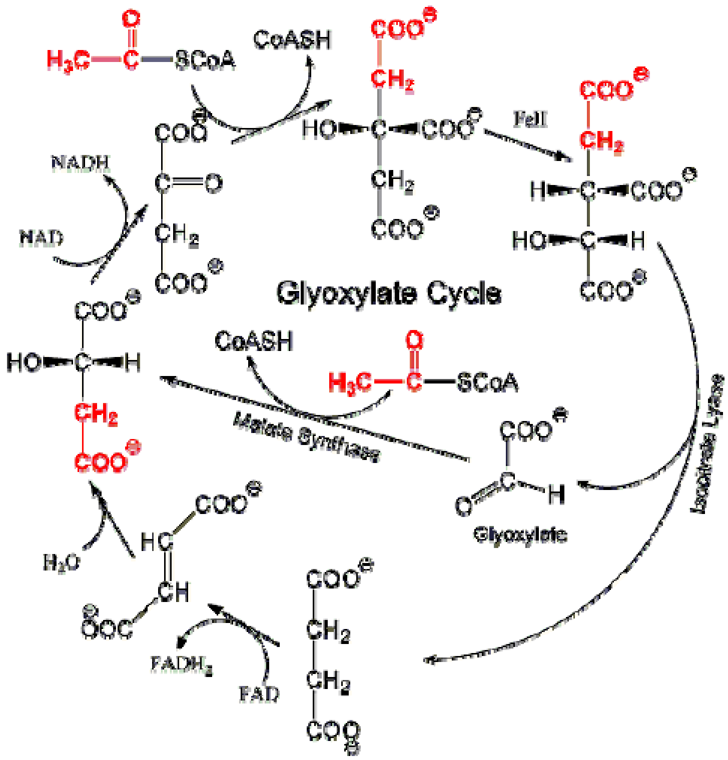
β-oxidation: In β-oxidation, the fatty acid is broken down to release acetyl-CoA. The process involves 4 main steps: dehydrogenation, hydration, oxidation, thiolysis. The process repeats until the fatty acid has been completely degraded to acetyl-CoA. Each round of β -oxidation yields 1 molecule of acetyl CoA and requires 1 molecule of NAD^+ and 1 molecule of FAD^+. Hence each round of β-oxidation releases 5 ATP molecules. For example, the β-oxidation of a C16 fatty acid will generate 8 molecules of acetyl CoA and 7 molecules of NAD+ and FAD+



Omega oxidation: It occurs in the endoplasmic reticulum rather than the mitochondria (the site of beta-oxidation). The omega carbon in a fatty acid is the carbon farthest in the alkyl chain from the carboxylic acid. In the omega oxidation pathway, this carbon is progressively oxidized first to an alcohol and then to a carboxylic acid, creating a molecule with a carboxylic acid on both ends.

R-CH2-CH2-CH2-COOH ------------------------ COOH-CH2-CH2-CH2-COOH

Glyoxylate cycle: In plants, the glyoxylate cycle occurs in special peroxisomes which are called glyoxysomes. This cycle allows seeds to use lipids as a source of energy to form the shoot during germination. The lipid stores of germinating seeds are used for the formation of the carbohydrates that fuel the growth and development of the organism. The two enzymes which regulates this cycle are isocitrate lyases and malate synthase.



Breakdown of fats

Active breakdown or degradation of fats takes place.

1. During the germination of fatty seeds and decomposition products may enter into glycolysis and krebs' cycle to release energy and also to synthesise soluble sucrose through glyoxylic acid cycle which is then translocated to the growing regions of the germinating seedlings.

2. In plants, when carbohydrates reserve declines, fats may form the respiratory substrates to release energy through oxidation.

Breakdown

Fats are first hydrolyzed in the presence of the enzymes lipases to yield fatty acid and glycerol.

$$\text{Tiglyceride} \xrightarrow{\text{Lipases}} \text{Fatty acids + Glycerol}$$

Oxidation of glycerol

Glycerol reacts with ATP under the influence of glycerol kinase to form glycerol 3-phosphate which is then oxidized to produce dihydroxy acetone phosphate by glycerol -3-phosphate dehydrogenase and NAD.

$$\text{Glycerol-3-P+ATP+NAD} \xrightarrow{\text{Glycerol-3-P dehydrongease}} \text{Dihydroxy acetone phosphate}$$

$$\text{Dihydroxy acetone phosphate} \longrightarrow \text{Pyruvic acid + 2ATP + NADH + } H^+$$

Glycolytic Pathway :

This conversion of glycerol to pyruvic acid, takes place in cytoplasm which yield 2ATP and 2NADH which in term produce 4 molecules of ATP. If pyruvic acid enters into Kreb's cycle (TCA), it will produce another 15 ATP mole. Thus a total of 2+4+15 = 21 ATPs with the consumption of one molecule of ATP in the presence of glycerol kinase enzyme. Therefore net gain of 20 ATP/glycerol.

Chapter-8

Plant growth regulators: Physiological roles and agricultural uses

Growth of the plant has for long been believed to be due to the minerals absorbed from the soil and the food materials synthesized by the plant. It is now however recognized that the growth of the plant is very much regulated by certain chemical substances known as growth regulators. These substances are formed in one tissue or organ of the plant and are then transported to other sites where they produce specific effects on growth and development.

Philips (1971) defined growth hormones as a substance which is synthesized and is transported to other cells where in extremely small quantities influences development processes.

The plants are known to produce mainly 5 classes of classical hormones namely auxins, gibberellins, cytokinins, abscisic acid and ethylene. However, some newly discovered hormones like Jasmonic acid, Salicylic acid and Brassinosteroides are also important.

A plant tissue may contain more than one of these growth regulators at the same time. The leaf primordial and developing seeds contain both auxin and gibberellins and in some plants ABA also. Both auxins and gibberellins cause stem elongation by different mechanisms while ABA and ethylene inhibits stem growth. Thus, two or more growth regulators may be similar in their action. When the effect is more than the sum of their individual effects it is called synergistic and when the action of two growth regulators is opposite it is called antagonistic. The final growth and development of the plant is the sum of total interactions of different growth regulators that are present in the plant.

1. Auxins:

Discovery

The idea of the existence of auxins in plants was for the first time conceived by Charles Darwin in 1881. He showed that coleoptile of canary grass could bend towards light when it is unilaterally illuminated. However, the coleoptile failed to bend when its tip was covered with an opaque cap. Most of the knowledge about auxins comes from the work on oat (*Avena sativa*) coleoptile.

Went (1926) demonstrated that coleoptile tips contain a substance capable of elongation of decapitated coleoptiles. He placed several freshly cut coleoptile tips on an agar block which was kept on a piece of inert material like glass. After several hours he cut the agar block into small cubes. He placed the agar cubes accentrically on decapitated coleoptile stumps for 2 hours in the dark. The effect of agar cube was similar to that of the tip as was shown by curvature of the coleoptiles (Avena coleoptile curvature test).

The first higher plant from which auxin could be extracted was maize kernels. It was identified as IAA. Indole Acetic Acid is the major auxin occurring in plants.

Occurrence:

All the parts of the plant body produce auxin. However, the major sites of auxin production are the shoot tip, developing seeds and buds. The amount of auxin present in different parts of the plant varies greatly. The amount is highest in the stem tip and coleoptile tip and decreased gradually down words.

Natural Auxin: Indole acetic acid (IAA)

Synthetic auxins: There are a number of synthetic chemicals which are similar to IAA in their biological activity. However, they do not occur in any plant. The important synthetic auxins are IBA (Indole Buteric Acid), NAA (Naphthalene Acetic Acid), 2, 4-D (2, 4 Dichloro Phenoxy Acetic Acid) and 2,4,5-T (2,4,5-Trichloro Phenoxy Acetic Acid).

Biosynthesis of auxins:

Indole acetic acid (IAA) is synthesized from an amino acid Tryptophan in 3 different pathways. These 3 pathways have different intermediate compounds and the pathway is named after the intermediate compound produced as follows

(a) Indole Pyruvic Acid Pathway, (b) Tryptamine Pathway, and (c) Indole Acetaldoxime Pathway.

Indole Pyruvic Acid Pathway:

The first reaction involves transmission of Tryptophan to Indole Pyruvic Acid. The enzyme tryptophan amino transferase transfers amino groups (NH_2) from Tryptophan resulting in the formation of Indole Pyruvic Acid.

In second step Indole pyruvic acid is decarboxylated to form Indole acetaldehyde. The enzyme involved is Indole pyruvic decarboxylase. A molecule of CO_2 is removed. In the final step Indole acetaldehyde is oxidized to IAA by 2 enzymes namely Indole acetaldehyde dehydrogenase and Indole acetaldehyde oxidase.

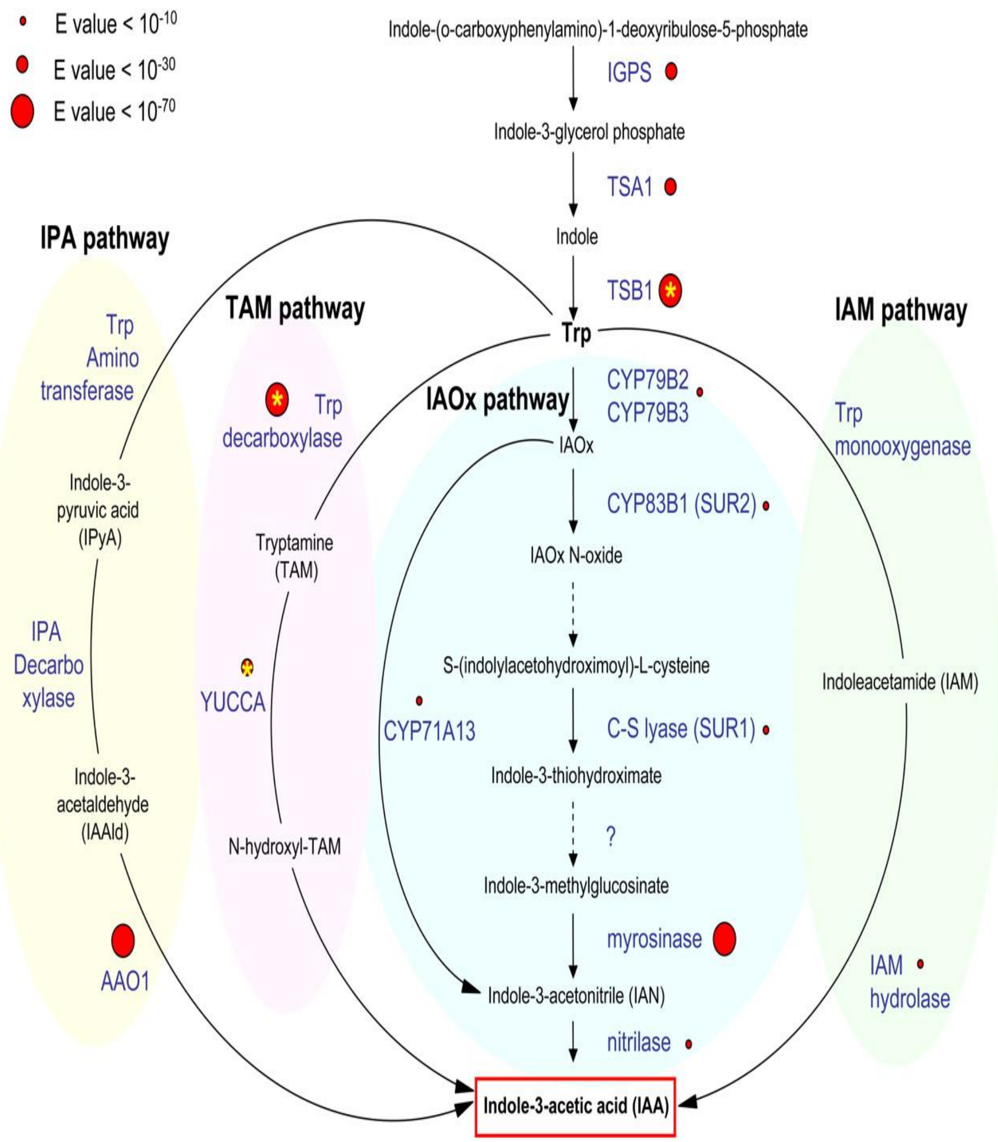
Tryptamine Pathway:

This pathway is of rare occurrence and was shown in tomato. Here tryptophan is first decarboxylated by the enzyme tryptophan decarboxylase forming tryptamine. Tryptamine then undergoes deamination forming indole acetaldehyde. The enzyme responsible for this reaction is amine oxidase. In the final step indole acetaldehyde is oxidized to IAA.

Indole Acetaldoxime Pathway:

This pathway is characteristic of the members of the family Brassicaceae. In this pathway tryptophan is first converted to indole acetaldoxime which in turn is further converted to indole aceto nitrile directly or through an intermediary compound glucobrassicin. Indole aceto nitrile is converted to indole acetic acid. Zinc is essential for biosynthesis of auxin since it activates the enzyme tryptophan synthetase.

Biosynthesis of Auxins:



Transport :

Auxin moves towards morphological basal end in stem cuttings. The movement of auxin is polar and basipetal. In roots it is polar but acropetal. In xylem it moves along the transpiration stream.

Mode of action :

It was observed that whenever auxin causes elongation of the coleoptile, the external medium in which the coleoptile was flooded, becomes acidic. The pH which was originally near neutral becomes decreased to 4.5. On this basis Hager, Menzel and Krans (1971) proposed acid growth hypothesis of auxin action. In this it was explained that the H^+ ions decreases P^H value and presumed to break the acid labile bonds or activate wall hydrolyzing enzymes and render the cell was soft. This will create suitable conditions for cell elongation.

Auxin and cell elongation :

The primary physiological effect of auxin is to promote the elongation of cells which may be due to increasing osmotic pressure and permeability of cytoplasm to water and decreasing cell wall pressure.

Auxin stimulates the production of hydrolyzing enzymes like β-1,3-gluconase, pectin methyl esterase and cellulase which soften cell wall and increase the plasticity resulting in reduction of wall pressure and cell elongation. Under the influence of auxin, cellulose synthetase increases and new wall material is synthesized within the cell wall resulting in extension or growth of the cell.

Physiological role of Auxins :

A. Cell division:

Auxin has been found to be responsible for initiating and promoting cell division in certain tissues eg. Cambium. Whenever wound is caused in the plant a swelling called callus is developed because of the proliferation of the parenchyma cells stimulated by auxin and a chemical substance traumatic acid. This can be put to practical use in grafting where the callus plays an important role in strengthening the union between stock and scion. Hence, during grafting of grapes, it was found that immersion of stock and scion in 0.1 percent of IAA resulted in quick growth of callus and success of graft union.

B. Root initiation:

The stem cuttings of some plants readily form adventitious roots when put in the soil. Adventitious root formation will takes place at the basal end of stem cuttings. Cuttings of such plants which do not readily root, form abundant roots when treated with auxins. Synthetic auxins like IBA, NAA are particularly very effective. In general cuttings of herbaceous plants readily respond to auxins while those of woody perennials like eucalyptus, mango and others fail to respond to auxin application.

In air layering of guava auxins like IBA at 500 ppm is used for root initiation.

C. Apical dominance :

The presence of apical bud causes a complete or partial inhibition of lateral buds. This is due to the presence of higher concentration of auxin at the apical bud, which causes a preferential movement of nutrients towards it.

In plants like sunflower, the main stem continues to grow and the lateral buds in the axils of leaves do not emerge and grow into branches. However, when the tip of the main stem is cutoff or when it terminates into an inflorescence, the lateral buds emerge into branches. In either case the influence of the shoot apex on the lateral bud is lost. This phenomenon of inhibition of laterals by the shoot apex is termed as apical dominance.

In plants like potato and tomato apical dominance is weak consequently the apical growing point of the main stem fails to suppress the emergence of lateral buds. Such plants are therefore extensively branched and bushy.

D. Inhibition of abscission layer:

Abscission is a process of dissolution of the middle lamella and primary walls of the cells at the base of the petiole, pedicle or peduncle. Abscission refers to detachment of plant organs. It is a balance between the inhibition of auxin and promotion of substances like ABA, Ethylene etc. Several auxins (2,4-D, IAA, NAA) inhibits the abscission of both leaves and fruits. Ex: application of NAA at 20 to 30 ppm twice at 15 days interval reduces flower and fruit drop in chillies and cotton. 2,4-D prevents defoliation in cabbage and cauliflower that often occurs during harvest.

E. Flower initiation :

Application of auxin inhibits flowering in several photoperiodically sensitive plants such as xanthium, soybean and others. But the exception is pineapple (*Ananas comosus*), a day neutral plant. This plant can be made to bloom promptly with the application of NAA or 2,4-D. However in this plant the effect of auxin is not direct but is mediated through ethylene formation.

Application of auxin also alters the sex ratio of flowers. In monoecious cucurbits increase the number of female flowers but the decrease the number of male flowers. Similarly in dioecious plants like cannabis, the male plants start producing female flowers when auxin is applied. Here again the effect of auxin is not shown to be direct but is mediated through ethylene formation.

F. Production of parthenocarpic fruits:

It is a general observation that in the absence of pollination and fertilization the ovary of the flower does not develop into the fruit, but the flower abscises and falls. However application of auxin causes development of ovary into the fruit in several plants such as tomato, brinjal and others. Such fruits are seedless as these have developed without the normal process of fertilization these are known as parthenocarpic fruits. The presence of large number of seeds in a fruit lowers its commercial value in the canning industry.

G. Eradication of weeds:

Plant roots are extremely sensitive to auxins. Very high concentration of auxins over stimulates growth promoting activities of root cells resulting in distorted roots with blocked sieve tubes. The roots ultimately decay and the plant are killed. 2,4D and 2,4,5-T are effective weedicides at higher concentration of 1 to 3 percent. 2,4D is selective weed killer. It is highly toxic to broad leaved plants or dicotyledons while relatively nontoxic to narrow leaved plants or monocot.

H. Growth in thickness:

The stem of dicots and gymnosperms not increase in length but also in thickness. Increase in thickness of the stem and root is due to radial growth. The process is termed as secondary growth.

I. Vascular differentiation:

Not only the activation of cambial rings but also the differentiation of cambial derivatives into xylem and phloem is also under the control of hormones. Interaction of both auxin and GA is involved in this. J. Prevention of lodging:

Naphthyl acetamide when applied on the base of oats and flax, they grow stiff, woody and erect. Thus, lodging in these crop plants is prevented.

2. Gibberellins

The discovery of Gibberellins was quite accidental. Japanese worker Kurosawa (1926) in Japan while conducting experiments on rice disease caused by *Gibberella fujikuroi* (causal organism for foolish seedling of rice or bakane disease) observed that the fungus caused excessive growth in rice. He applied the fungal extracts to intact healthy plants and observed enhanced growth. Later Yabuta and Sumuki (1938) named the active principle as gibberellin. Further it was purified, crystallized and named as gibberellic acid (Curtis and Cross 1954). Now gibberellins are designated as GA_1, GA_2 and so on. The common gibberellic acid is GA_3. At present 112 types of gibberellins are known.

Occurrence and Site of synthesis:

Gibberellins are synthesized in the young leaves (major site), shoot tip, root tip and the developing seeds.

Biosynthesis:

Acetyl Co-A is the precursor for the biosynthesis of gibberellins. Three molecules of Acetyl Co-A are linked together to form a molecule of mevalonic acid. Mevalonic acid in turn is activated in the presence of ATP, Mn and Mg and is converted to isopentenyl phosphate (IPP). This is a 5 carbon compound. Four molecules of IPP undergo stepwise condensation, first to Trans geranyl pyrophosphate (GPP) then to trans farnesyl pyrophosphate (FPP) and finally to form a diterpene called geranyl geranyl pyrophosphate (GGPP). This is 20 carbon compound. This GGPP is converted to kaurene. The conversion of GGPP to kaurene is carried out by the enzyme kaurene synthetase in two steps.

First GGPP is converted to copalyl pyrophosphate. In the second step copalyl pyrophosphate in turn is converted to kaurene. Kaurene undergoes oxidation and a series of reactions resulting in the formation of gibberellins.

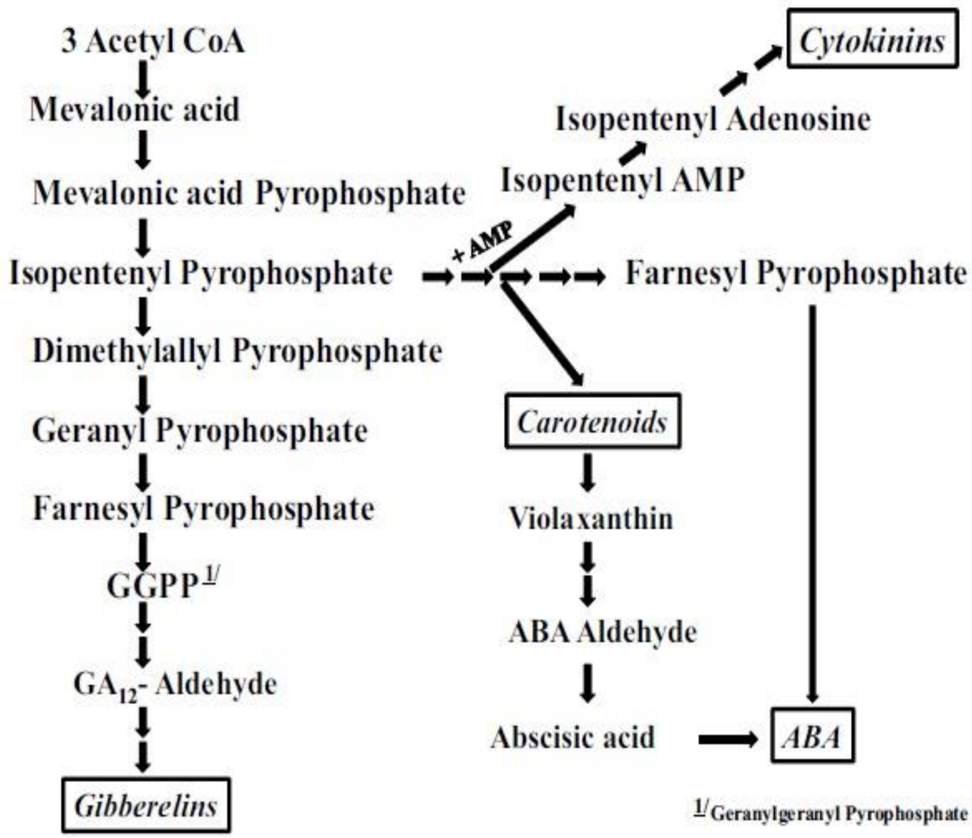


Figure 2 - Mevalonic acid via for the biosynthesis of gibberellins, cytokinins, and abscisic acid.

Transport :

Transport of gibberellins is passive and non-polar. Gibberellins move both in xylem and phloem and vice versa through vascular cells.

Mode of action :

There are several hypotheses to explain the mechanism of GA in the plants, in which the most important is:

1. Increase in the endogenous auxin content:

Whenever GA causes cell enlargement, the effect is not considered to be direct. The effect is indirectly mediated through formation of auxin, in turns is held responsible for the cell elongation.

Gibberellin has been shown to cause synthesis of amylase in barley aleurone cells. This enzyme converts starch to reducing sugars resulting in an increase of osmotic pressure, causing entry of water into the cells and cell enlargement.

Physiological effects :

A. Stimulation of stem growth:

The most important effect of GA is the stem elongation. When GA is applied the stem elongates markedly. As a result, such plants grow taller. GA caused stem elongation, has the following characteristic features (1) enhanced stem growth is not due to increased formation of nodes and internodes but results from rapid elongation of internodes. Therefore, GA treated plants do not differ from control plants in the number of nodes and internodes. Elongation of internodes is due to both cell division and cell elongation. Younger internodes respond better than older ones and plants grown in light respond better to GA than those grown in the dark. However, not all plants respond equally to GA application. It is only the genetic dwarf and rosette plants which show marked stem elongation.

Genetic dwarfs: From the field grown maize four mutants were isolated. These mutants grow only up to about one fourth the heights of normal tall varieties. When gibberellin is applied, the dwarf plants respond readily and show marked stem elongation. These become comparable in height to corresponding tall varieties. Here the degree of stem elongation is proportional to the concentration of GA that is applied.

B. Bolting:

Production of floral axis is called bolting. Bolting and flowering are induced normally after photo induction or vernalisation. Bolting however can be induced without vernalisation by the treatment of the plant with gibberellins.

Many plants require a period of low temperature for flowering. Application of GA replaces the vernalization (0-5^{0}C) requirement for the flowering of carrot, beetroot, chicory and others. Vernalization or low temperature requirement is usually met with when the plants pass through natural winter. However, this low temperature requirement can be completely overcome, and plants can be made to flower in high temperatures by applying GA. Therefore, low temperature requirement of plants can be replaced with GA.

C. Flowering in long day plants:

Gibberellins promote flowering in long day plants under unfavorable SD conditions. Ex: Niger.

D. Parthenocarpic fruits:

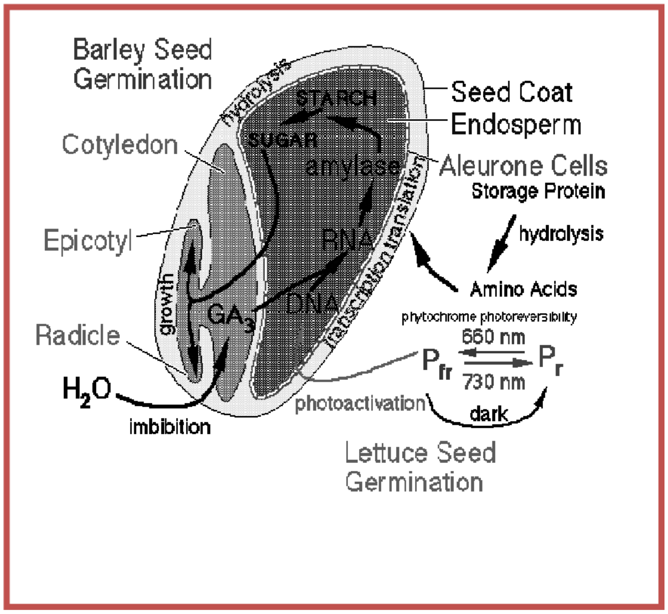
Gibberellins have been found to be more effective than auxins in causing parthenocarpic development of fruits in plants like tomatoes, apples, pears and stone fruits. Gibberellin application promotes panicle exertion. Generally, 30% of the panicle is covered by leaf sheath. Application of GA + Brassinosteroids is practically used in commercial seed production of Rice.

E. Breaking of dormancy:

Gibberellins are effective in breaking the dormancy in potato tubers and in tree buds in winter. In potato the tubers remain dormant for weeks after harvest. However, when GA is applied the buds sprout soon after the tubers are harvested. This will be useful to use the freshly harvested tuber for sowing. The seed material has to be dipped in 0.5 to 1.0 g of GA/lit of water.

Role of GA in germination of seeds :

Gibberellin has been shown to cause synthesis of amylase in barley aleurone cells. This enzyme converts starch to reducing sugars resulting in an increase of osmotic pressure, causing entry of water into the cells and cell enlargement. GA3 is also known to increase permeability of aleurone cells to sucrose. It increases the activity of membrane synthesizing enzymes. Synthesis of phospholipids is also increases due to GA application. The gearing up of all these metabolic activities results in cell elongation.



3. **Cytokinins:**

Skoog and his coworkers discovered cytokinins when they were trying to identify a compound to initiate and sustain the proliferation of cultured tobacco pith tissue. Crystals of a cell division inducing substance was later isolated for the first time by Miller, from an autoclaved herring sperm DNA in 1951 and named it as Kinetin. The liquid endosperm of coconut (coconut milk) is also found to be rich in cell division causing factors. Letham (1963) extracted, purified and crystallized cytokinin from immature kernels of maize and named it as zeatin.

Occurrence:

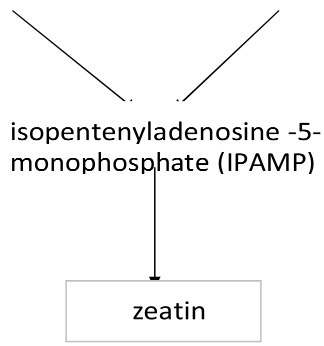
Naturally occurring cytokinins are N^6- substituted adenine derivatives. Usually Zeatin is the most abundant naturally occurring free cytokinin. There are also synthetic cytokinin compounds that have not been identified in plants, most notably of which are the diphenyl urea type cytokinins, such as thidiazuron, which is used commercially as a defoliant and an herbicide.

Cytokinins, occur freely and also as a component of RNA of plants, microorganisms and animals. In higher plants root tips, shoot tips, developing fruits, xylem sap and germinating seeds are rich sources of cytokinins. Root tips synthesize cytokinins and transport them through the xylem to all parts of the plants. This might explain their accumulation in young leaves, fruits and seeds in to which xylem transport occurs.

Biosynthesis:

The biosynthetic pathway of free cytokines is not completely understood. There are two methods in which they may be produced. The first is the direct pathway, involving formation of Isopentenyladenosine-5-monophosphate (IPAMP) from AMP and dimethylallyl pyrophosphate (DMAPP), to form zeatin-type compounds.

AMP (Adinosine mono phosphate) Dimethylallyl pyrophosphate (DMAPP)



Another possibility is that they may be released by the hydrolysis of tRNA, first to mono nucleotides and then to free cytokinins. In spite of extensive effort having been focused on these pathways information is still highly fragmented.

Transport:

When cytokinin is applied to leaves and stems, the hormone does not move and the effect is localized. Cytokinin is carried passively along the transpiration stream in xylem from root. It moves in phloem in a basipetally polar direction in very small quantities.

Mode of action:

Cytokinin is a structural component of transfer RNA molecule. They may help in binding of mRNA with tRNA - amino acid complex during protein synthesis. Cytokinins increase the synthesis of nucleic acid by increasing the enzyme tRNA synthetase and decrease the degradation by reducing the activity of ribonuclease. Cytokinin increases the incorporation of phosphorous in to nucleic acids and adenine into RNA.

Physiological role:

1. Cell division

Cytokinins are known to be regulators of cell division in mature cells. The most important effect of cytokinins is stimulation of cell division in excised tissues. The number of cell divisions increases proportionally to the concentration of added cytokinin when auxin is not limiting. Cytokinins alone does not promote cell division. When both auxin and cytokinins are added together, cells divide rapidly, and the callus tissue grows.

2. Morphogenesis (Root and bud differentiation)

Cytokinins in interaction with auxins control morphogenesis. The cells of tobacco pith do not either grow or differentiate when only auxin or only cytokinin is added to the medium. However, when the medium contains both auxin and kinetin in the ratio of 10:1 pith cells grow and forms a mass of unorganized cells (callus).

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.

3. Anti Senescence hormone (Richmond - Lang effect)

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 delay of senescence of leaves and other organs of the plants by cytokinins is called as Richmond - Lang effect.

In an experiment, one of the two primarily opposite leaves of a bean plant was treated with Benzyl adenine. This treatment accelerated senescence of untreated leaf. This is because of mobilization of organic metabolites and minerals from untreated leaf to cytokinin treated leaf because of cytokinin acts as mobilizing centers.

Retardation of senescence of vegetables can be achieved by cytokinins. Green vegetables like cabbage, lettuce and celery deteriorate rapidly after harvest. Post-harvest spray of Benzyl adenine at 10 to 40 ppm or post-harvest dip of 10 ppm increase shelf life of these vegetables.

4. Promotion of lateral bud growth

Application of cytotinins 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* causes a 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.

5. Breaking of dormancy

Cytokinins can replace the red light (660 nm) requirement in seed germination of lettuce and tobacco. Lettuce seeds require the presence of red light for germination in addition to moisture, air and suitable temperature. However, the seeds can be made to germinate in the dark by applying Kinetin. Thus, Kinetin replaces the red-light requirement for germination.

In cocklebur, each fruit (but) contains two seeds which are of unequal in size. The lower one in largest and germinates while the upper seed is dormant. Here the dormancy is due to the presence of germination inhibitors. This dormancy is overcome by the application of kinetin.

6. Cell enlargement

Cortical cells of tobacco root were observed to enlarge four times of their normal size in the presence of kinetin.

4. Abscisic Acid (stress hormone) :

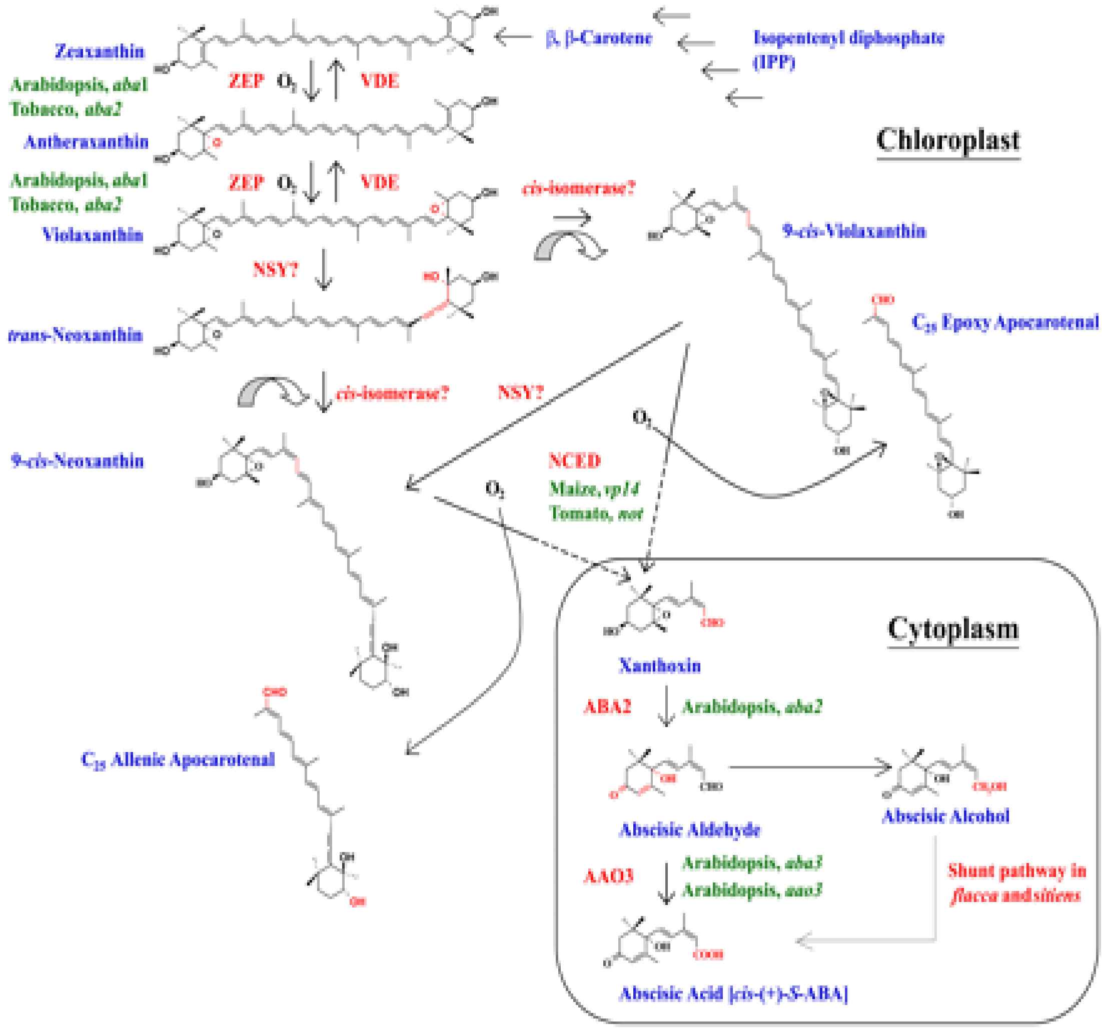
Occurrence:

The plant growth regulator ABA is one of the widespread and naturally occurring inhibitor found in plants. Addicot and his colleagues (1964) isolated this abscission causing compound from cotton bolls and named it as abscisin I & abscisin II. It is now known that ethylene is the hormone that triggers abscission and that ABA induced abscission of cotton bolls is due to ABA's ability to stimulate ethylene production. In higher plants ABA occurs in all parts of the plant body. It has been reported from the leaves of birch (Betula sps) and tubers of potato. ABA is found in all parts of the seed namely the seed coat, embryonic axis, cotyledons and endosperm.

Biosynthesis:

It is a sesquiterpenoid (15-carbon) which is partially produced via the mevalonic pathway in chloroplasts and other plastids.

ABA is synthesis in plants involves two pathways (1) carotenoid pathway
(2) Mevalonic acid pathway or Isoprenoid pathway.



Site of synthesis :

All parts of the plants such as stem, root and leaves. Fruits and seeds are also capable of ABA synthesis.

Transport :

ABA is transported by both the xylem and phloem, but it is normally much more abundant in the phloem sap.

Mode of action :

ABA is involved in the short term physiological effects (e.g. stomatal closure), as well as long term developmental processes (e.g. seed maturation). Rapid physiological responses of ABA frequently involve alteration in the fluxes of ions across membranes and may involve some gene regulation as well.

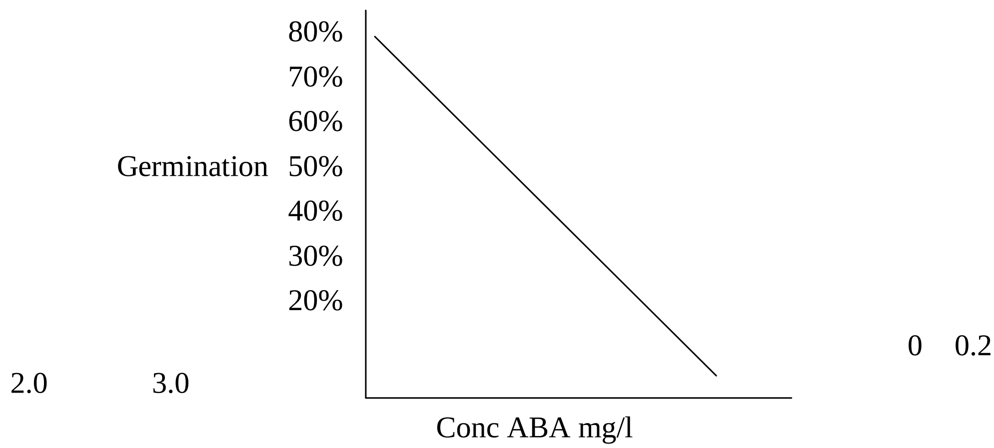
Mode of action of ABA in causing various physiological effects can be seen in three different ways:

1) ABA brings about changes in membrane permeability for different ions (Like K^+, Ca^{++} etc.). It plays a role in stomatal closure.
2) ABA inhibits DNA & RNA synthesis (transcription) finally leads to senescence.
3) It inhibits translation (Protein synthesis) thus formation of enzymes is blocked.
4) Germination process is affected ultimately leads to dormancy.

Physiological role :

A. Seed dormancy:

Application of ABA inhibits seed germination in several species.



ABA concentration

Similarly seeds which are dormant are shown to contain ABA. Seeds of apple remain dormant and fail to germinate till they are exposed to a period of stratification. such seed show the presence of ABA. When the seeds are stratified the ABA content falls with a corresponding increase in GA content. Thus, it can be concluded that the seed dormancy is controlled by GA-ABA balance at least in some species.

ABA helps in inhibiting precocious germination and vivipary. This is very important because dormancy caused by ABA do not allow the seed to germinate while it is still on its mother plant.

B. Bud dormancy

In woody species, dormancy is an important adoptive feature in cold climates. When a tree is exposed to very low temperatures in winter it protects its meristems with bud scales and temporarily stops bud growth. Bud dormancy is seen in Acer, betula and other temperate tree sps. This is accompanied by buildup of ABA within these plants.

C. Effect of stomata

Application of ABA causes rapid closure of stomata. The stomatal aperture progressively decreases with the concentration of ABA.

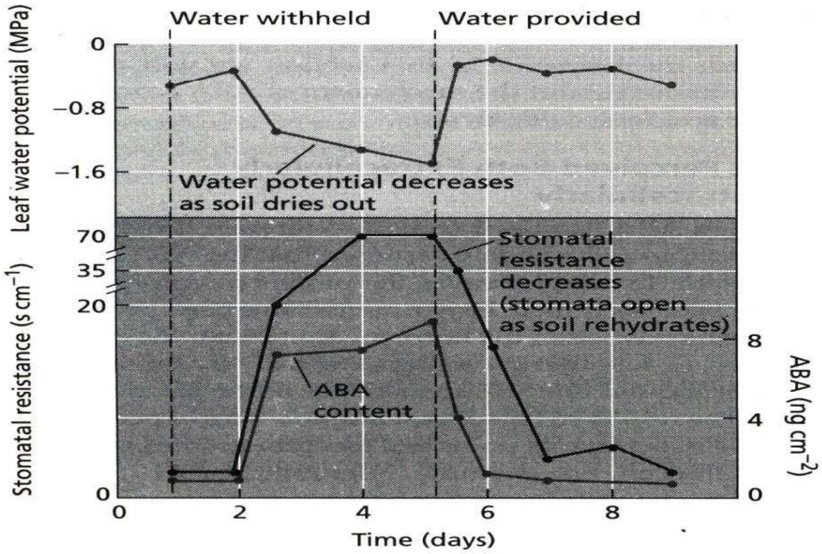


FIGURE Changes in water potential, stomatal resistance (the inverse of stomatal conductance), and ABA content in maize in response to water stress. As the soil dried out, the water potential of the leaf decreased, and the ABA content and stomatal resistance increased. The process was reversed by rewatering. (After Beardsell and Cohen 1975.)

Reference: Lincoln Taiz and Eduardo Zeiger 2006, Plant Physiology, Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.

ABA accumulates in higher concentration in wilting leaves. This accumulated ABA closes stomata. ABA might inhibit the formation of enzymes which are responsible for the conversion of starch into sugar and formation of organic acids. It reduces the osmotic concentration and causes closure of stomata.

D. Senescence

ABA is quite effective in promoting senescence of excised leaf discs of both monocots and dicots. Although leaf discs are affected, when sprayed on the corresponding intact leaf, ABA is not effective even at higher doses in inducing senescence.

E. Flower initiation

ABA induces flowering in SD plants and inhibits the same in LD plants. Here the hormone inhibits vegetative growth and causes apical bud dormancy. In such plants onset of dormancy precedes flowering. Therefore, effect of ABA on flowering is indirect.

F. Antagonism

ABA inhibits GA stimulated growth in various forms. Therefore ABA is known as Antigibberellin.

5. Ethylene :

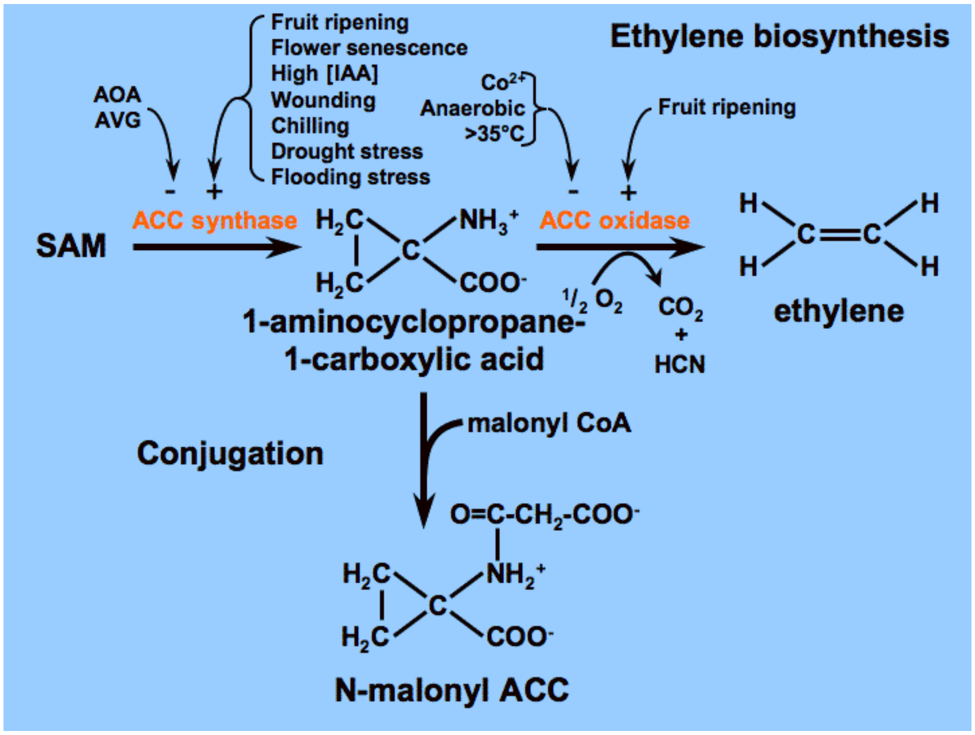
Neljubow (1901) a Russian plant physiologist was the first to show the importance of ethylene present in the illuminating gas as a growth regulator of plants. He observed that dark grown pea seed lings growing in the laboratory (illuminated with coal gas) exhibited symptoms that were later termed as triple response: reduced stem elongation, increased lateral growth, and abnormal horizontal growth. Denny (1924), reported that ethylene is highly effective in inducing fruit ripening. Gane (1934) established that ethylene is produced by the apple fruits during ripening in storage.

Occurrence :

In higher plants, all most the parts of the plant body produce ethylene. In general, meristematic regions and nodal regions are most active in ethylene biosynthesis. However, ethylene production also increases during leaf abscission and flower senescence, as well as during fruit ripening. This is otherwise called as phytogerontological hormone.

Biosynthesis :

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. Methionine activated by ATP gives rise to S-adenosyl Methionine (SAM). This reaction is mediated by enzyme Methionione adenosyl transferase. In the next step, SAM breaks into 5'-methyl thio adenosine (MTA) and amino cyclo propane carboxylic acid (ACC). This reaction is carried by the enzyme ACC synthase. ACC is oxidized to ethylene with the release of HCN (Hydrogen cyanide) and CO_2.



Amino ethoxy vinyl glycine (AVG), and Amino oxy acetic acid (AOA) block the conversion of SAM to ACC. Silver Nitrate and Silver thiosulphate are the specific inhibitors of ethylene action.

Ethylene Transport :

Ethylene being a gas can easily diffuse into plant tissues through the intercellular spaces. From ripening fruits, ethylene diffuses out into the atmosphere through the cut end of pedicel and fruit surface.

Mode of action:

There are several theories to explain the mechanism of action of ethylene. Amongst them most important are:

1. Membrane permeability: Ethylene is considered to dissolve in cell membranes altering their permeability. Ethylene is highly soluble in lipids which are constituents of cell membranes.
2. Another hypothesis is by regulating auxin metabolism. Ethylene treatment results in a reduction in the content of diffusible (free) auxin. This may result from (1) decreased synthesis (2) decreased transport and (3) increased binding. Ethylene has been shown to inhibit transport of auxin from the site of production to the site of action.

Physiological roles (Includes both positive and negative effects) :

1. Fruit ripening :

   Broadly fruits can be 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 sharp decline.

   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.

2. Abscission and senescence :

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

3. Roots on stem cuttings :

   Application of ethylene promotes callus formation and initiation of adventitious roots on the stem cuttings. Sometimes adventitious roots may arise on the stem of intact plant as well. 4. Root and shoot growth

   Ethylene inhibits linear growth of the stem and root of dicots. The effect increases with increasing concentration.

5. Flowering and sex expression:

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

6. 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.

7. Thinning in apple :

   Thinning of fruits in apple eliminates biennial bearing and also improves fruit size and quality. Application of ethephon at 100 to 300 ppm reduces fruit set. In cotton also ethylene induces fruit thinning.

8. Exudation of sap and latex :

When ethereal is applied to rubber plants, flower of latex continues for a longer duration. Etherel probably prevents coagulation of latex and consequent blocking of laticiterious ducts.

Plant growth regulators :

Novel plant growth regulators :

1. **Jasmonates:**

   Jasmonic acid (JA) and its methyl ester (MEJA) occur in several plants and also in the oil of jasmine.

   Physiological roles:

   - JA and MeJA inhibit the germination of non-dormant seeds and stimulate the germination of dormant seeds.
   - JA plays a role in the formation of flowers, fruit and seed. It is suggested by the relatively high levels of this compound in developing plant reproductive tissues.
   - Reported a role in insect and disease resistance of plant.
   - JA stimulates tomato and apple fruit ripening.

2. **BRASSINOSTEROIDS (brs):**

   Brassinolide, a potent plant growth stimulator, was the first BR isolated; it was discovered in rape (*Brassica napus*) pollen in 1979. So far, more than 40 brassinolide analogues, collectively known as brassinosteroids, have been identified and characterized from many different plant species.

   - Immature seeds and pollen contain the highest concentrations of brassinosteroids.
   - When applied exogenously to intact plants at micromaolar or nanomolar concentrations BRs can induce a variety of physiological responses, including seed germination, pollen tube growth, stem elongation, leaf unrolling and bending, vascular differentiation, induction of ethylene biosynthesis, altered gene expression, and stress response modulation. They help to Increase flowering fruit set and yield.
   - Foliar spray of 0.3 ppm BR at panicle initiation and flowering stage increases yield in rice

3. **Salicylic Acid:**

   Salicylic or ortho hydroxy benzoic acid belongs to a diverse group of plant phenolics. The interest in salicylic acid began with the discovery that this compound is a natural trigger for the metabolic explosion which raises the temperature of the thermogenic inflorescences of Arum Lillie.

   - SA increase flower longevity by inhibiting ethylene biosynthesis by blocking the conversion of ACC to ethylene.

- SA regulates some aspects of disease resistance in plants called SAR(Systemic acquired resistance).

4. **Triacontanol:**

Saturated primary alchohol isolated from shoots of alfalfa. Response is very rapid in increasing growth. Enhanced growth in rice and maize is reported.

Growth Retardants :

The term growth retardant refers to the chemicals that slow down cell division and cell elongation of shoot tissue and regulate plant height physiologically without formative effects. They do not occur naturally in plants.

Examples: AMO 1618,

CCC (Chloro choline chloride) (2-Chloroethyl–trimethyl ammonium chloride)

Chlormequat chloride (Cycocel)

Alar or B9 paclobutrazol
Mepiquat chloride

Growth Inhibitors :

Growth inhibitors suppress the growth of plants. ABA and ethylene are called as natural growth inhibitors. They bring about certain formative changes in plants. There are synthetic growth inhibitors also. Examples: Malichydrazide (MH)

2,3,5-T or Triiodo benzoic acid (TIBA)

Commercial application of Plant growth regulators in Agriculture and Horticulture:

A) **Auxins**

a) IBA (@250 ppm) and NAA were found to increase root development in the propagation of stem cuttings.
b) 2,4-dichlorophenoxy acetic acid (2,4-D) stimulates excessive uncontrolled growth in broad leaf plants for which it is used as a herbicide.
c) Application of NAA (Napthalene Acetic Acid) reduces flower and fruit drop in Mango.
d) NAA application brings uniform flowering and fruit set by inducing ethylene formation in Pineapple.
e) NAA application @ 10-100 ppm during fruit setting period controls boll shedding in cotton crop.

B) Gibberellins:

a) GA is used extensively on seedless grape varieties to increase the size and quality of the fruit. Pre- bloom spray of 20 ppm induces rachis of the fruit cluster to elongate. This creates looser clusters that are less susceptible to disease during the growing season.

b) GA is used to increase the yield of barley malt and to decrease the time required for this process to occur. Application of GA to germinating barley supplements the endogenous content of this hormone and accelerates the production and release of hydrolytic enzymes. They can easily degrade the stored carbohydrates.

c) Foliar spray of GA_3, at 100 ppm during panicle initiation stage enhances the panicle exertion and increases seed weight and yield in hybrid rice.

d) GA has also has been used to control flower sex expression in cucumbers and squash. GA application tends to promote maleness in these plants.

e) Gibberellic acid is also applied to citrus crops, though the actual use depends on the particular crop. For example GA_3 is sprayed onto oranges and tangerines to delay or prevent rind-aging, so that fruit can be harvested later without adverse effects on rind quality and appearance. For lemons and limes, GA_3 synchronizes ripening and enhances fruit size.

f) Gibberellic acid is used extensively to increase the sucrose yield of sugarcane. Sugarcane, a normally fast-growing C_4 member of the Poaceae (grass) family, is sensitive to cooler winter temperatures, which reduce internode elongation and subsequent sucrose yield. The adverse effects of cooler temperatures can be counteracted by the application of GA_3.

C) Ethylene:

a) Because ethylene regulates so many physiological processes in the plant development, it is the most widely used plant hormones in agriculture. Auxins and ACC can trigger the natural biosynthesis of ethylene and in several cases are used in agricultural practice.

b) Because of its high diffusion rate, ethylene is very difficult to apply in the field as a gas, but this limitation can be overcome if an ethylene releasing compound is used. The most widely used such compound is ethephon or 2chloro ethyl phosphonic acid (CEPA) (trade name: ethrel).

c) Ethrel @ 100-250 ppm sprayed at 2-3 leaf stage induce femaleness in cucumber and melons.

d) It helps in degreening of citrus and banana which increases its market acceptability.

e) Storage facilities developed to inhibit the ethylene production and promote preservation of fruits have a controlled atmosphere of low 0_2 concentration and low temperature that inhibits ethylene biosynthesis. A relatively concentration of CO_2 (3-5%) prevents ethylene action as a ripening promoter.

D) Other growth regulators:

- AMO 1618 (a quaternary ammonium salt) is used in the cultivation of ornamental plants and causes a bushy shape and a sturdy growth of the treated plants.

- paclobutrazol: Reduces the problem of biennial bearing in Mango.
- Mepiquat chloride, Chlormequat chloride (Cycocel) : used in ornamental plants for shorter internodes and thicker stems (used in poinsettias), it also prevents lodging and increases tillering in cereals.
- Malichydrazide (MH): prevents premature sprouting of onion and potato.
- 2,3,5-T or Triiodo benzoic acid (TIBA): Increases flowering in chrysanthemum.

Chapter-9

Physiological aspects of growth and development of major crops: Growth analysis, Role of Physiological growth parameters in crop productivity

Growth and Development :

Growth and development are the most fundamental and conspicuous characteristics of all living organisms. According to dictionary, growth is the advancement towards maturity and development is a gradual increase in size. The plant physiological definition of growth is ‘an irreversible increase in mass, weight or volume of a living organism, organ or cell.

Physiological aspects of growth and development :

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 cellulose organelles.

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 must pass through the following 3 phases:

1. Cell formation phase
2. Cell elongation phase
3. 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 with or without 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 large vacuoles. In the last phase or cell maturation, secondary walls are laid down and cell matures and gets differentiated into permanent tissue.

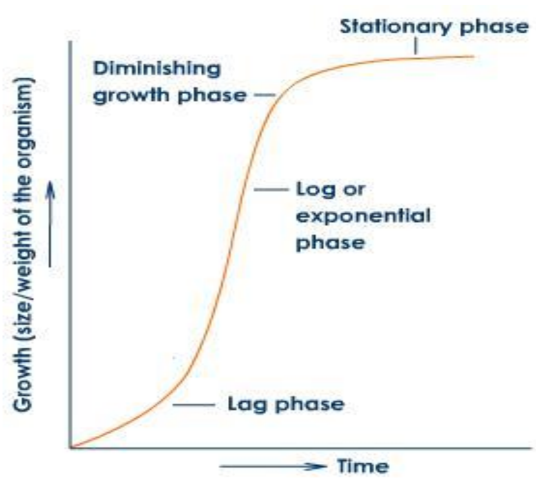
Growth curve :

Typical growth pattern of an annual plant is divided into three phases.

1. Lag period of growth: during this period the growth rate is quite slow because it is the initial stage of growth.

2. Log period of growth: during this period, the growth rate is maximum and reaches the top because at this stage the cell division and physiological processes are quite fast.

3. Senescence period or steady state period: during this period the growth is almost complete and become static. Thus, the growth rate becomes zero.



Growth and development :

Measurement of growth: growth can be measured by a variety of parameters as follows

A. Fresh weight:

Determination of fresh weight is an easy and convenient method of measuring growth. For measuring fresh weight, the entire plant is harvested, cleaned for dirt particles if any and then weighed.

B. Dry weight:

The dry weight of the plant organs is usually obtained by drying the materials for 21 to 48 h at 70 to 80°c and then weighing it. The measurements of dry weight may give a more valid and meaningful estimation of growth than fresh weight.

However, in measuring the growth of dark grown seedling it is desirable to take fresh weight.

C. Length:

Measurement of length is a suitable indication of growth for those organs which grow in one direction with almost uniform diameter such as roots and shoots.

The length can be measured by a scale. The advantage of measuring length is that it can be done on the same organ over a period of time without destroying it.

D. Area: it is used for measuring growth of plant organs like leaf. The area can be measured by a graph paper or by a suitable mechanical device. Nowadays modern laboratories use a photoelectric device (digital leaf area meter) which reads leaf area directly as the individual leaves is fed into it.

Growth analysis:

Growth analysis is a mathematical expression of environmental effects on growth and development of crop plants. This is a useful tool in studying the complex interactions between the plant growth and the environment. Growth analysis in crop plants was first studied by british scientists (Blackman, 1919; Briggs, Kidd and West, 1920; William; 1964; Watson; 1952; Blackman, 1968). This analysis depends mainly on primary values (dry weights) and they can be easily obtained without great demand on modern laboratory equipment.

The basic principle that underlie in growth analysis depends on two values (1) total dry weight of whole plant material per unit area of ground (w) and (2) the total leaf area of the plant per unit area of ground (a).

The total dry weight (w) is usually measured as the dry weight of various plant parts *viz.*, leaves, stems and reproductive structures. The measure of leaf area (a) includes the area of other organs *viz.*, stem petioles, flower bracts, awns and pods that contain chlorophyll and contribute substantially to the overall photosynthesis of the plants.

According to the purpose of the data, leaf area and dry weights of component plant parts have to be collected at weekly, fortnightly or monthly intervals. These data are to be used to calculate various indices and characteristics that describe the growth of plants and of their parts grown in different environments and the relationship between assimilatory apparatus and dry matter production. These indices and characteristics are together called as growth parameters. Some of the parameters that are usually calculated in growth analysis are crop growth rate (CGR), relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), leaf weight ratio (LWR), Specific leaf area (SLA), leaf area index (LAI) and leaf area duration (LAD). Accuracy in calculations of these parameters and their correct interpretation are essential aspect in growth analysis.

Advantages of growth analysis :

A) We can study the growth of the population or plant community in a precise way with the availability of raw data on different growth parameters.

B) These studies involve an assessment of the primary production of vegetation in the field i.e. at the ecosystem level (at crop level) of organization.

C) The primary production plays an important role in the energetics of the whole ecosystem.

D) The studies also provide precise information on the nature of the plant and environment interaction in a particular habitat.

E) It provides accurate measurements of whole plant growth performance in an integrated manner at different intervals of time.

Drawbacks of growth analysis

In classical growth analysis sampling for primary values consist of harvesting (destructively) representative sets of plants or plots and it is impossible to follow the same plants or plots through out whole experiment.

Growth characteristics - definition and mathematical formulae

The following data are required to calculate different growth parameters in order to express the instantaneous values and mean values over a time interval. In the following discussion w, w_l, w_s and w_r are used to represent the dry weights of total plant (w), dry leaves (w_l), stem (w_s) and roots (w_r) respectively. Whereas a is the leaf area and p is the land area.

1. Crop growth rate (CGR) :

D.j. watson coined the term crop growth rate. It is defined as the increase of dry matter in grams per unit area per unit time. The mean CGR over an interval of time t_1 and t_2 is usually calculated as show in the following formula

$$CGR = \frac{1}{p} \times \frac{w_2 - w_1}{t_2 - t_1} \ (g\ m^{-2}\ day^{-1})$$

Where CGR is the mean crop growth rate, w_1 and w_2 are the dry weights at two sampling times t_1 and t_2 respectively.

2. Relative growth rate (RGR):

The term RGR was coined by Blackman. It is defined as the rate of increase in dry matter per unit of dry matter already present. This is also referred as 'efficiency index' since the rate of growth is expressed as the rate of interest on the capital. It provides a valuable overall index of plant growth. The mean relative growth rate over a time interval is given below

$$RGR = \frac{\log_e w_2 - \log_e w_1}{t_2 - t_1} \quad (g\ g^{-1} day^{-1})$$

3. Net assimilation rate (NAR):

The NAR is a measure of the amount of photosynthetic product going into plant material i.e. it is the estimate of net photosynthetic carbon assimilated by photosynthesis minus the carbon lost by respiration. The NAR can be determined by measuring plant dry weight and leaf area periodically during growth and is commonly reported as grams of dry weight increase per square centimeter of leaf surface per week. This is also called as unit leaf rate because the assimilatory area includes only the active leaf area in measuring the rate of dry matter production.

The mean NAR over a time interval from t_1 to t_2 is given by

$$NAR = \frac{w_2 - w_1}{t_2 - t_1} \times \frac{\log_e a_2 - \log_e a_1}{a_2 - a_1} \quad (gcm^{-2}wk^{-1})$$

4. Leaf area ratio (LAR):

The LAR is a measure of the proportion of the plant which is engaged in photosynthetic process. It gives the relative size of the assimilatory apparatus. It is also called as capacity factor. It is defined as the ratio between leaf area in square centimeters and total plant dry weight. It represents leafiness character of crop plants on area basis.

$$LAR = \frac{a}{w} \quad (cm^2 g^{-1})$$

5. Leaf weight ratio (LWR):

It is one of the components of LAR and is defined as the ratio between grams of dry matter in leaves and total dry matter in plants (g). Since the numerator and denominator are on dry weight basis LWR is dimensionless. It is the index of leafiness of the plant on weight basis.

$$LWR = \frac{w_l}{w} \text{ (unitless)}$$

6. Specific leaf area (SLA):

It is another component of LAR and defined as the ratio between leaf area in cm^2 and total leaf dry weight in grams. This is used as a measure of leaf density.

The mean SLA can be calculated as

$$SLA = \frac{a}{w_l} \ (cm^2 g^{-1})$$

7. Specific leaf weight (SLW):

The reversal of SLA is called as SLW. It is defined as the ratio between total leaf dry weight in gm and leaf area in cm^2. It indicates the relative thickness of the leaf of different genotypes.

$$SLW = \frac{w_l}{a} \ (g\ cm^{-2})$$

8. Leaf area index (LAI):

D.j. watson coined this term. It is defined as the functional leaf area over unit land area. It represents the ieatiness in relation to land area. At an instant time (t) the LAI can be calculated as

$$LAI = \frac{w_l}{a} \text{ (unit less)}$$

For maximum production of dry matter of most crops, LAI of 4-6 is usually necessary. The leaf area index at which the maximum CGR is recorded is called as ‘optimum leaf area index'.

9. Leaf area duration (LAD):

It is usually expressed as a measure of leaf area integrated over a time period. Some takes into account both the magnitude of leaf area and its persistence in time; it represents the leafiness of the crop growing period. Thus the unit of measurement of lad may be in days or weeks or months.