FUNDAMENTALS OF CROP PHYSIOLOGY

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Chapter 1
SYNOPSIS OF THE COURSE INTRODUCTORY CROP PHYSIOLOGY

Definition:

Crop physiology is the study of physiological changes in crop plant, like movement of water, light, nutrient, CO_2 from external environment to the plant system for producing essential products. Crop physiology tends to understand, describe and explain a process comprises a series of sequential events operating under natural conditions. Some of the most important processes operating in plants are stomatal mechanism; water and mineral absorption; photosynthesis, respiration, etc. A plant physiologist tends to understand, describe and explain such processes.

The natural activity of the cells, tissues, organs and organisms is referred to as their function. These functions are explained at the cellular and molecular levels. In crop physiology, an attempt is also made to study the factors which modify growth and development. In brief, in crop physiology, comprehensive information on the structure, processes and functions operative at the cells, tissues, organs is desired in order to explain the processes of growth and development in an organism.

Indeed the development of these sciences has contributed to the evolution and discovery of several instruments and techniques which helped in the elaboration of plant anatomy. A sound knowledge of plant anatomy is essential for interpreting the functions and processes operating in a plant. Several of the plant processes could only be revealed by the use of sophisticated instruments and techniques provided by the chemical and physical sciences. The availability of the techniques of radioisotopes, anti-metabolites, chromatography, scanning, transmission electron microscopy, and mass spectrometry has helped in the elaboration of several processes. In fact, elaboration of cell structure has considerably aided in understanding plant structure in relation to function. The discovery in plants of lysosomes, peroxisomes and other organelles in recent years is the outcome of combined technology of biochemists, biophysicists and anatomists.

Clearly crop physiology is not a static discipline but shall always change and be enrichened by the discovery and usage of new methodology and instruments. This is also a reason why for one process divergent opinions exist. The observations are dependent upon techniques employed while the interpretations are dependent upon understanding of divergent chemical and physical

phenomena. It is an enlivened science which will change with time and be enriched with more and increased experiences over the years. Take, for instance, the process of photosynthesis and even the discovery of photorespiration or the processes operating in C_4 plants. Since plants possess different patterns and habitats as compared to animals it is important to study plant physiology. Plants are also static and possess the capacity to manufacture their own food. Therefore, they possess the capacity to grow and add cells throughout their life span. The control of plant development is the outcome of action of several hormones, environments and nutritional factors.

Most of these factors interact with each other and cause effective growth of the plant. As compared to animals plants possess special features which point towards their unique physiological characteristics. These have non-motile habit, autotrophy, and dependence on soil for mineral supply; possess several devices in the terrestrial habitat to protect against excessive evaporation, heating and transport of water from the soil to the plant apex. The presence of rigid cell wall, occurrence of circadian rhythms for several metabolic processes, development of devices to overcome drought, frost-injury, also add to the uniqueness of plants. These characteristics are best studied and explained on the basis of physiological organization. A successful plant must have the ability to compete in its environments to its best advantage. Thus, plants have undergone physiological evolution and adaptation to the environments.

Need for the Study of Crop Physiology:

The knowledge of crop physiology will help in forging several advances in agriculture, horticulture, forestry, plant pathology and other disciplines of botany. In fact, researches in plant physiology have been and are likely to contribute immensely to crop improvement. Increase in crop production is based on exploiting maximal levels or plant metabolic processes. The production of new varieties and strains shall have to take into account the physiological attributes of basic material or genotypes. The control of soil fertility, overcoming presence of excessive salts in the soil through the knowledge of plant physiology has helped in increased crop production.

A basic knowledge of plant metabolic processes can help in the increase of photosynthetic conversion of solar energy for the production of food materials that are utilized by human beings. Basic knowledge on nitrogen fixation will help in increased utilization of atmospheric nitrogen by different plant species. In the past few years several tissue culture techniques have been developed which have shortened the life cycle of several plant species, helped in raising plants from seeds with shrunken endosperm, and have increased our understanding of cell wall formation and mineral uptake. The detailed knowledge of plant hormones, their synthesis and mode of action,

has considerably facilitated their application in checking water loss, manipulating growth and development of certain crops and improving the quality of food materials. The usage of certain hormonal weedicides has minimized the occurrence of weeds in the crop fields.

Crop yields have increased by the judicial usage of auxins, cycocel, gibberellins, phenols and aliphatic alcohols, etc. 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. In recent years most of the breeding projects also seek the help of the plant physiologists. Physiological processes play a significant role in several interactions between plant and animals. Man changes his environments deliberately or unintentionally and this change affects the physiological behaviour of plants. The physiological reactions of plants due to their introduction and cultivation are an admitted fact. Increased urbanization, development of industry and excessive utilization of land has led to the modifications of the environments.

As a result of these modifications there is a drastic effect on the accompanying fauna. For successful agricultural practices a sound physiological base is a must. Plant cultivation or agriculture was a crude art or a native effort and plant physiological studies have developed into a regular science. Modern understanding of the physiological mechanisms of growth and development is being increasingly exploited for increased quality and quantity of crops. This insight has also increased the survival and or extending the range of desirable plants. Controlled fertilizer application and proper water management have been exploited to the best advantage of limited resources. Practices as crop rotation, green crop ploughing, usage of selective fertilizers, use of growth hormones, inhibitors, etc., are all based on our understanding of plant physiological concepts.

Recently computer technology has been used in aiding plant growth and manipulating plant responses to varied environments. With the growing population there are greater demands on production of various food crops. Agriculture and agricultural produce are becoming industrialized and the role of plant physiologist is ever increasing. Environmental engineering in all aspects including usage of barren lands, growing increased number of crops, and exploitation of solar radiations by the existing and newly bred species shall involve plant physiologists. In the coming years, teaching and research in crop/plant physiology will occupy a pivotal place in our institutions. The search for deeper understanding and insight of how plants absorb water and minerals, utilize and conserve them will continue with added scientific techniques

Chapter 2
Introduction to crop physiology and its importance in Agriculture

The meaning of Plant Physiology refers to “the science of properties and functions in normal conditions”. The aim of the Plant Physiology has been described as early as the early 20th Century by the Russian Plant Physiologyist, V.I. Palladin as : “Which is to gain a complete and thorough knowledge of all the Phenomena occurring in plants, to analyse complex life processes. So as to interpret them in terms of simpler one and reduce them finally to the principles of physics and chemistry”. Nevertheless, Noggle and fritz (1983) described the Plant Physiology as “the science concerned with processes and functions, the response of plants to changes in environment and the growth and development that results from responses Crop physiology is concerned with the processes and functions of the crops at cellular, sub-cellular and whole plant levels in response to environmental variables and growth. In short, **physiology is the study of functional aspects of crop plants.**

Photosynthesis

Through photosynthesis green plants are able to manufacture their food themselves. Certain plants like maize, sugarcane and sorghum possess C_4 pathway which have higher adaptability to drought, high temperature and high light intensity. Such plants also lacking photorespiration. Therefore, there is a great need to reduce photorespiration through breeding program. The yields of many crops are poor due to poor translocation of photosynthates to the sink. Therefore, there is an urgent need to identify the varieties with better translocation of photosynthates towards reproductive parts.

Mineral nutrition

There is an urgent need to identify the fertilizer requirements, mineral application and proper stage of application. Application of excess nitrogenous fertilizers in cotton resulted in decreased yield. In pulses application of nitrogenous fertilizers at an early stages inhibits the development of nodules leading to poor yield. In later stages, at flowering nodule activity decreases, this is the time for proper application of nitrogen. Through hydroponics plants can be grown without soil. Through foliar application many elements which gets precipitated are made available. There is an urgent need to identify the nodule activity, survival period etc. The efforts are being made to transfer nitrogen fixing genes (nif genes) in cereals also so that cereals could also be able to fix atmospheric nitrogen. It has been identified through physiological research that during pod development the nodule activity is very slow. Therefore, application of nitrogenous fertilizers during this time is a must.

Stress Physiology

Plants absorb large quantities of water and 98% is lost through transpiration. Therefore, there is need to check the excessive loss by using antitranspirants. Efforts are being made to identify the drought resistant varieties. Critical stages have to be identified where maximum loss may occur due to water stress. Plant responses to environmental extremes such as excess or deficiency of water , mineral salts , high and low temperature and atmospheric pollutants have been worked out.

Improvement of plant type

Indeterminate cotton varieties are not suitable for mechanical harvesting. Therefore, by using growth retardants such as CCC such varieties can be made compact which would facilitate

easy harvesting. Lodging in tall varieties of wheat can also be checked by spraying growth retardants. Many tall trees can be grown in pots. In grapes ring of bark (phloem) above soil level is removed which cuts down the translocation of photosynthates to the underground parts. Consequently, the fruits become bigger in size.

Plant growth substances

Use of growth regulators change the plant architecture, make the plants to bloom, hasten the rooting of stem cuttings and ripening of fruits, extend the shelf life of cut flowers, vegetables and fruits.

Rooting of cuttings

Many fruits and timber yielding plants are slow growing when propagated through seeds. Such plants do not resemble the parent trees in vigour and quality of fruits. This problem can be overcome by dipping the stem cuttings in 100 to 500 ppm of synthetic auxins like NAA and IBA. Seridax is a commercially available auxin mixture which is used extensively.

Breaking of dormancy

Seeds of some species of grapes, apple and peach remain dormant and do not germinate till they pass through winter. Soaking of seeds in GA breaks the dormancy of such seeds and such seeds can germinate soon after harvesting.

Inhibition of sprouting

Potato tubers and onion bulbs sprout when stored which leads to loss of weight and deterioration of quality. This can be prevented by treating the tubers with methyl ester of NAA (MENA) which is an auxin and onion bulbs with melic hydrazide (MH).

Controlling the size of the plant

In sugarcane GA is used to increase the length of internode and sugar content. Many ornamentals are grown by using growth retardants like Cycocel, B-Nine and Phosphon D in pots. In tobacco flowering leads to emergence of suckers (short lateral branches) due to this quality of tobacco leaf is deteriorated. This problem can be overcome by using MH.

Promotion of flowering

Application of NAA causes uniform flowering in pineapple leading to development of uniform sized fruits. Recently ethephon (ethrel) is used for this purpose in pineapple orchards. This is also used to increase the number of female flowers and consequently yield. Normally in cucurbits the flowers are unisexual and yield is limited by number of female flowers produced by the plant.

Control of abscission

In apple, mango and others fruit falls before maturity causing reduction in yield. This is prevented by spraying NAA and 2, 4–D which are used to prevent pre harvest fall in citrus fruits.

Fruit development and ripening

Normally ovary after pollination form fruits and ovules seed. Sometimes due to non formation of pollen grains fertilization is failed and ovary withers and falls down. In tomato this can be checked by using Para chloro phenoxy acetic acid (PCPA) .This also helps in fruit setting without fertilization. This is called parthenocarpic fruit development. Artificial ripening in mango, banana and oranges can be induced by spraying the fruits with calcium carbide which releases acetylene and ethephon which releases the ethylene.

Weed control

Synthetic auxin like 2, 4-D (2,4 Dichloro phenoxy acetic acid) when sprayed kills dicot weeds like chenopodium. They act on living cells of vascular ray cells, increase the respiratory rate leading to death of cells.

There are five classes of herbicides.

- Phenoxy compounds Ex. 2,4-D, 2,4-5 T.
- Triazines Ex. Simazine and Atrazine.
- Substituted ureas Ex. Monuron and Diuron.
- Carbonates Ex. Barban.

Tissue culture

Through tissue culture technique plants can be developed from tissues like parenchyma, phloem and pollen in synthetic media. This technique is employed to raise the haploid plants through pollen culture.

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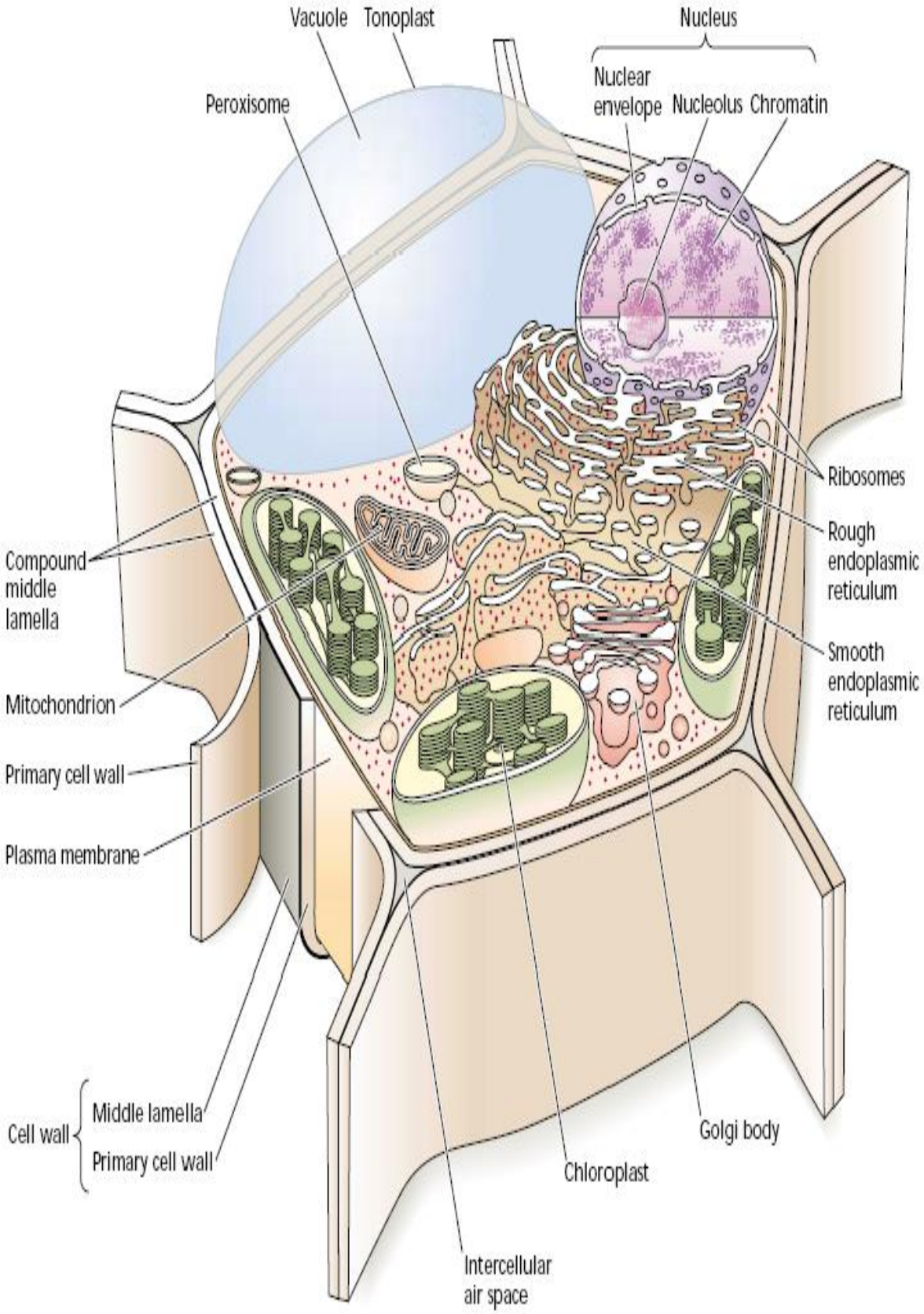
Cell

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

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


Vacuole
Tonoplast
Nucleus
Nuclear envelope
Nucleolus
Chromatin
Peroxisome
Ribosomes
Rough endoplasmic reticulum
Smooth endoplasmic reticulum
Compound middle lamella
Mitochondrion
Primary cell wall
Plasma membrane
Cell wall
Middle lamella
Primary cell wall
Golgi body
Chloroplast
Intercellular air space

2. Diffusion and osmosis

Role and significance of water

Water is said to be the liquid of life. Because, life is originated in organs, environmental and in the course of evolution it became fully dependent upon water in a number of ways. Water is one of the most plentiful chemicals available in the earth and the chemical formula is H_20. It is a tiny V-shaped molecule contains three atoms do not stay together as the hydrogen atoms are constantly exchanging between water molecules

The water molecule consists of an oxygen atom covalently bonded to two hydrogen atoms. The two O—H bondsform an angle of 105°. Because the oxygen atom is more **electronegative** than hydrogen, it tends to attract the electrons of the covalent bond. This attraction results in a partial negative charge at the oxygen end of the molecule and a partial positive charge at each hydrogen. Water has special properties that enable it to act as a solvent and to be readily transported through the body of the plant. These properties derive primarily from the polar structure of the water molecule.

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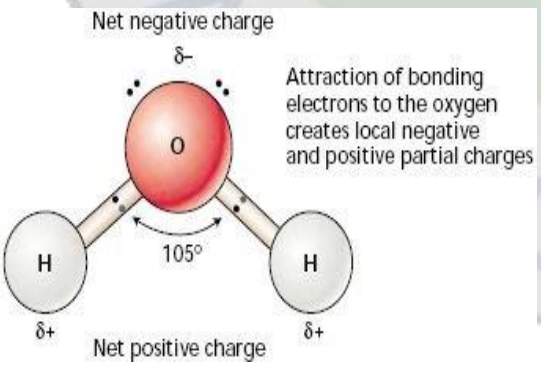
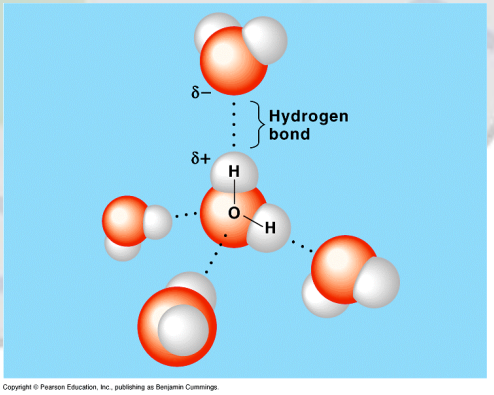
Structure of the water molecule.

- The Polarity of water molecules gives rise to hydrogen bonds
- The Polarity of water makes an excellent solvent
- The Thermal properties of water result from hydrogen bonding
- The Cohesive and adhesive properties of water are due to hydrogen bonding



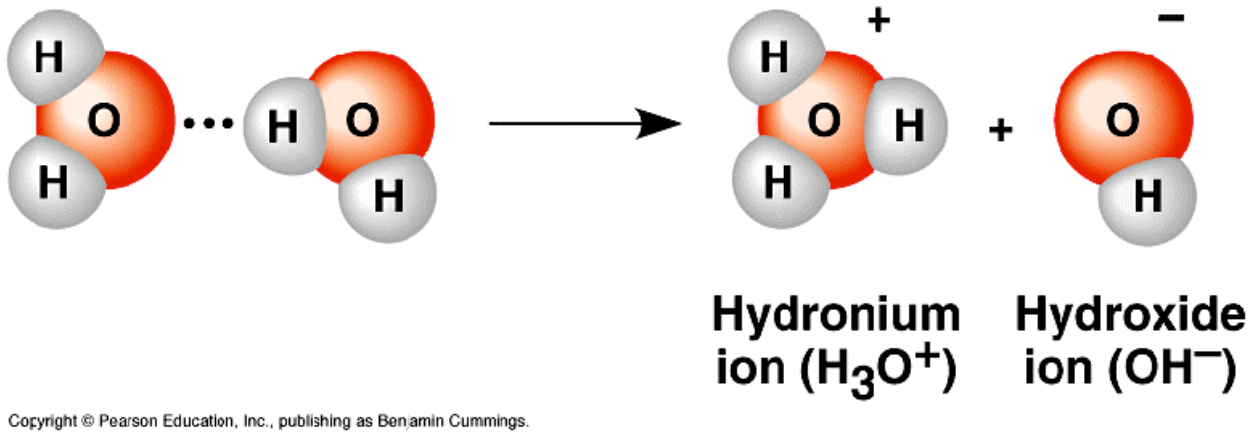


Importance of water to plants

- Water typically constitutes 80 to 95% of the mass of growing plant tissues.
- Water is the main constituent of protoplasm comprising up to about 90-95 per cent of its total weight. In the absence of water, protoplasm becomes inactive and is even killed.

- Different organic constituents of plants such as carbohydrates proteins, nucleic acid and enzymes etc. Lose their physical and chemical properties in the absence
- Water participates directly in many metabolic processes. Inter conversion of carbohydrates and organic acids depend upon hydrolysis and condensation reaction.
- Water increases the rate of respiration. Seeds respire fast in the presence of water.
- Water is the source of hydrogen atom for the reduction of CO_2 in the reaction of
- photosynthesis.
- Water acts as a solvent and acts as a carrier for many substance. If forms the medium
- in which several reactions take place.
- Water present in the vacuoles helps in maintaining the turgidity of the cells which is a must for proper activities of life and to maintain this from and structure.
- Water helps in translocation of solutes
- In tropical plants, water plays a very important role of thermal regulation against high temperature.
- The elongation phase of cell growth depends on absorption of water.

Dissociation of water



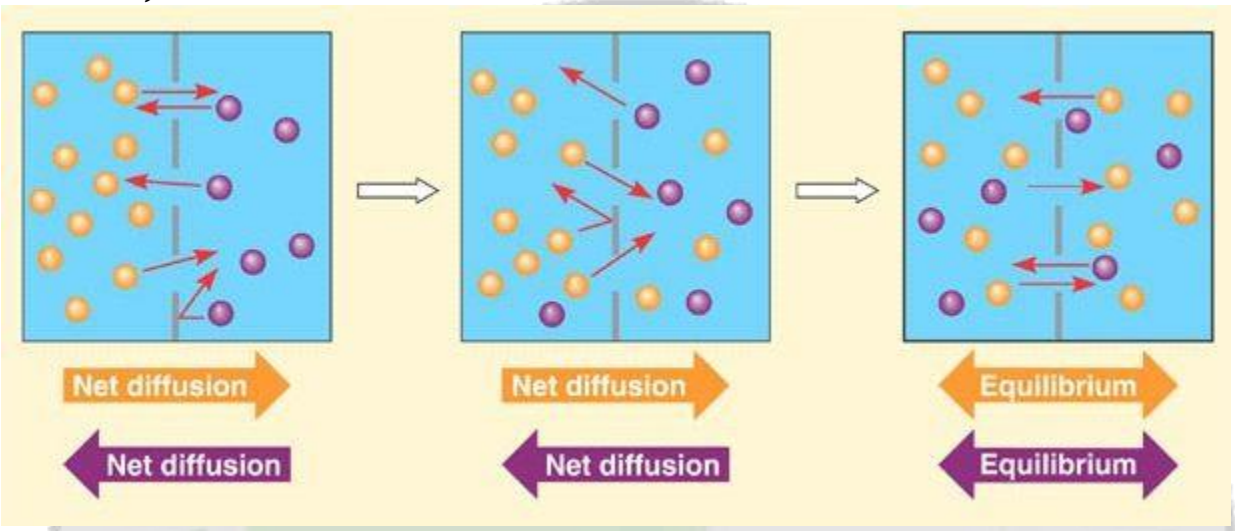
Properties of water

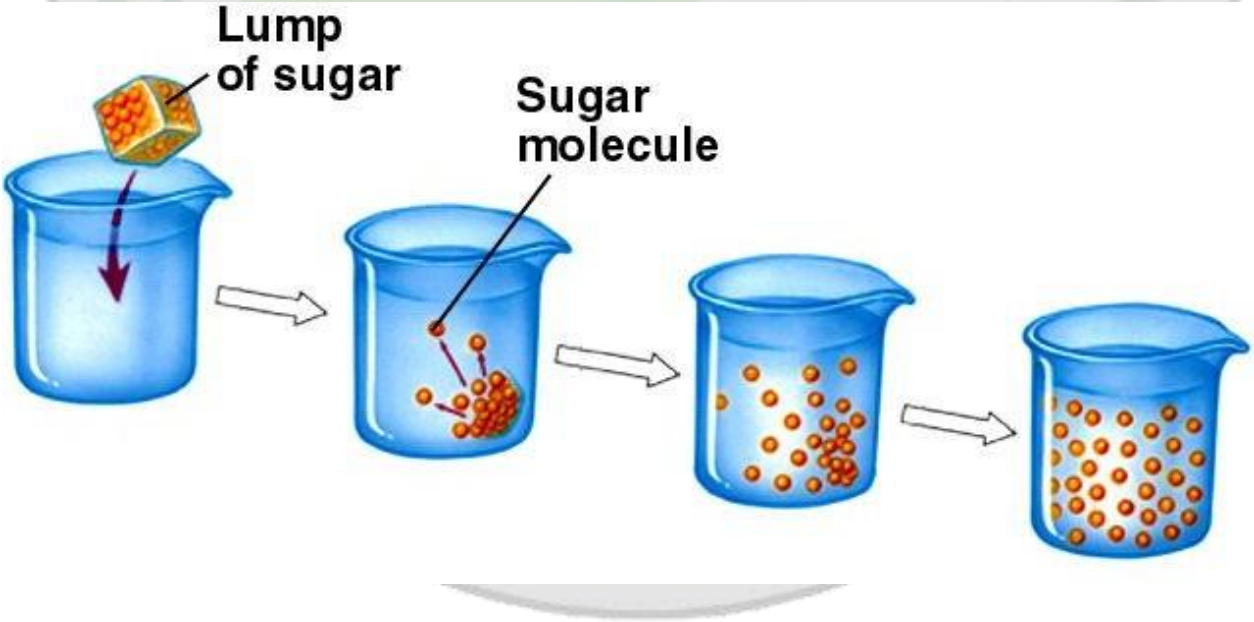
1. Solvent for electrolyte & non electrolyte
2. High specific heat
3. High latent heat of vaporization (540 cal g-1)
4. Cohesive and Adhesive Properties
5. High surface tension
6. High Tensile Strength
7. Stabilizes temperature
8. Transparent to visible radiation
9. Low viscosity

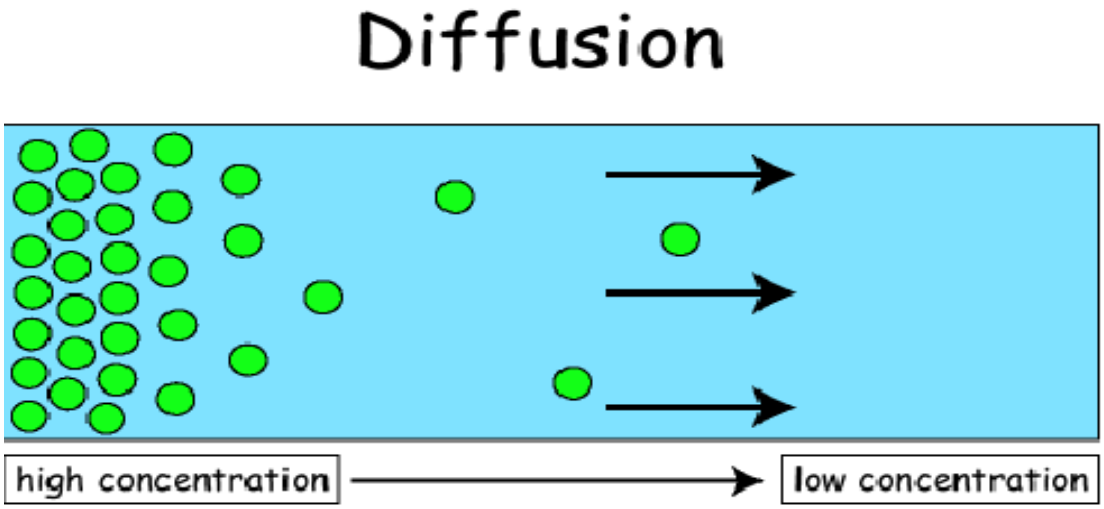
HOW IT HELPS IN PLANTS?

WATER PLAYS A CRUCIAL ROLE in the life of the plant. For every gram of organic matter made by the plant, approximately 500 g of water is absorbed by the roots, transported through the plant body and lost to the atmosphere. Even slight imbalances in this flow of water can cause water deficits and severe malfunctioning of many cellular processes. Thus, every plant must delicately balance its uptake and loss of water.

Diffusion, osmosis and imbibitions







The movement of materials in and out of the cells in plants has taken place in a solution or gaseous form. Although the exert process of this is not very clear, three physical process are usually involved in it. They are diffusion, osmosis and imbibition.

The movement of particles or molecules from a region of higher concentrations to a region of lower concentration is called as diffusion. The rate of diffusion of gases is faster than liquids or solutes. The diffusion particles have a certain pressure called as the diffusion pressure which is directly proportional to the number as concentration of the diffusing particles. These forms the diffusion takes place always from a region of higher diffusion pressure to a region of lower diffusion pressure (i.e) along a diffusion pressure gradient. The rate of diffusion increases if,

i) Diffusion pressure gradient is steeper
ii) Temperature is increased
iii) Density of the differing particles is lesser
iv) Medium through which diffusion occurs is less concentrated.

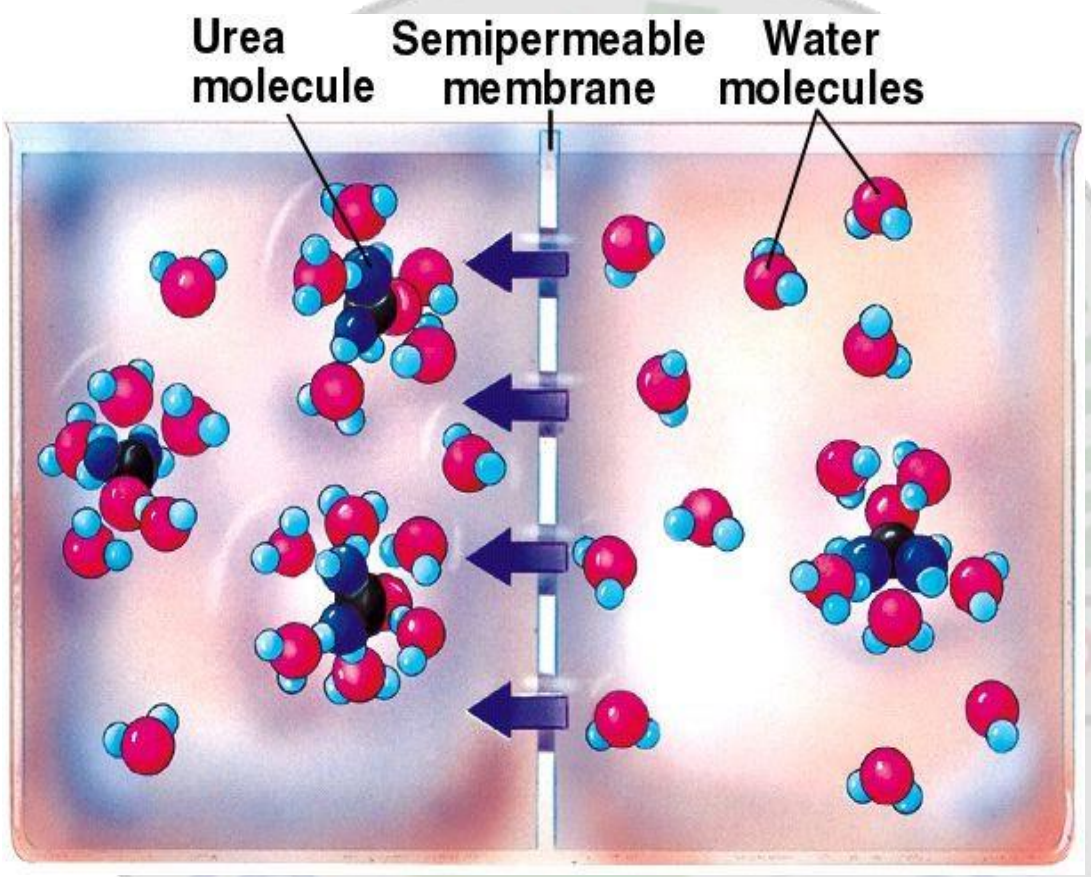
Diffusion of more than one substance at the same time and place may be at different rates and in different direction, but is independent of each other. A very common example of this is the gaseous exchange in plants. Beside osmotic diffusion the above mentioned simple diffusion also plays a very important role in the life of the plants.

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

- It is important in stomatal transpiration as the last step in the pollen, where diffusion of water vapour from the interrelation space into the outer atmosphere occurs through open stomata.

Osmosis

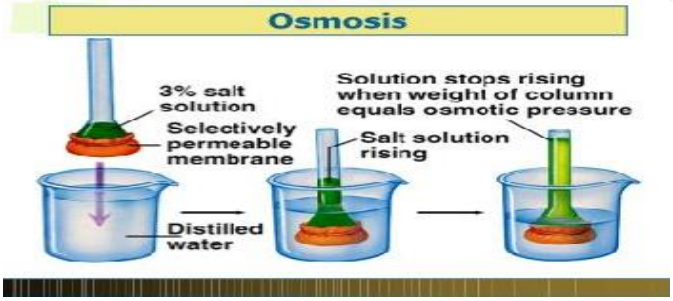
The diffusion of solvent molecules into the solution through a semi permeable membrane is called as osmosis (some times called as *Osmotic diffusion*). In case there are two solutions of different concentration separated by the semi permeable membrane, the diffusion of solvent will take place from the less concentrated suitable into the more concentrated solution till both the solutions attain equal concentration.



Osmosis is the diffusion of water across selective permeable membrane

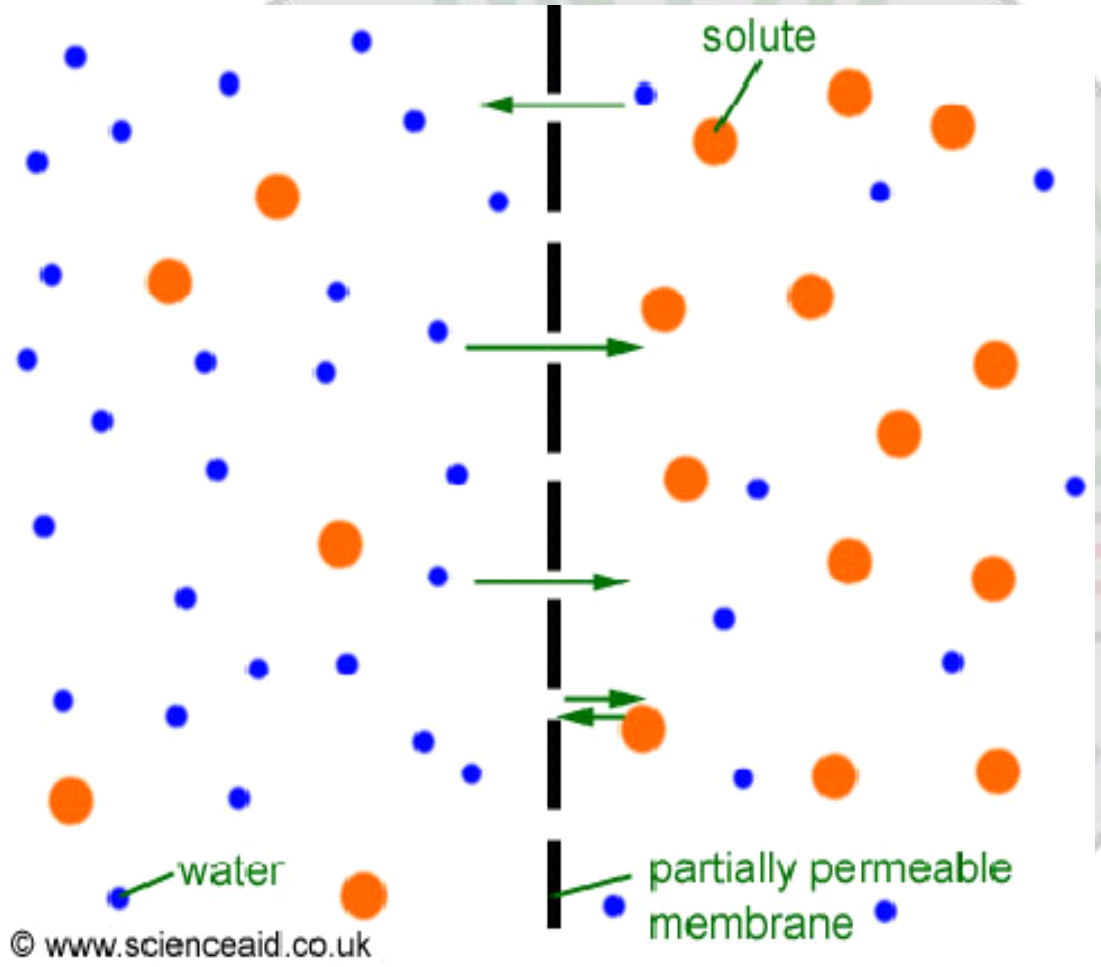
Osmotic pressure

As a result of the separation of solution from its solvent (or) the two solutions by the semi permeable membrane, a pressure is developed in solution to the pressure by dissolved solutes in it. This is called as osmotic pressure



(O.P). OP is measured interms of atmospheres and is directly proportional to the concentration of dissolved solutes in the solution. More concentration solution has higher O.P. O.P of a solution is always higher than its pure solvent.

During osmosis, the movement of solvent molecules taken place form the solution whose osmotic pressure is lower (i.e less concentration as hypotonic) into the solution whose osmotic pressure is higher (i.e, more concentrated as hypertonic). Osmotic diffusion of solvent molecules will not take place if the two solutions separated by the semipermeable membrane are of equal concentration having equal *Osmotic pressures* (i.e., they are isotonic). In plant cells, plasma membrane and tonoplant act as selectively permeable or differentially permeable membrane.



End-osmosis

Of a living plant cell is placed in water or hypotonic solution whose O.P is lower than cell sap, water in-terms into the cell sap by osmosis and the process is called end osmosis. As a result of entry of water with the cell sap, a pressure is developed which press the protoplasm against the cell wall and become turgid. This pressure is called a turgor pressure. Consequence of the turgor pressure is the wall pressure which is exerted by the elastic cell wall against the expanding protoplasm. At a given time, turgor pressure (T.P) equals the wall pressure (W.P).

T.P = W.P

Exosmosis

If on the other hand, the plant cell is placed in hypertonic solution (whose O.P is higher than cell sap) the water cover out the cell sap into the outer solution and the cell becomes flaccid. This process is known as exosmosis. Cell (or) tissue will remain as such in isotonic solution.

Significance of osmosis in plants

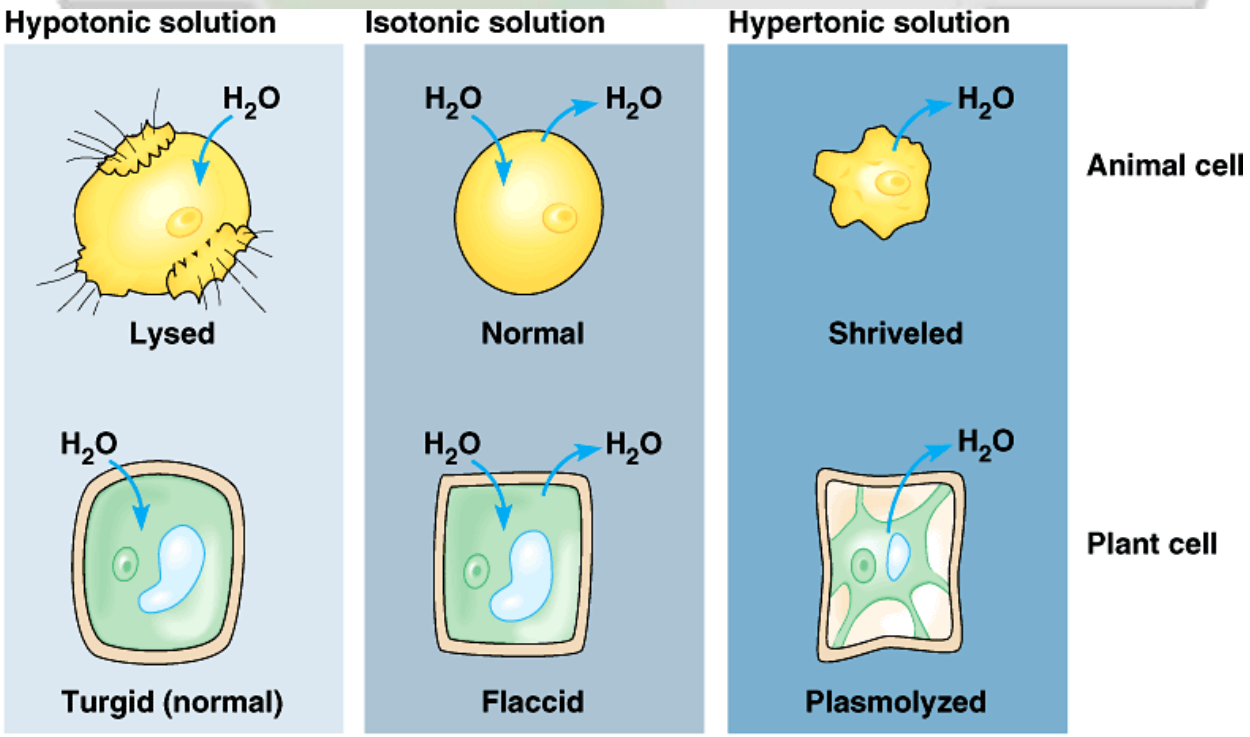
1. Large quantities of water are absorbed by roots from the soil by osmosis
2. Cell to cell movement of water and other substances dissolve is involves osmosis
3. Opening and closing of stomata depend upon the turgor pressure of guard cells
4. Due to osmosis, the turgidity of the cells and hence the shape or from of them organs is maintained.
5. The resistance of plants to drought and frost increases with increase in osmotic pressure to later cells
6. Turgidity of the cells of the young seedling allows them to come out of the soil.

hypotonic - solution whose osmotic pressure is lower (less concentration)

hypertonic - solution whose osmotic pressure is higher (more concentration)

isotonic - diffusion of solvent molecules will not take place

Osmotic effects on cell



Imbibition

Certain substances if placed in a particular liquid absorb it and swell up. For example, when some pieces of grass or dry wood or dry seeds are placed in water they absorb the water quickly and swell up considerably so that their volume is increased. These substances are called as imbibants and the phenomenon as imbibition, certain force of attraction is existing between imbibants and the involved substance. In plants, the hydrophilic colloids *viz*., protein and carbohydrates such as starch, cellulose and pectic substance have strong altercation towards water. Imbibition plays a very important role in the life of plants. The first step in the absorption of water by the roots of higher plants is the imbibition of water by the cell walls of the root hairs. Dry seeds require water by imbibition for germination.

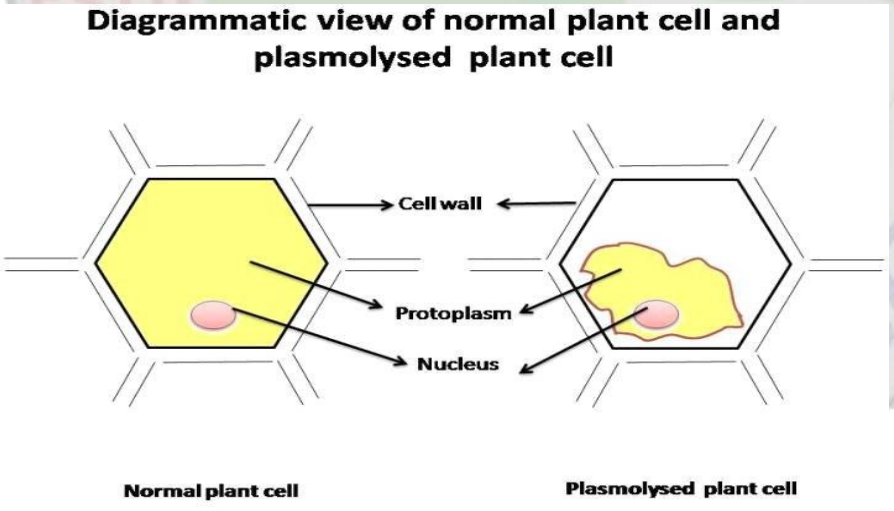
As a result of imbibition, a pressure is developed which is called as imbibitions pressure or matric potential (ψ_m). It is analogous to the osmotic potential of a solution. With reference to pure water, the values of ψ_m are always negative. The water potential of an imbibant is equal to its matric potential plus any turgor or other pressure (pressure potential) which may be imposed upon the imbibant.

$\psi_w = \psi_m + \psi_P$

If the imbibant is unconfined to turgor or such pressure, the equation can be significant to $\psi_w = \psi_m$

Plasmolysis

When a plant cell or tissue is placed in a hypertonic solution water cover out from thecell sap into the outer solution of exosmosis and the protoplasm begins to sprinkler or contract. The protoplasm separate from the cell wall and assures a spherical form and them phenomenon is called plasmolysis. Incipient plasmolysis is stage where protoplasm begins to contract from the cell wall. If a plasmolysed cell in tissue is placed in water, the process of endosmosis take place. Water enters into the cell sap, the cell becomes turgid and the protoplasm again assumes it normal shape and position. This phenomenon is called deplasmolysis.



Advantages of plasmolysis

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

3. Absorption of water

Terms contacted with soil water

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

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

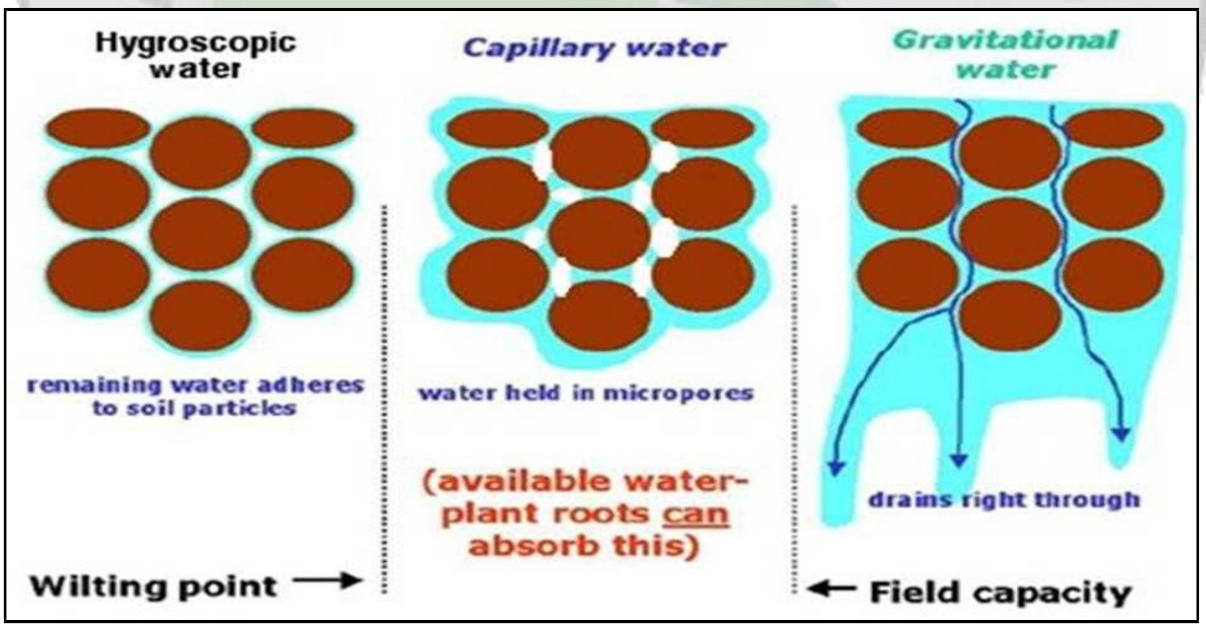
Gravitational water: Some part of water percolates downward through larger pores between soil particles under influence of gravitational force till it reaches the water table, not available for plant growth.

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

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

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

Field capacity or water holding capacity



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

Water table

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

Water use efficiency

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

Wilting coefficient or wilting point

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

Temporary or transient wilting

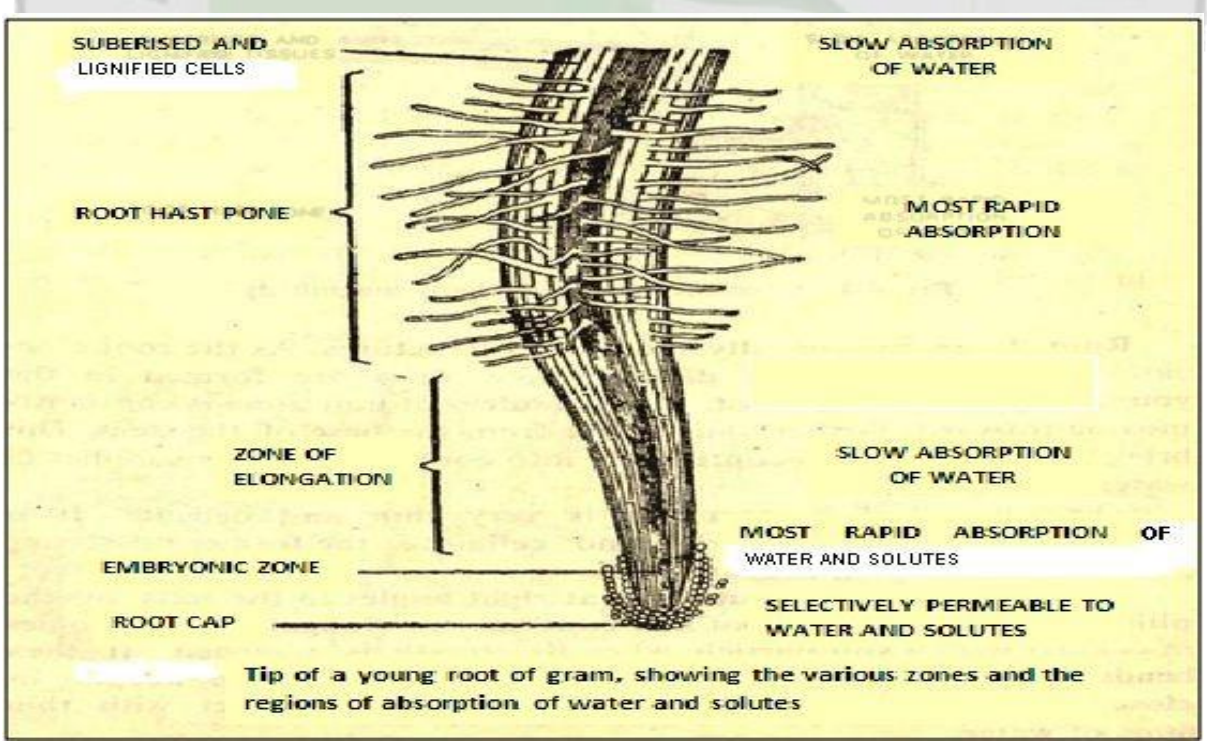
Despite of sufficient moisture in soil plants may exhibit sign of willing. This happens generally in a afternoon of a hot summer day when transpiration exceeds absorption but plants recover again at night automatically when transpiration is reduced and absorption is still continued. This type of wilting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

Permanent wilting

Wilting caused by an actual lack of water in soil. A plant will not recover unless water is added to the soil. In most herbaceous plants the leaf water potential is then - 14 to -15 bars.

Water absorbing system of plant

The main part of the plant which is concerned with water absorption is the root. The depth of roots varies with different species and greatly affected by factors like water, mineral nutrients, O_2 and temperature. Some species are deep feeders possess deep root system, whereas, some are surface feeders associated with shallow root system.



Tip of a young root of gram, showing the various zones and the regions of absorption of water and solutes

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

More number of tips in roots favours higher absorption. Extensively developed root system is associated with higher absorption rates in view of more number of active tips in roots.

At the extreme tip of root, root cap exists which consists of mass of cells forming a protective sheath around growing point. The growing points are about one mm in length and made up of embryonic cells which are rapidly dividing. This region is called embryonic zone. This zone is associated with higher absorption rate. Behind this lies a zone of elongation where growth of root takes place in length. This region favours very slow absorption rate. This is followed by root hair zone which is covered with root hairs. Root hair zone extends from 1 to 4 cm. Behind this region mature part of root lies where cell walls become lignified and suberized. This zone is associated with slow absorption rate. Most of the water absorption takes place through the root hairs. A root hair is the tubular extension of epidermal cells. As the root elongates the older root hairs die and new one come up in younger parts of roots. As a result the root hair zone is constantly moving forward farther and farther. This brings the root hairs continuously come into contact with new supply of water in the soil.

Anatomy of xylem tissue

Xylem has been recognized as tissue involved in the water translocation. Several different type of cells, living and nonliving, comprise xylem tissue. Of these, the tracheary elements are most prevalent and through these cells water translocation takes place. Xylem also consists of xylem fibers (scleranchyma) and living parenchyma cells.

Tracheids and vessels

1. The vessel elements and tracheids are the cells most involved in the water translocation.
2. Both are more or less elongated, have lignified secondary wall and are dead when they are mature and functional.
3. Since vessel elements and tracheids are dead at maturity, there is no interfering protoplast in the lumina of the cells, a situation that allows for the efficient translocation of relatively large amounts of water.
4. Perforated and walls are characteristic of vessel elements but do not occur in tracheids. However, tracheids are well supplied with bordered pits.
5. In the more developed vessel elements, the end walls may be entirely missing leaving nothing to obstruct the passage of water through the cell.
6. These structures formed from a series of vessel elements attached to one another by their end walls is called vessel or xylem duct.

7. This network is important not only for the maintenance of turgor but also for the translocation of other substances that may be carried from cell to cell by the moving water(e.g. essential mineral elements).

Xylem fibres

The xylem fibre is a long, thin, tapering cell with a very thick, lignified cell wall and is dead at maturity. The primary function of the xylem fibre is support, but it is also possible for some water to pass through xylem fibres since they are in association with each other and with the tracheids and vessel elements via pit pairs.

Xylem parenchyma

Living parenchyma cells may be found interspread in the conducting cells or as components of xylem rays and are generally referred to as a wood and ray parenchyma, respectively. One obvious function of xylem parenchyma is the storage of food. The living parenchyma cells of the xylem may have a vital role in the translocation of water.

Mechanism of water absorption

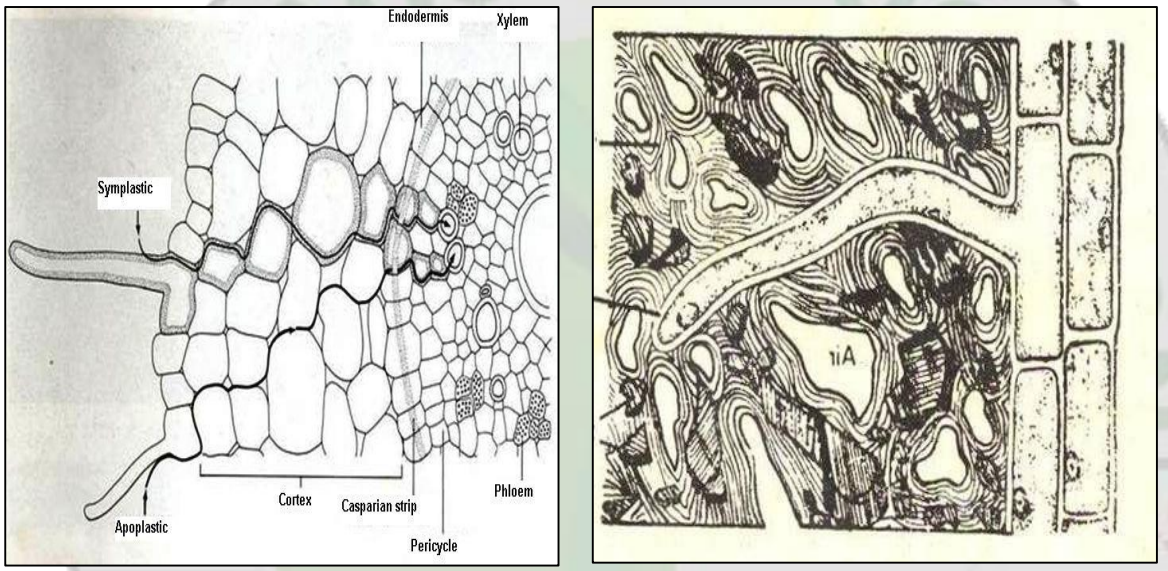
1. The absorption of water takes place through root hairs which are in contact with water films on soil particles. Inside the root hair is a thin lining of cytoplasm which encloses a large vacuole filled with cell sap.
2. The cell wall of a root hair is a permeable membrane. Its cytoplasmic line is a semipermeable membrane
3. The capillary movement of water in soil takes place through cohesive force of water molecules. Root hair cells are in contact with cortical cells which extends to endodermis.
4. Internal to endodermis is a single layer of parenchymatous cells which lies opposite to protoxylem. This arrangement offers a direct channel for the passage of water to xylem.
5. These are known as passage cells. These cells allow very easy movement of water across endodermis.
6. Due to this its suction pressure will fall below that of adjacent cortical cell b, with the result water will pass from a to b.
7. The diffusion of water into b reduces its suction pressure which falls below that of cortical cell c. With the result water passes from b to c, by this way water reaches to endodermal and pericycle cells which then becomes trurgid. It will exert no suction pressure, hence will give up water to xylem vessels.

8. The walls of xylem vessels are inelastic, so there is no turgor pressure, and the whole of osmotic pressure of xylem sap constitutes its suction pressure. This being higher than the reduced suction pressure of parenchyma (pericycle) cells, water will be drawn into xylem vessels. The force with which water is drawn in from the soil depends upon difference between osmotic pressure of xylem sap and soil solution.

9. The water is pushed into xylem vessels by surrounding cortical cells with certain force. This force is called root pressure. Conditions which hinder water uptake are poor aeration, cold or dry soils, higher concentration of salts in soil, presence of toxic substances etc.

Absorption of water by aerial parts of the plant

The absorption of water both in liquid and vapour forms occurs to a small extent



through aerial parts of most plants. The extent to which this occurs depends on the water potential of leaf cells and the permeability of the cutin layer (Gessner 1956). Roberts, South and Palmiter (1948) found that the cutin layer on the leaves of the McIntosh apple was not continuous but occurred in lamellae parallel to the outer epidermal walls. Interspread with the parallel layers of cutin, they found parallel layers of pectinaceous material of good water absorbing capacity. Not only was this material present with the cutin layer at the surface of the leaf, but it extended vertically to the vein extensions within interior of the leaf. It thus formed a continuous path for water from the surface to the vascular tissue. The permeability of the cutin layer of the apple leaf to water is rather good.

Some investigations believe that water absorbed by the leaves can travel in a negative direction through the plant and can actively diffuse through the roots into the soil. Breazeale, McGeorge and Breazeale (1950, 1953,1951) demonstrated that both tomato and corn plants are

capable of moving water absorbed by the leaves back into the soil. The activity only occur along water potential gradients favouring movement in this direction.

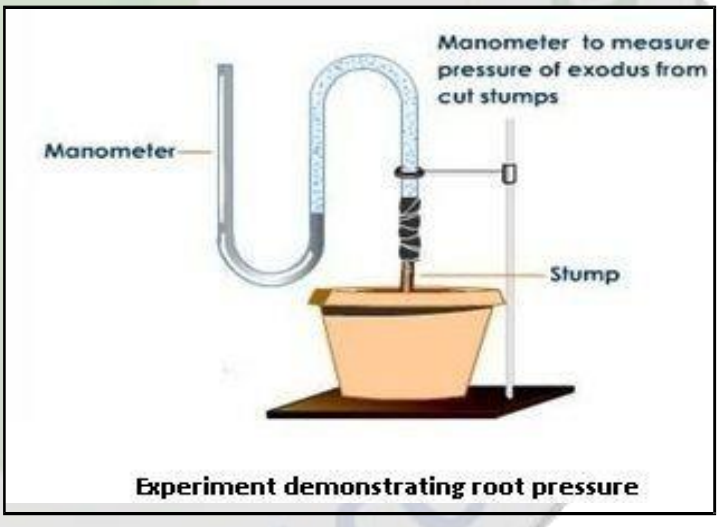
Apoplast and Simplast

1. Apoplast refers to the dead parts of plant cells viz; all walls, xylem vessels, intercellular spaces, whereas, symplast includes all living parts of plant cells viz; plasmodesmata and elements within cytoplasmic membrane.

2. The terms apoplast and simplast were originally introduced by Munch (1931) in his studies on the flow of water and solution in plants. The terms are convenient for describing the path of absorbed and translocated water and solution. Water may be translocated across the root cortex through a system of interconnecting cell walls and intercellular spaces before reaching the casparian strip of the endodermal walls..

Movement of water and solutes into the living cells of the plant is due to osmosis (water), free diffusion (passive uptake of solutes) or active uptake (solutes). This living continuum in the plant, including the plasmodesmata and elements within the cytoplasmic membrane, Munch termed the symplast.

Root pressure



Experiment demonstrating root pressure

In the root pressure we may observe xylem sap under pressure exuding from the cut end of the stump. If a well watered tomato plant is detopped and the stump is attached with a rubber sleeve to a manometer, a rise in the level of liquid in manometer can be demonstrated as a result of root pressure. Stocking (1956) defined the root pressure as a pressure developing in the tracheary elements of xylem resulting from the metabolic activities of roots. Root pressure is referred to as an active process. The movement of water up to the stem as result of root pressure is due to osmotic mechanisms that are created as a result of the active absorption of salts by the roots.

Root pressure which is developed due to the accumulation of solute in the xylem ducts appears to be affected by factors that affect respiration Viz; oxygen tension, narcotics, auxin and respiration inhibitors. Several investigations have observed an automatic, diurnal fluctuation in

exudations caused by root pressure. Dropped tomato plants with their root systems immersed in solutions of different concentrations exhibit different exudation rates.

Factors affecting water absorption

Soil temperature: Low temperature reduces the absorption of water due to following reasons.

- Increased viscosity of water which retards the water movement to the plant.
- Decrease in rate of root elongation and roots fail to reach new areas.
- Alternation in properties of protoplasm. Due to low temperature viscosity of protoplasm increases and permeability of plasma membrane to water decreases. As a consequence the movement of water across the living cells of roots is slowed down.
- Reduction in metabolic activities of living cells**.**

Soil air: Low availability of O_2 in soil affects water absorption adversely. Soil lacking O_2 favours the action of anaerobic bacteria like Clostridium which decomposes soil matter and release poisonous gases like H_2S, ethylene, ammonia etc. which are toxic to roots and inhibit their development.

Soil water: Increase in water content of soil increases water absorption to a certain limit, thereafter it declines due to decrease in aeration of soil.

Mineral salts: Absorption of water is also retarded by higher concentration of salts in soil because they increase the osmotic pressure of soil solutions. Saline soils and salt marshes are physiologically dry for the plants.

5. Transpiration and Stomatal Physiology

Although large quantities of water are absorbed by plant from the soil but only a small amount of it is utilized. The excess of water is lost from the aerial parts of plants in the form of water vapours. This is called as transpiration.

Transpiration is of three types

1. Stomatal transpiration

Most of the transpiration takes place through stomata. Stomata are usually confined in more numbers on the lower sides of the leaves. In monocots. Eg. Grasses they are equally distributed on both sides. While in aquatic plants with floating leaves they are present on the upper surface.

2. Cuticular transpiration

Cuticle is impervious to water, even though, some water may be lost through it. It may contribute a maximum of about 10% of the total transpiration.

3. Lenticular transpiration

Some water may be lost by woody stems through lenticells which is called as lenticular transpiration.

Mechanism of stomatal transpiration

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

i. Osmotic diffusion of water in the leaf from xylem to intercellular space above the stomata through the mesophyll cells.

ii. Opening and closing of stomata (stomatal movement)

iii. Simple diffusion of water vapours from intercellular spaces to other atmosphere through stomata.

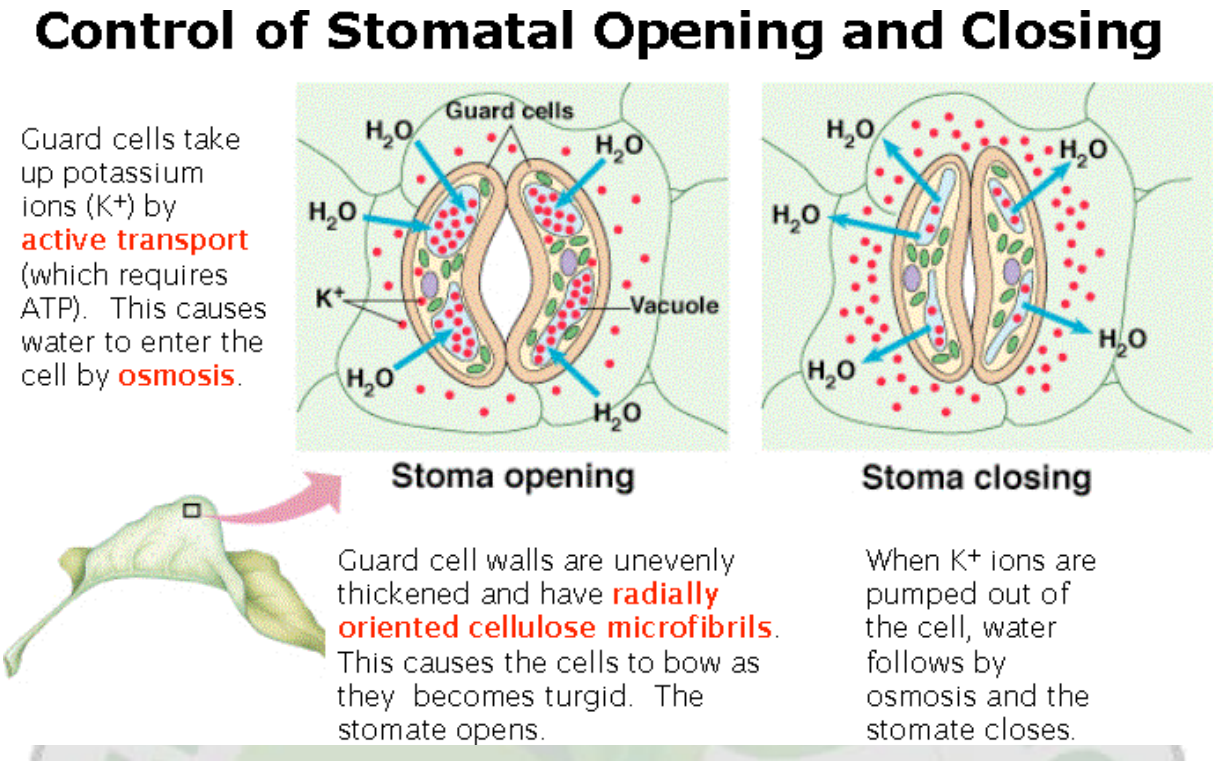
♦ Inside the leaf the mesophyll cells are in contact

♦With xylem, and on the other hand with intercellular space above the stomata

♦When mesophyll cells draw water from the xylem they become turgid and their diffusion pressure deficit (DPD) and osmotic pressure (OP) decreases with the result that they release water in the form of vapour in intercellular spaces close to stomata by osmotic diffusion. Now in turn, the O.P and D.P.D of mesophyll cells become higher and hence, they draw water form xylem by osmotic diffusion.

Opening and closing of stomata (Stomatal movement)

The stomata are easily recognized from the surrounding epidermal cells by their peculiar shape. The epidermal cells that immediately surround the stomata may be similar to other epidermal cells or may be different and specialized. In the latter case, they are called as subsidiary cells. The guard cells differ from other epidermal cells also in containing chloroplasts and peculiar thickening on their adjacent surface (in closed stomata) or on surfaces. Consequent to an increase in the osmotic pressure (OP) and diffusion pressure deficit (DPD) of the guard cells (which is due to accumulation of osmotically active substances), osmotic diffusion of water from surrounding epidermal cells and mesophyll



cells into guard cells follows. This increase the turgor pressure (TP) of the guard cells and they become turgid. The guard cells swell, increase in length and their adjacent thickened surfaces starch forming a pore and thus the stomata open. On the other hand, when OP and DPD of guard cells decrease (due to depletion of osmotically active substances) relative to surrounding epidermal and mesophyll cells, water is released back into the latter by osmotic diffusion and the guard cells become flaccid. The thickened surfaces of the guard cells come close to each other, thereby closing the stomatal pore and stomata.

Osmotic diffusion of water into guard cells occur when their osmotic pressure increases and water potential decreases (i.e become more negative) related to those of surrounding epidermal and mesophyll cells. The guard cells become flaccid when their osmotic pressure decreases relative to the surrounding cells (Movement of water takes place from a region of higher water potential to a region of lower water potential. These may be several different agents or mechanisms which control stomatal movements. Hydrolysis of starch into sugars in guard cells Synthesis of sugars or organic acids in them

The active pumping of K^+ ions in the guard.

1. Hydrolysis of starch into sugars in guard cells

Starch – sugar Inter conversion theory

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

Light high pH

Starch +Pi ---------- Glucose-1-phosphate

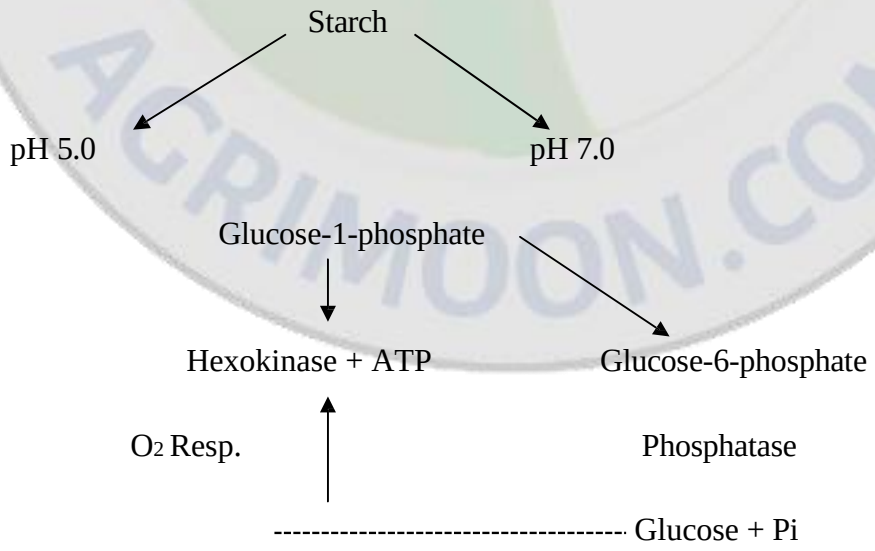
(Insoluble) Dark low pH (Soluble)

According to Steward 91964), the conversion of starch and inorganic phosphate into glucose-1-phosphate does not cause any appreciable change in the osmotic pressure because the inorganic phosphate and glucose-1-phosphate are equally active osmotically.

In this scheme he has suggested that,

Glucose-1-phosphate should be further converted into glucose and inorganic phosphate for the opening of stomata.

Metabolic energy in the form of ATP would be required for the closing of stomata which probably comes through respiration.



2. Synthesis of sugars or organic acids in Guard cells

During day light photosynthesis occurs in guard cells as they contain chloroplast. The soluble sugars formed in this process may contribute in increasing the osmotic potential of guard cells and hence resulting in stomatal opening. However, very small amounts of soluble sugars (osmotically active) have been extracted from the guard cells which are insufficient to affect water potential.

As a result of photosynthesis CO_2 concentration in guard cells decreases which leads to increased pH up of organic acids, chiefly malic acid during this period in guard cells. The formation of malic acid would produce proton that could operate in an ATP-driven proton K^+ exchange pump moving protons into the adjacent epidermal cells and K ions into guard cells and thus may contribute in increasing the osmotic pressure of the guard cells and leading to stomatal opening.

Reverse process would occur in darkness.

3. ATP –Driven proton (H^+) – K exchange pump mechanism in Guard cells

According to this mechanism, there is accumulation of K^+ ions in the guard cells during day light period. The protons (H^+) are 'pumped out' from the guard cells into the adjacent epidermal cells and in exchange K^+ ions are mediated through ATP and thus are an active process. ATP is generated in non-cyclic photophosphorylation in photosynthesis in the guard cells. The ATP required in ion exchange process may also come through respiration.

The accumulation of K ion is sufficient enough to significantly decrease the water potential of guard cells during day light. Consequently, water enters into them from the adjacent epidermal and mesophyll cells thereby increasing their turgor pressure and opening the stomatal pore. Reverse situation prevails during dark when stomata are closed. There is no accumulation of 'K' in g cells in dark.

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

Significance of Transpiration

Plants waste much of their energy in absorbing large quantities of water and most of which is ultimately lost through transpiration. Some people thin that – Transpiration as advantageous to plant. Others regard it as an unavoidable process which is rather harmful.

Advances of transpiration

1. Role of movement of water

Plays an important role in upward movement of water i.e Ascent of sap in plants.

2. Role in absorption and translocation of mineral salts

Absorption of water and mineral salts are entirely independent process. Therefore transpiration has nothing to do with the absorption of mineral salts. However, once mineral salts have been absorbed by the plants, their further translocation and distribution may be facilitated by transpiration through translocation of water in the xylem elements.

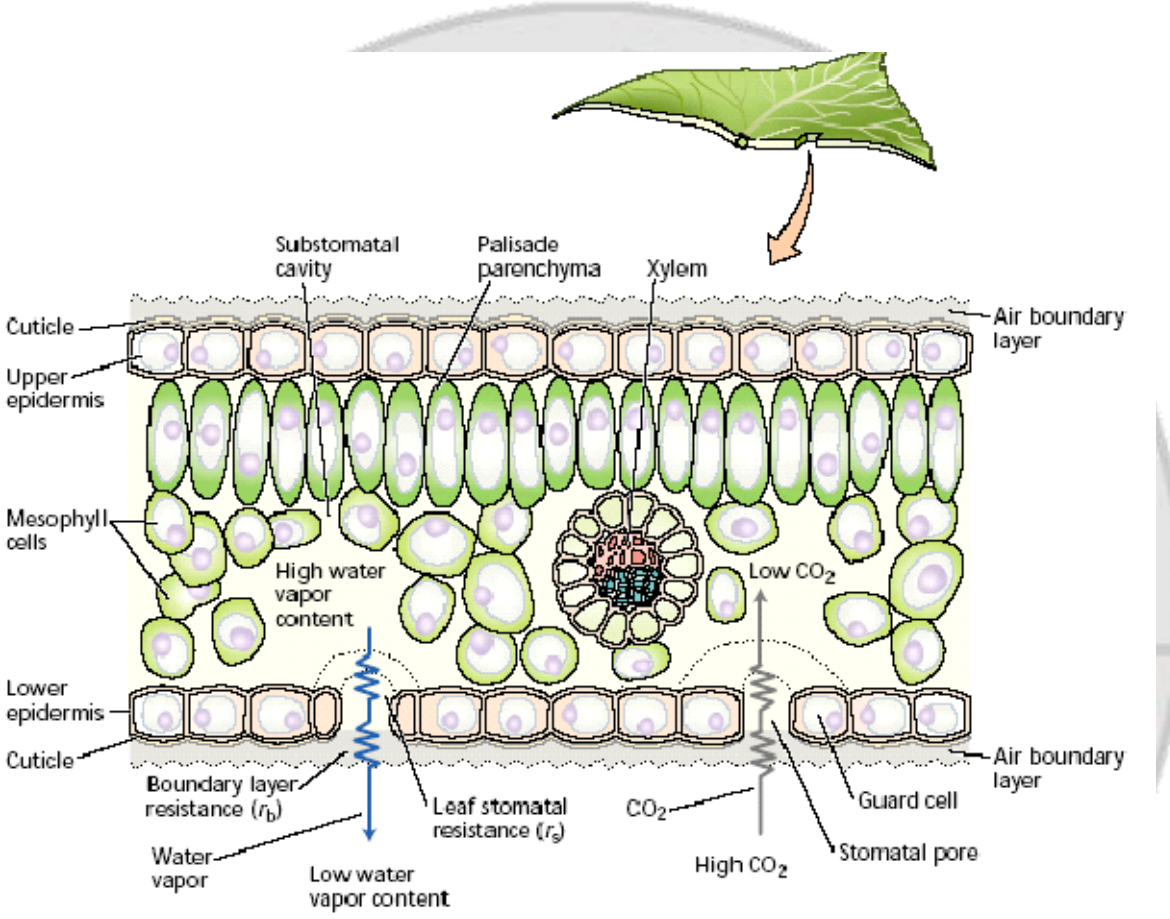
3. Role of regulation of temperature

Some light energy absorbed by the leaves is utilized in photosynthesis; rest is converted into heat energy which raises their temperature. Transpiration plays an important role in controlling the

temperature of the plants. Rapid evaporation of water from the aerial parts of the plant through transpiration brings down their temperature and thus prevents them from excessive

Transpiration as a necessary evil

1. When the rate of transpiration is high and soil is deficient in water, an internal water deficit is created in the plants which may affect metabolic processes
2. Many xerophytes have to develop structural modification and adaptation to check transpiration.



3. Deciduous tress has to shed their leaves during autumn to check loss of water. But, in spite of the various disadvantages, the plants cannot avoid transpiration due to their peculiar internal structure particularly those of leaves. Their internal structure although basically mean for gaseous exchange for respiration, P.S. etc. is such that it cannot check the evaporation of water. Therefore, many workers like Curtis (1926) have called transpiration as necessary evil.

Factors affecting transpiration rate

The rate of transpiration is affected by a number of internal (plant factor) and external factors.

I. Internal or Plant Factors:

(i) Root - shoot ratio:

If all other conditions are favourable for transpiration, the water absorbing capacity of roo surface and transpiring capacity of the leaf suface regulate the rate of transpiration if transpiration is greater than absorption, a water deficit results, causing reduction in transpiration rate. In other words a low root/shoot ratio decreases the rate of transpiration.

(ii) Leaf area:

The greater the leaf area, the higher will be the water loss due to transpiration.

(iii) Stomatal frequency:

Stomatal frequency means the total number of stomata per unit leaf area. Stomatal frequency varies with different species. Greater the frequency of stomata, faster is the rate of transpiration.

(iv) Structure of leaf:

Presesncc of thick cuticlc, wax layers and trichomes on the surface of leave reduce the rate of transpiration.

II. External or Environmental Factors

(i) Light: There is a close relationship between the opening of stomata and presence of light. Light affects the rate of transpiration in two ways. Firstly, light causes stomata to open. As a result of wide opening of stomata, the saturated interior cells of leaf arc exposed to the outer atmosphere. Consquently, the rate of transpiration is increased in bright sunlight. Secondly, it increases the temperature of leaf and thus affects the rate of transpiration. In a nut shell, the combined affect of light causes opening of stomata and incerases the rate of vaperisation of water.

(ii) Wind:

The increase in the wind velocity increases the rate of transpiration by remouing the water vapour of the atmosphere from the vicinity of transpiring surface and lowering relative humidity. The transpiration is faster in mild wind. The winds of much higher velocity retard the rate of transpiration.

(iii) Temperature:

The increase in temperature increases the rate of transpiration. This is due to increase in the rate of evaporation of water from cell surface and decrease in the humidity of the external atmosphere. However, there is a limit in rise of temperature in relation to loss of water by transpiration. At very high temperature, usually beyond 35°C the rate of transpiration gradually falls due to inactivity of the protoplasm.

(iv) Humidity of the air:

Humidity is expressed as the percentage of water vapour present in the atmosphere. The relative humidity of the atmosphere affected the rate of transpiration to a great extent because it influences the DPD gradient between the intercellular spaces and outside atmosphere.

The higher the relative humidity of the outside atmosphere the lower will be the rate of transpiration. Conversely the lower the relative humidity of the outside atmosphere the higher will be the rate of transpiration.

(v) Atmospheric pressure:

The reduction of atmospheric pressure reduces the density of the external atmosphere. This allows more rapid diffusion of water. The plants growing on hills show higher rate of transpiration because of low atmospheric pressure and thus they develop xerophytes characters

(vi) Water Supply:

Deficiency of water in soil decreases the rate of transpiration. This is due to low absorption of water from the soil.

Antitranspirants

A number of substances are known which when applied to the plants retard their transpiration. Such substances are called as antitranspirants. Some examples of antitranspirants are colourless plastics, silicone, oils, low viscosity waxes, phenyl mercuric acetate, abscisic acid, CO2, etc. Colourless plastic, silicone oils and low viscosity waxes belong to one group as these are sprayed on the leaves, form after film which is permeable to O2 and CO2 but not to water. Fungicide phenyl mercuric acetate, when applied in low concentration (10-4 m), it exercised a very little toxic effect on leaves and resulted in partial closure of stomatal pores for a period of two weeks. Similarly ABA a plant hormone also induces stomatal closure. CO2 is an effective antitranspirants. A little rise in CO2 concentration from the natural 0.03% to 0.05% induces partial closure of stomata. Its higher concentration cannot be used which results in complete closure of stomata affecting adversely the photosynthesis and respiration.

GUTTATION

In some plants such as garden nasturtium, tomato, colocasia etc, water drops ooze out from the uninjured margins of the leaves where a main vein ends. This is called as guttation and takes place usually early in the morning when the rate of absorption and root pressure are high while the transpiration is very low. The phenomenon of guttation is associated with the presence of special types of stomata at the margins of the leaves which are called as **water stomata or hydathodes**. Each hydathode consists of a water pore which remains permanently open. Below this there is a small cavity followed by a loose tissue called as epithem. This epithem is in close association with the ends of the vascular elements of veins. Under high root pressure the water is given to the epithem by the xylem of the veins. From epithem water is released into the cavity. When this cavity is completely filled with watery solution, the later begins to ooze out in the form of watery drops through the water pore.

Difference between transpiration and Guttation

Transpiration	Guttation
1. Water is lost from aerial parts of plants in the form of invisible water vapours	Watery solution oozes out from uninjured margins of aerial leaves only
2. Transpiration occurs mostly through stomata. It may also takes place through cuticle and lenticels	It occurs only through hydathodes (water stomata)
3. It takes place throughout the day, its rate being maximum at noon.	It takes place only early in the morning when root pressure and the rate of water absorption are higher

6. Mineral nutrition of Plants, Functions and deficiency symptoms of nutrients

Essential elements

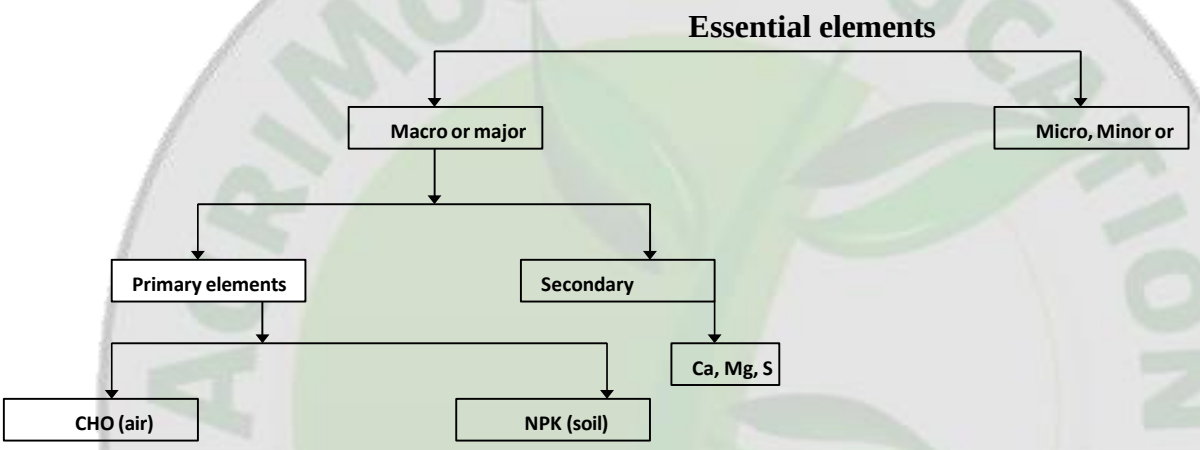
Macro or major elements: Elements required by plants in huge quantities i.e. 1000 mg/kg of dry matter. Examples – CHO NPK Ca Mg S**.**

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

Beneficial and other elements

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

Classification of elements



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

Plant nutrients have been divided into four basic groups.

1. The first group of essential elements form the organic (carbon) compounds of the plant. Plants assimilate these nutrients via biochemical reactions involving oxidation and reduction.
2. The second group is important in energy storage reactions or in maintaining structural integrity. Elements in this group are often present in plant tissues as phosphate, borate and silicate esters in which the elemental group is bound to the hydroxyl group of an organic molecule (i. e. sugar- phosphate).
3. The third group is present in plant tissues as either free ions or ions bound to substances such as pectic acid present in the cell wall of particular importance or their roles as enzyme cofactors and in the regulation of osmotic potentials.
4. The fourth group has important roles in reactions involving electron transfer.

Chapter 3
Classification of plant mineral nutrients according to biochemical functions

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

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

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

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

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

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

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

Group 3: Nutrients that remain in ionic form.

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

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

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

Chlorine: Required for the photosynthetic reactions involved in O_2 evolution.

Manganese: Required for activity of some dehydrogenase, decarboxylase, kinases, oxidases and peroxidases. Involved with other cation- activated enzymes and photosynthetic O_2 evolution.

Sodium: Involved in the regeneration of phospho enol pyruvate in C_4 and CAM plants . Substitute for potassium in some functions.

Group 4: Nutrients that are involved in redox reactions.

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

Zinc: Constituent of alcohal dehydrogenase, carbonic anhydrase etc.

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

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

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

Essential elements for most higher plants and internal concentrations considered adequate

Elements	Chemical symbol	Form available to plants	Atomic Wt.	Concentration in Dry Tissue		Relative No. of Atoms Compared to Molybdenum
				Mg/Kg	(%)	
Molybdenum	Mo	MoO_4^{2-}	95.95	0.1	0.00001	1
Nickel	Ni	Ni^{2+}	58.71	?	?	?
Copper	Cu	Cu^{+}, Cu^{2+}	63.54	6	0.0006	100
Zinc	Zn	Zn^{2+}	65.38	20	0.0020	300
Manganese	Mn	Mn^{2+}	54.94	50	0.0050	1,000
Boron	B	H_3BO_3	10.82	20	0.002	2,000
Iron	Fe	Fe^{3+}, Fe^{2+}	55.85	100	0.010	2,000
Chlorine	Cl	Cl^{-}	35.46	100	0.010	3,000
Sulfur	S	SO_4^{2-}	32.07	1,000	0.1	30,000
Phosphorus	P	$H_2PO_4^{-}$ HPO_4^{2-}	30.98	2,000	0.2	60,000
Magnesium	Mg	Mg^{2-}	24.32	2,000	0.2	80,000
Calcium	Ca	Ca^{2+}	40.08	5,000	0.5	125,000
Potassium	K	K^{+}	39.10	10,000	1.0	250,000
Nitrogen	N	NO_3^{-}, NH_4^{+}	14.01	15,000	1.5	1,000,000
Oxygen	O	O_2, H_2O	16.00	450,000	45	30,000,000
Carbon	C	CO_2	12.01	450,000	45	35,000,000
Hydrogen	H	H_2O	1.01	60,000	6	60,000,000

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

Nitrogen

It is absorbed in the form of NO_3, $\overline{NO_2}$ and NH_4^{+}.

Functions

It is very important due to its presence in protein molecule. Nitrogen is found in important molecules as purines, pyrimidines, porphyrins and cytochromes. Purines, pyrimidines are found in nucleic acids, RNA and DNA which are important for protein synthesis. The porphyrin structure

is found in metabolically important compounds as the chlorophyll pigments and the cytochromes essential in photosynthesis and respiration.

Deficiency symptoms

1. Most important symptom is yellowing of leaves (chlorosis) due to loss of chlorophyll. The symptoms appear first in older leaves then in new or growing leaves due to its high mobility. The younger leaves retain their nitrogen and obtain N from older leaves as well through translocation.
2. Under severe conditions of nitrogen deficiency the lowermost leaves on plants like in tobacco or beans dries. It also indirectly involved in anthocyanin pigment synthesis besides chlorophyll. In tomato due to nitrogen deficiency purple colour in leaf petioles and veins was noticed

Phosphorus

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

Functions

It is constituent of nucleic acid, phospholipids, the coenzyme NAD & NADP, constituent of ATP and other high energy compounds. Meristematic cells show high P concentration where P is involved in nucleoprotein synthesis. It is also involved in activation of amino acids through ATP for the synthesis of protein moiety.

Deficiency symptoms

1. Phosphorus deficiency may cause premature leaf fall and purple and red anthocyanin pigmentation.
2. Its deficiency causes development of necrotic areas on the leaves, petioles or fruits. The parts become stunted in appearance, leaves may have dark to blue green colouration. 3.Older leaves show the deficiency symptoms first. Symptoms of zinc and phosphorus deficiencies may sometimes look alike for example, lack of either one of these elements may cause distortion in shape of leaves of some plants (Hewitt, 1963).

Potassium

It is absorbed in K^+ form.

Functions

Deficiency affects processes like respiration, photosynthesis, chlorophyll development and water content of leaves. The important function of potassium is opening and closing of stomata. It is found in abundance in meristematic region. It activates the enzymes involved in the formation of certain peptide bonds. Apical dominance is lacking in K deficient plants.

Deficiency symptoms

1. Due to K deficiency chlorosis first occurs on the leaves followed by the development of necrotic areas at the tip and margin of leaf. The symptoms are first seen on the older leaves due to translocation of K into new leaves.

2. There is a tendency for the leaf tip to curve downward in potato and French bean. Marginal regions may roll inward towards the upper surface. Stunted growth due to shortening of internode was also noticed in K deficient plants.

Calcium

It is absorbed as Ca^{++}.

Functions

It is constituent of cell wall in the form of calcium pectate. The middle lamella is composed of calcium and magnesium pectates. Therefore, it is essential for formation of cell membranes and lipid structures. Calcium in small amounts is necessary for mitosis. It plays a role as an activator of enzyme phospholipase in cabbage leaves (Davidson and Long 1958).

Deficiency symptoms

1. Due to deficiency of calcium meristematic regions of stem, leaf and root tips are greatly affected and die. Roots may become short, stubby and brown in calcium deficient plants (Kalra 1956).
2. Chlorosis occurs along the margins of younger leaves, areas become necrotic. Malformation or distortion of the younger leaves was also noticed in calcium deficient plants. Hooking of leaf tip is also seen.
3. Deficiency symptoms appear first in younger leaves and growing points due to immobility of calcium

Magnesium

It is absorbed as Mg^{++}.

Functions

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

Deficiency symptoms

1. It is constituent of chlorophyll, hence deficiency causes interveinal chlorosis in leaves. Initially yellowing is seen in the basal leaves, as the deficiency becomes more acute the yellowing is seen in new leaves also.
2. Chlorosis is sometimes followed by the appearance of anthocyanin pigments in leaves. At more acute deficiency necrotic spots may be seen over leaves.

Sulphur

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

Functions

Its main function is its participation in protein structure in the form of sulphur bearing amino acids Viz; cystine, cysteine and methionine. It is taken up by the plants as sulphate (SO_4^{2-})

which is later on reduced via an activation step involving the compound 3 phosphoadenosine – 5 – phosphosulphate (PAPS) and ATP. It is also important in Fe – S proteins in photosynthesis, N metabolism and ferredoxin synthesis.

Deficiency symptoms

1. Deficiency symptoms of S are similar to N deficiency in certain respects like in N deficient plants, there is a chlorosis in leaves followed by production of anthocyanin pigments in some species (Easton 1951).
2. Unlike N deficient plants sulphur deficient plants show chlorosis on the younger leaves first. However, under severe conditions all leaves may be affected (Gilbert 1951).
3. Hall and co-workers (1972) found sulphur deficiency results in decrease in stroma lamellae and increase in grana stakking in corn plants.

Manganese

It is absorbed as Mn^{++}.

Functions

It acts as an activator of enzymes involved in the respiration and nitrogen metabolism. Enzymes of Krebs cycle, malic dehydrogenase and oxalo succinic decarboxylase requires the presence of manganese as an activator. Manganese acts as an activator for enzyme nitrate reductase and hydroxyl amine reductase (Nason 1956; Sadana and Mcelroy 1957). It is also involved in the destruction or oxidation of IAA (Goldacre 1961; Kenton 1955).

Deficiency symptoms

1. Deficiency of Mn^{++} is characterized by the appearance of chlorotic and necrotic spots on the interveinal areas of the leaves.
2. Symptoms first appear on young leaves in some species, whereas in some species on older leaves.
3. Hewitt (1945) and Piper (1942) noted brown necrosis in cotyledons of pea and bean seeds in Mn deficient plants

Iron

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

Functions

Iron is directly incorporated into cytochromes as well as in compounds necessary for the electron transport in the mitochondria and into ferredoxin which is important for light reaction in photosynthesis. It is essential for chlorophyll synthesis. It is required in the synthesis of chloroplast proteins and enzymes involved in chlorophyll synthesis (Gauch and Duggar 1954). It is also found in iron – porphyrin proteins, like cytochromes, peroxidases and catalases.

Deficiency symptoms

1. Important symptom is interveinal chlorosis in leaves. The younger leaves are most affected.
2. More mature leaves show no chlorosis because of the immobility of iron in plants. 3.Chlorosis sometimes followed by chlorosis of veins so that whole leaf becomes yellow. 4. In severe cases

the young leaves even become white with necrotic lesions. Lack of iron may inhibit formation of chloroplasts through inhibition of protein synthesis.

Copper

Copper is absorbed as Cu^{++}.

Functions

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

Deficiency symptoms

1. Deficiency brings Exanthema disease that is characterized by Gummosis (Gummy exudates) accompanied by dieback and glossy brownish blotches on leaves and fruits.
2. Its deficiency also causes reclamation that is disease of cereals and characterized by chlorotic leaf tips and failure to set seeds.
3. Copper deficiency causes a necrosis of tip of young leaves that proceeds along the margin of leaf and gives it a withered appearance.

Zinc

It is absorbed as Zn^{++}.

Functions

It is involved in biosynthesis of auxins. Skoog (1940) observed a decrease in auxin content in zinc deficient tomato plants. Scientists also concluded that zinc deficiency reduces auxin content through its involvement in the synthesis of tryptophan, a precursor of auxin (Tsui 1948). It participates in the metabolism of plants as an activator of several enzymes like carbonic anhydrase which converts carbonic acid into carbon di oxide and water. Zn deficiency causes accumulation of soluble nitrogen compounds such as amino acids and amides (Possingham 1956).

Deficiency symptoms

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

Boron

It is absorbed as H_3BO_3.

Functions

Gauch and Duggar (1954) observed that boron is involved in carbohydrate transport within the plant. Uptake and translocation of sugar is retarded in Boron deficient plants. It also plays an

important role in DNA synthesis in meristems.. It is involved indirectly through translocation of sugar.

Deficiency symptoms

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

Molybdenum

It is absorbed as MoO_4^{2-}.

Functions

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

Deficiency symptoms

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

Chlorine

It is absorbed as Cl^-.

Functions

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

Deficiency symptoms

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

Nickel

It is absorbed as Ni^{++}.

Functions

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

Deficiency symptomes

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

Hydroponics It is the technique of growing plants with their roots immersed in nutrient solution without soil. The method of growing plants in aqueous nutrient solutions is known as hydroponics culture or hydroponics. The growing of higher plants with their roots in dilute solutions of mineral salts instead of in soils has led to a vastly increased understanding of plant nutrition. The use of water culture technique for growing plants permits precise control of the supply of nutrient ions in the root environment. The first recorded use of water culture technique was in the year 1699 by John
Woodward. Latter the process of growing plants in water culute was named as hydroponics by Gericke 1930.

The water culture technique is extensively used to grow certain high value crops (eg.Tomato, lettuce and strawberry) in glass houses during off seasons in metropolitan areas of developed countries. In addition, vegetables are grown in plastic houses in a few coastal desert regions, where sea water is desalinated and supplied to roots in precisely the amounts required by the growing plants

Advantages

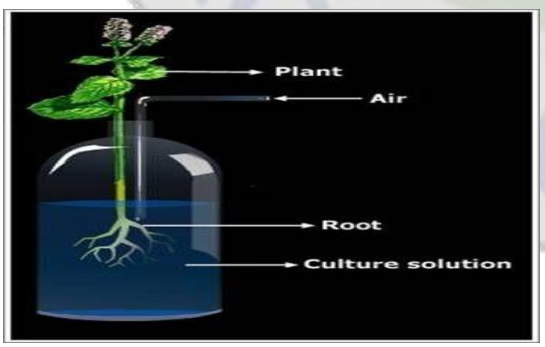
- Mineral salts can be provided in the desired requirements.
- By using distilled water in the nutrient solution contamination can be avoided.

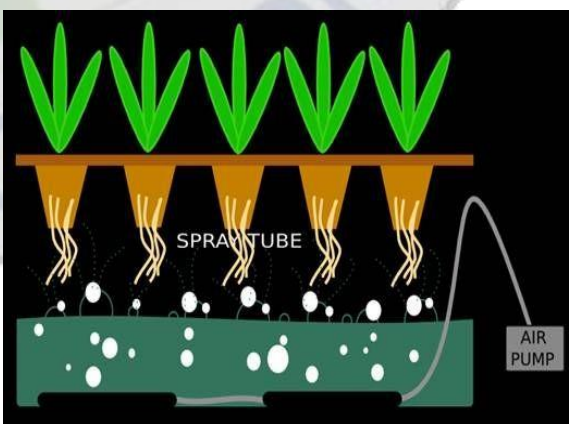
Technique of using

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

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

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





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Hydroponics	Aeroponics

Chapter 4
Nutrient uptake mechanisms

Absorption and translocation of solutes

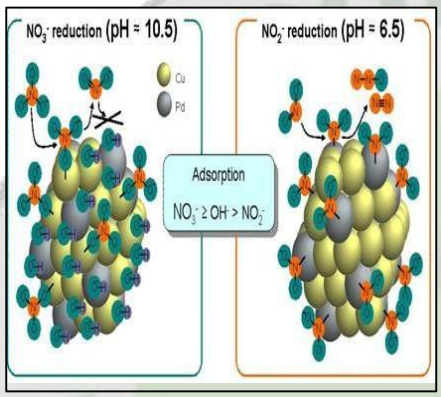
Certain terms are associated with the uptake of substances.

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

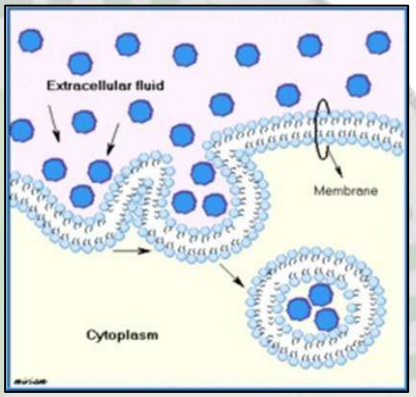
Divided into two parts

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

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



Adsorption



Absorption

Absorption- It can be divided in two parts:

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

Passive absorption can be divided in two parts

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

Some scientists believe that ions can move inside roots along with the mass flow of water due to transpirational pull. According to this theory an increase in transpiration increases the absorption of ions (Russel and Barber 1960). Lopushinsky (1964) noted in the experiments with tomato using radioactive isotopes 32 P and 45 Ca that increase in absorption increases the salt absorption. Accumulation of ions against a concentration gradient is possible under mass flow

mechanism due to an ion exchange mechanism or Donnan effect and equilibrium. The mass flow of ions through root tissue may also be possible with the aid of transpirational pull.

Outer space and apparent free space

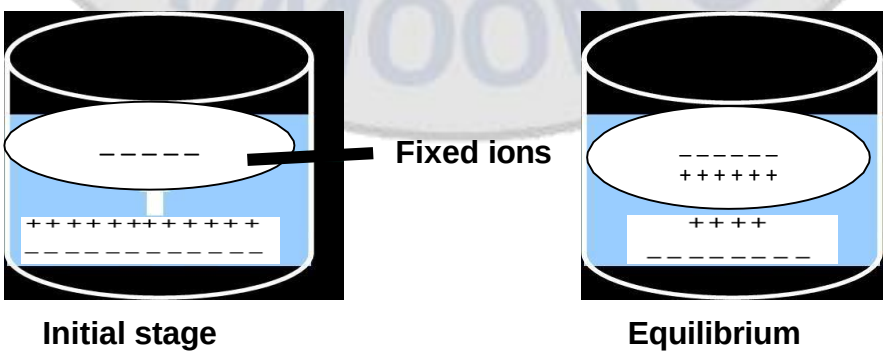
The outer space is defined as part of plant cell or tissue which allows free diffusion to take place, whereas apparent free space is the apparent volume of plant tissue for accommodating the diffusion of ions freely.

Salt absorption takes place through the intimate contact of the root system with the soil colloids or soil solution. Investigations have shown that ions are also absorbed through passive process also called nonmetabolic absorption. It has been observed that when a plant cell or tissue is transferred from a medium of low salt concentration to a medium of high salt concentration, there is an initial rapid uptake of ions, followed by a slow steady uptake that is under metabolic control. The initial rapid uptake is not affected by temperature or metabolic inhibitors indicates noninvolvement of metabolic energy. In response to the concept of outer space researchers turned to the task of calculating the volume of plant cell or tissue involved. They immersed a tissue in a solution of known concentration, allowed it to come to equilibrium and then determined the amount of salt taken up. Hope and Stevens (1952) found that bean root tips, when immersed in KCl solution, reached equilibrium in 20 minutes. The term apparent free space was introduced to describe the apparent volume accommodating the free diffusion of ions.

Theories (Passive absorption)

Donnan's equilibrium:

The absorption takes place in response to fixed ions.



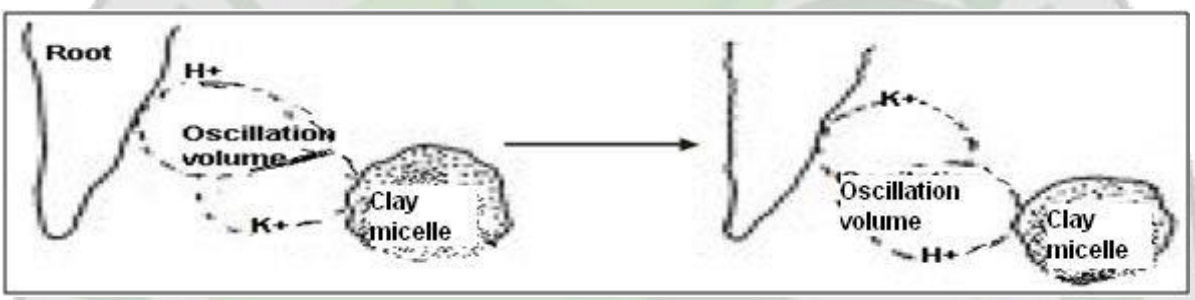
Suppose one cell is placed in the nutrient solution. Inside the cell membrane there is concentration of anions to which the membrane is impermeable. Suppose this membrane is permeable to anions and cations of the outer solution equal number of anions and cations will move across the membrane till equilibrium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

At equilibrium - $Ci^{+}\ Ai^{-} = CO^{+}\ AO^{-}$

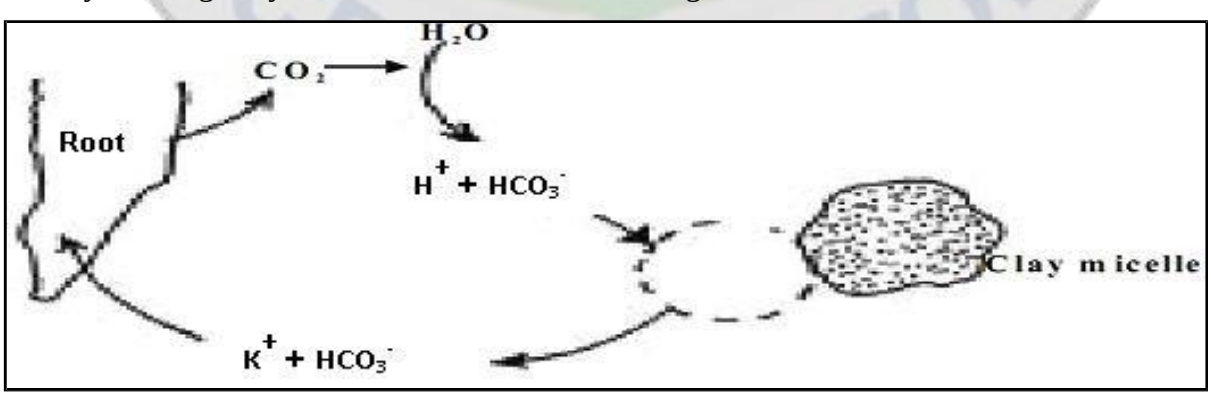
Ci = Cations Inside, Ai= Anions Inside

Co = Cations outside Ao = Anions outside

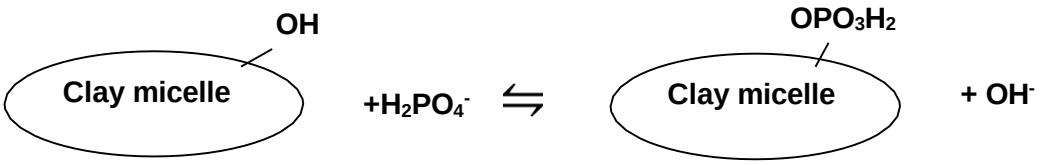
Contact exchange theory: Exchange of ions of the same charge held on the surface of soil colloids or root surface.



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



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



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

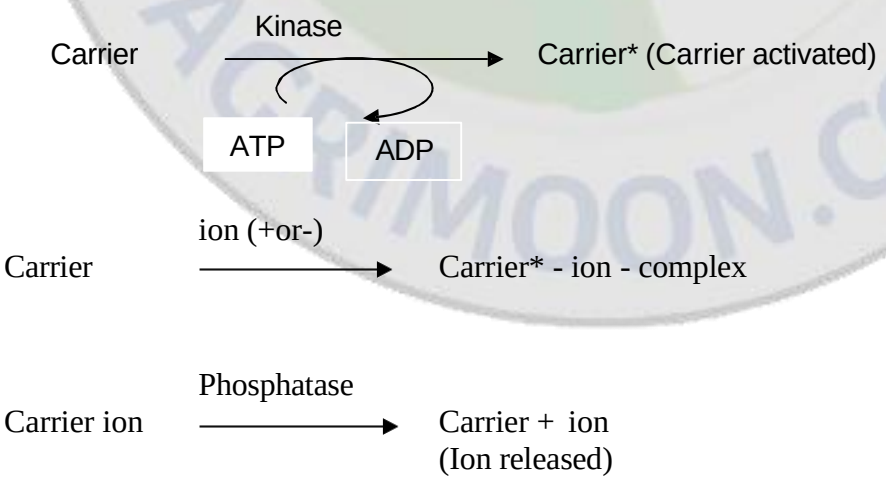
Carbonic exchange theory

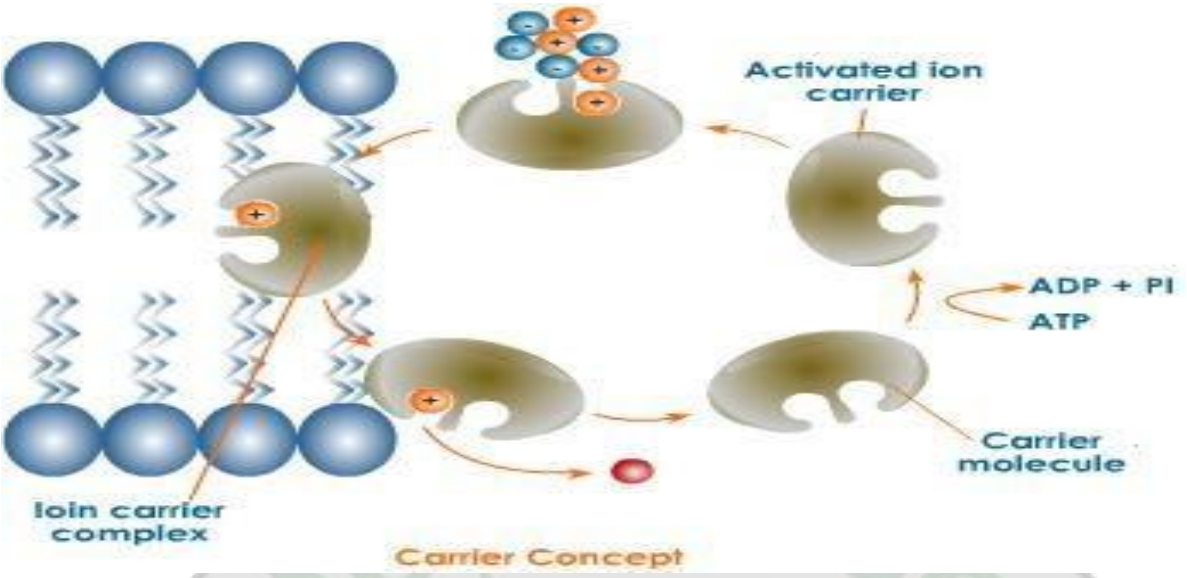
It is assumed that ions first dissolve in the soil solution, CO_2 released during the respiration dissolves in soil water forming carbonic acid (H_2CO_3) which is a week acid, it is converted into H^+ and HCO_3^- ions. H^+ ions reach the clay particles and release other cations like K^+ from clay by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

Theories of active absorption

Carrier hypothesis

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





Isotopic exchange

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

Saturation effects

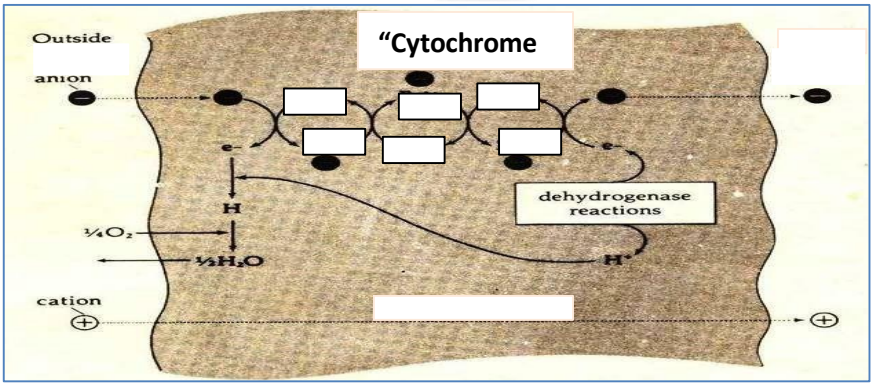
The concept presence of carriers in cell wall can also be demonstrated by experiments when there is a high salt concentration, absorption rate decreases due to engagement of all active sites or carriers.

Specificity

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

Ion pump mechanism: (Lundegardh and Burstrom 1933)

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



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

Later on Lundegardh (1950, 54) concluded:

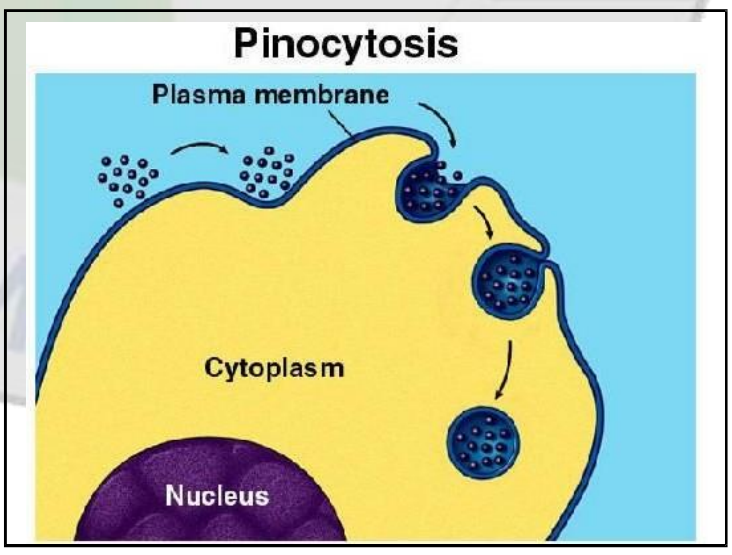
1. Anion absorption is independent of cation absorption and takes place by different mechanism.
2. An oxygen gradient exists from the outer surface to the inner surface of the membrane which favours oxidation at the outer surface and reduction at the inner surface.
3. Actual transport of anion occurs through a cytochrome system.

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

ATP Carrier Mechanism

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

Pinocytosis: It is the phenomenon which accounts for transport of larger molecules across the membrane like proteins, viruses etc. The plasma membrane is not smooth. The larger molecules first adhere to its surface. At this point the membrane inviginates and surrounds the particles on all sides forming a tiny vesicle or vacuole like structure around the particle. The vesicle is then pinched off from the membrane and molecules are released into the cytoplasm. Here the vesicle membrane dissolves releasing the particle into the cytoplasm.



Membrane transporters

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

Channels

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

Pumps

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

Carriersdf

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

Symporters

An integral membrane protein involved in movement of two or more different molecules or ions across a phospholipid membrane against the concentration gradient in the same direction. The phenomenon is called symport and proteins are called symporters.

Antiporter

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

Aquaporins

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

Factor affecting salt absorption

Temperature

An increase in temperature increases the salt absorption. However, beyond 40^0C temperature there was a decrease in salt absorption which was mainly due to denaturation of enzymes involved in salt absorption. Temperature changes affect both passive and active absorption processes. The rate of free diffusion depends on kinetic energy of diffusing molecules which is dependant on temperature. Low temperature also reduces rate of biochemical reactions required for active transport.

pH of soil: The availability of ions in the soil solution is greatly affected by hydrogen ion concentration or pH of the soil. For example monovalent phosphate $H_2PO_4^-$ which is readily taken up by the plants is common in acidic soils. However, as soil approaches towards alkaline medium HPO_4^{-2} and PO_4^{-3} forms are available. $H_2PO_4^-$ form is easily taken up by the plants, HPO_4^{-2} form is not easily taken up by the plant and PO_4^{-3} form is not absorbed by the plants. Hence soils having low pH values are associated with higher absorption of phosphorus.

Light

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

O_2 tension

The salt absorption is retarded in absence of O_2 due to decrease in ion pump mechanism and oxidation reduction processes.

Interaction of ions

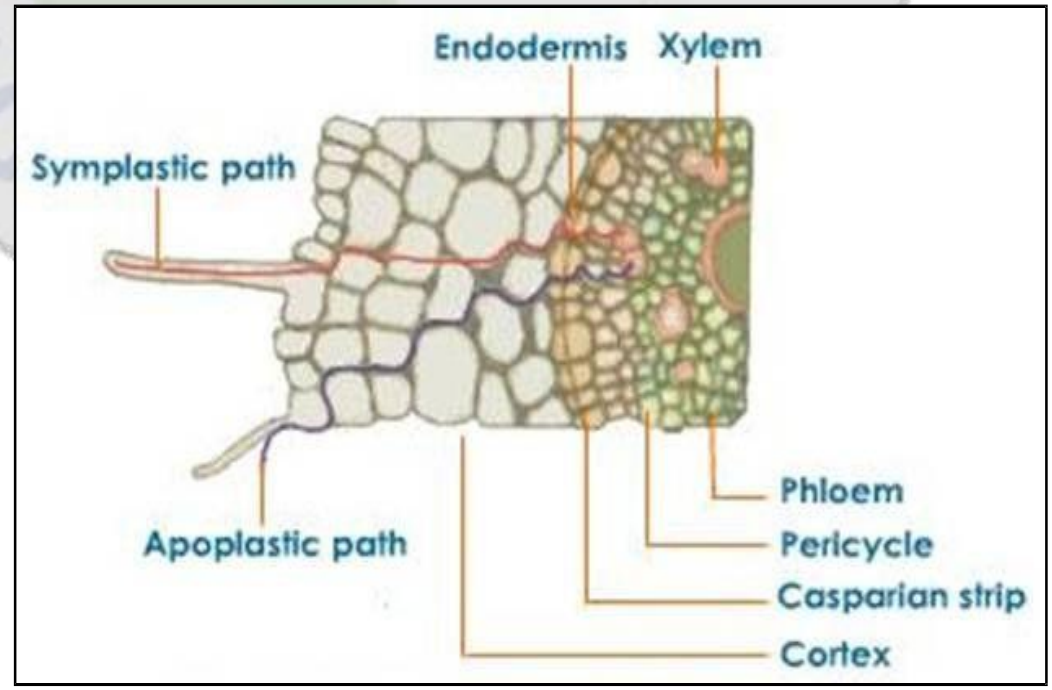
Absorption of one element is affected by presence of others. Viets (1944) found that the K^+ absorption is affected by presence of Ca^{++}, Mg^{++} and other polyvalent cations in external medium. He found that uptake of K^+ and bromine is less in absence of Ca^{++}, but it further decreases after the calcium concentration is increased past a maximum point.

Growth

The growth of plant increases surface area, number of cells, synthesis of new binding sites or carriers, factors etc. thereby increasing salt absorption.

Mechanism of ion uptake

The actual absorption of salts by roots is both passive and active. The movement of salts into apparent free space is passive allowing for free diffusion of ions. Apparent free space may confined to cell walls and part of cytoplasm. The absorbed ions move freely up to endodermis where further penetration is retarded by casparian strip. Diffusing ions move unhindered through wet cell wall



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

Translocation of salts in the xylem

The salts accumulated in the xylem ducts of roots are carried upward with the transpiration stream. The salts move upward in the xylem tissues has been demonstrated in several ways. Experiments have shown that the upward translocation of salts is retarded if xylem is removed. It is thought that the salts move upward through the transpiration stream. Arnon, Stout and Sipos (1940) noted in the tomato plants that radioactive phosphate travelled upward to the tip of tomato plants more rapidly in transpiring plants. Sutcliffe (1962) showed that if transpiration by a leaf is inhibited by covering the leaf with a polythene bag it also inhibited salt absorption. Stout and Hoagland (1939) showed using radioactive isotopes that potassium is translocated upward in the xylem tissue. However, lateral interchange of potassium between xylem, cambium and phloem may takes place.

Lateral translocation of salts

Generally xylem tissue is separated from the phloem tissue by a layer of cells called cambium. The cambial tissues regulate upward, lateral and downward movement of salts. Suppose if a particular element is present in phloem in high concentration, the cambium maintains balance by accommodating some salts inside. If a element is present in low concentration in phloem cambium enhances lateral translocation into the phloem.

Translocation of salts into the phloem

It is thought that upward movement of salts takes place through xylem from where they are translocated into the phloem.

Outward movement of salts from the leaves

Studies have shown that in deciduous plants prior to leaf abscission there is a movement of mineral nutrients out of leaves. Among nutrients moving out are N, P, K, S, Cl, Fe and Mg. Those remaining are Calcium, Boron, Manganese and Silicon. The withdrawal of mineral nutrients from leaves takes place in the phloem tissue.

Circulation and reutilization

Mason and Maskell (1931-36) concluded that minerals are taken up through the transpiration stream and moved to the leaves and excess quantities are retranslocated downward into the phloem. The mineral salts could be laterally transported into the xylem tissue where upward translocation could take place again.

Chapter 5
GROWTH AND DEVELOPMENT, GROWTH ANALYSIS

Growth

Growth is defined as an irreversible increase in size and volume of plant accompanied by increase in dry weight.

Development

It refers to the qualitative changes in plant parts. Example - Formation of flowers and fruits, falling of leaves etc.

Vegetative growth

Growth occurs from seed germination till before initiation of floral primordia. Important events are germination, seedling emergence, leaf and stem growth.

Reproductive growth

Growth that occurs from initiation of floral primordia till completion of seed formation. The important events are initiation of floral primordia, flower emergence, anthesis, pollination, fertilization, seed development and maturation.

Phases of growth

The growth is mainly accomplished in three main phases:

Cell division

In this phase cells divide and increase in number. If the plane of the cell division is transverse, the organ elongates as in stem and root. If the plane is longitudinal then girth or diameter increases.

Cell elongation

Enlargement is not symmetric in all planes, but enlargement is predominantly takes place in longitudinal direction. During the process the young cells absorb water due to higher osmotic pressure. As a result turgor pressure increases, cell wall stretches. Before cell division and enlargement nucleic acids, proteins, lipids, carbohydrates and other metabolites are synthesized in the cells which are utilized for synthesis of protoplasm and constituents like mitochondria, ribosomes, plastids, membrane and others. During this time protoplasm forms only a thin layer lining of the cell wall and a large vacuole filled with cell sap occupies the centre. Stretching of cell wall is made permanent by addition of cell wall materials to the original wall which becomes thicker and capable of further extension. New molecules may be inserted between original molecules known as intussusception. Other method in which new material is deposited by the protoplasm as a lining to the cell wall layers already present is known as apposition.

Cell differentiation

After cell division and enlargement cells change their shape, cell wall thickens. Cell differentiation is the process of specialization of cells to perform different functions. Examples - formation of xylem tracheids, sieve elements of phloem, guard cells of stomata from simple parenchymatous cells are some of the examples of differentiation.

Measurement of growth

Linear measurement

Generally growth is measured in terms of increase in length of root and shoot. Stem length is taken from soil surface to the tip of uppermost node.

Short comings

Variation in seedling growth. In dark stem elongates more but remains thin, whereas in light seedling is short and thick.

Fresh weight measurement

Weight of freshly harvested plant is taken as fresh weight.

Short comings

- Plant has to be removed while measuring fresh weight.
- It depends on water content of tissue which keeps on changing due to variation in rate of absorption and transpiration.

Dry weight measurement: Plant material is dried at 80^0C temperature for about two to three days till constant weight.

Short comings: Dry matter may increase due to deposition of food material like starch or may decrease due to oxidation of food material as a result of excess heating.

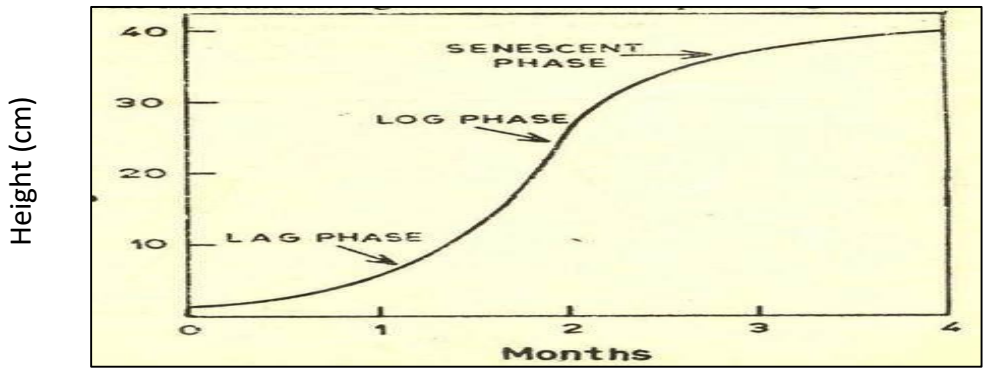
Leaf area measurement: Measurement of leaf area is also taken as an index of growth.

Short comings: Accurate determination is difficult without having sophisticated instruments.

Volume measurement: Volume of plant part or plant can be measured by water displacement method.

Short comings: It is not accurate due to difference in compactness of tissue.

Growth curve: When growth of the plant is plotted against time usually sigmoid (S shaped) curve is obtained. The growth curve remains same whether it is measured in terms of increase in height, fresh weight, dry weight or volume. In the initial stage growth rate remains slow called lag phase. Then growth increases very fast known as log phase, exponential growth phase or grand growth period. Towards the later stages growth becomes slowed down called senescent phase.

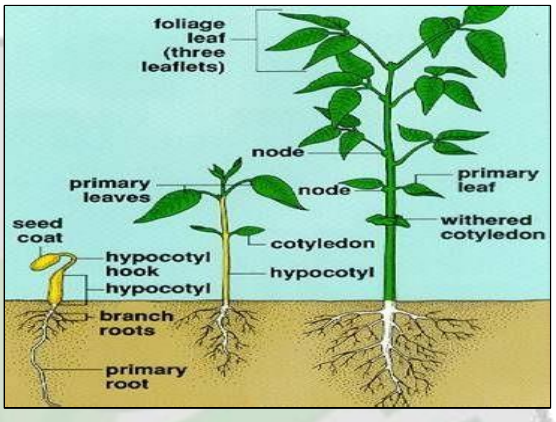


Determinate growth

Apical meristem soon becomes differentiated into an inflorescence and growth in length becomes stopped. Examples - most of the cereals, sunflower.

Indeterminate growth

In most of the plants shoot apical meristem remains active throughout the life of the plant and continues to form new tissues and organs. Such plants are called indeterminate plants. Examples - most of the pulses.



Growth Analysis

It is the method of estimating net photosynthetic production. It was worked out by (Blackman 1919; Briggs, Kidd and West 1920; Williams 1946; Watson 1952; Coombe 1960; Blackman 1968; Nichiporovich et al. 1961; Brike 1965 and Necaw 1965).

Net photosynthetic production: Amount of photosynthates left after the respiration and other biochemical reactions.

Biomass: Dry weight of whole plant including roots, also expressed as organic matter or energy content of plant.

Primary productivity: Rate of plant production over a certain period of time. It is also measured in terms of increase in dry weight, organic matter, CO_2 assimilation and solar energy fixed.

Growth Analytical Parameters

Relative Growth Rate (RGR)

Rate of increase in biomass per unit of biomass present. (Blackman 1919; Fisher 1921).

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

Weight Weight^{-1} Time^{-1}

W_1 & W_2 are dry weight of plants at two successive intervals t_1 & t_2, Ln - Natural log.

Importance:

Represents increment in existing biomass**.**

Crop Growth Rate (CGR)

Daily increment in plant biomass per unit ground area per unit time.

Watson (1952) CGR = $\frac{W_2 - W_1}{P(t_2 - t_1)}$ Weight Area^{-1} Time^{-1}

Where P = ground area which a plant occupies or group of plants occupy.

Importance:

Represents the daily increment in stand biomass**.**

Net Assimilation Rate (NAR):

- Rate of plant biomass increment per unit leaf area; synonym is unit leaf rate (ULR).
- Rate of measurement of photosynthesis per unit leaf area per unit time.

Gregory (1926) NAR = $\frac{W_2 - W_1}{A_2 - A_1} \times \frac{\text{Ln } A_2 - \text{Ln } A_1}{t_2 - t_1}$ Weight Area^{-1} Time^{-1}

Where A_1 & A_2 are leaf area at two intervals

Importance

Describes the per unit area efficiency of assimilatory apparatus.

Leaf Area Index (LAI)

- Total leaf area per unit area of ground.
- Ratio of assimilatory surface area to the ground area.

Watson (1952) LAI = $\frac{A}{P}$

For two stages Gardner *et al.* 1985 LAI = $\frac{\frac{A_1 + A_2}{2}}{P}$

Importance:

Represents canopy cover of that plant which is important in photosynthesis.

Optimum LAI

LAI values beyond which photosynthetic production or crop growth rate declines.

Leaf Area Duration (LAD)

Active period of leaf growth or persistence of leaf area.

Watson (1952) LAD = $\frac{A_1 + A_2}{2} \times t_2 - t_1$ Area Time

Importance:

Longer persistence of leaf area will facilitate the photosynthesis for a longer period of time.

Leaf Area Ratio (LAR)

- Ratio between total leaf area and total plant biomass.
- Ratio between leaf area and total plant dry weight.

Gregory (1926):

$$LAR = \frac{A}{W} \text{ Area weight}^{-1}$$

For two stages Gardner *et al.* (1985) $LAR = \frac{\frac{A_1}{W_1} + \frac{A_2}{W_2}}{2}$

Importance:

It characterizes the relative size of assimilatory apparatus which plays an important role in photosynthesis.

Specific Leaf Area (SLA):

- Leaf area per unit leaf dry Mass.
- Ratio of leaf area to leaf dry weight indicates relative proportion of assimilatory, conductive or mechanical tissues in leaves.

Watson (1952) $SLA = \frac{A}{WL}$ Area Weight $^{-1}$

For two stages Gardner *et al.* (1985) : $SLA = \frac{\frac{A_1}{WL_1} + \frac{A_2}{WL_2}}{2}$

Importance

It represents relative proportion of assimilatory, conductive or mechanical tissues in leaves which is important in photosynthesis, translocation of food material and providing mechanical strength to the leaves.

Leaf Weight Ratio (LWR)

Ratio between leaf dry weight and plant dry weight.

Watson (1952) $LWR = \frac{WL}{W}$

For two stages Gardner *et al.* (1985): $LWR = \frac{\frac{WL_1}{W_1} + \frac{WL_2}{W_2}}{2}$

Importance

Represents the relative proportion of leaf dry weight to the total dry weight.

Specific leaf weight (SLW) / Specific leaf mass

- Leaf dry mass per unit leaf area (LMA).
- Ratio between leaf dry weight and leaf area.

Beadle (1982) $SLW = \frac{WL}{A}$ Weight Area $^{-1}$

For two stages Gardner *et al.* (1985): $SLW = \frac{\frac{WL_1}{A_1} + \frac{WL_2}{A_2}}{2}$

Importance

Represents dry matter production per unit of leaf area.

Biomass Duration (BMD)

Parameter represents dry weight losses or gains during a unit time period.

Kvet (1962) $BMD = \frac{W_2 - W_1}{\text{Ln } W_2 - \text{Ln } W_1} (t_2 - t_1)$ Weight Time

Importance

Represents gain or loss of photosynthetic products during a particular period of time which may be crucial for growth and development.

Harvest Index (HI)

Ratio of economic yield to the biological yield (Synder and Carlson 1984).

$$HI (\%) = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

Economic yield refers to the yield of economic valuable part of plant and biological yield total dry weight of plant.

Importance:

HI refers to the potentiality of plant to transfer dry matter to the sink from the source.

Merits of Growth Analysis

- Represents a promising experimental approach in the initial stages of analyzing the complex characters of yield capacity.
- More suitable for smaller plants.
- Represents the first step in the analysis of primary production.
- Primary values on which it is based are easy to obtain without much demand and laboratory equipments.
- It can also be used to investigate the ecological phenomenon such as success of species in various habitats, competition among species or genetic differences in yielding capacity and effects of agriculture treatment on crop growth.
- Production efficiency of crop stand can also be measured.

Demerits of growth analysis

- It gives no direct indication of respiratory loss and this index does not necessarily serve as a direct measure of inherent photosynthetic capacities.
- It is not applicable for big trees.
- Does not measure real photosynthesis since it represents the net result of photosynthetic gain over respiratory loss.

Factors affecting growth

Temperature

Temperature affects biochemical reactions and thereby growth. Generally growth increases with increase in temperature up to optimum limit, thereafter it declines and stopped at 60^0 C. The optimum temperature differs from species to species. Rate of photosynthesis has lower temperature coefficient (Q10 = 2) and respiration has the higher (Q10 >2). The temperature coefficient is the ratio of rate of reaction at particular temperature and 10^0 lower. Therefore, when temperature increases, the rate of respiration increases more as compared to photosynthesis.

Thermoperiodicity

It is defined as response of plants to varying diurnal and nocturnal temperature. Ex. Tomato plants don't grow well if kept at 25^0 C during day and night but grow better if day temperature is 25^0 C and night temperature is 17- 20^0 C. In some plants vernalization is required for certain period to break the bud and seed dormancy.

Effect of low temperature and frost

Prolonged exposure to low temperature below the minimum kill the plants. The minimum temperature varies from species to species. Due to low temperature ice crystals form in the intercellular spaces. Ice crystals increase in size and withdraw water from the cells. As a result the protoplasm becomes desiccated and cell sap becomes concentrated. This may cause coagulation and precipitation of cell colloids resulting in death. In severe frost ice crystals may be formed within the cells which disrupt the physical structure of protoplasm by mechanical stresses. More number of colloids in the cells are associated with frost resistance due to following regions. 1. Lowers the freezing point. 2. Reduces the likelihood of internal ice formation. 3. Decreases the amount of water that can be withdrawn from the cell. 4. Reducing the amount of water that can be frozen.

Effect of high temperature

Above optimum temperature growth and other phenomenon of life are affected adversely. The ability to withstand high temperatures varies with the species. Certain bacteria which live in hot springs can withstand temperature even up to 70-80^0C. Generally lethal temperature lies between 50 - 60^0 C. High temperature causes coagulation of protoplasmic proteins. The high temperature increases the respiration rate and plant becomes starved and susceptible to attack of pathogens.

Effect of Light

The visible part of spectrum lies from 400-700 nm wavelength. From violet extending up to red region of spectrum affects growth. The chlorophyll absorbs light mostly from red (660 nm) and blue (400 nm) part of visible spectrum but absorbs very little from green part (490 nm). Therefore chlorophyll appears green. The radiations of longer wavelengths such as x rays, cosmic rays and ultra- violet rays are not required for growth. Light affects plant growth mainly because of its effect on chlorophyll synthesis, photosynthesis, phototropism, stomatal opening and transpiration.

Intensity of light

Generally in absence of light the seedling becomes weak, long and thin and leaves become minute. Such a seedling is said to be etiolated. Chlorophyll pigment does not develop and seedling remains pale yellow. On the other hand the seeds germinated in light produces a stout, short and healthy seedling with short internodes.

Duration of light

The duration of light in many plants decides when the plant should stop vegetative growth and start the reproductive phase of growth. Soybean plants when grown in Aug.-Sep. starts flowering shortly when natural duration of sunlight is relatively short.

Quality of light

Blue light (400-510 nm) is absorbed by the pigments Beta carotene and riboflavin and causes bending of plant organs towards or away from light, while red is absorbed by phytochrome Pfr. These pigments mediate a number of photomorphogenetic responses such as seed germination and flowering.

Moisture and water deficit

Water provides turgidity to plant cells and due to this the stem becomes erect and leaves are horizontal. Water deficit occurs when transpiration rate exceeds absorption rate. Temporary water deficit occurs in plants during the noon but plant recovers during the night. Prolonged water deficit occurs in most of the plants due to inadequate water in soil or due to impaired water absorption because of excess of dissolved salts in soil solution. Water deficit causes reduced turgor pressure which affects cell division and enlargement adversely resulted in reduced growth. It also causes closure of stomata which inhibits photosynthesis and respiration rates. Water deficit causes decreased hydration of proteins which changes the physiochemical properties of protoplasm like viscosity, permeability etc. Water deficit decreases the synthesis of growth promoting hormones like auxins, gibberellins and cytokinins but promotes the synthesis of growth inhibiting hormones abscisic acid and ethylene. Water deficit also causes inhibition of protein and nucleic acid synthesis, but promotes hydrolysis of starch and proteins leading down to accumulation of sugars and amino acids. Among amino acids proline accumulates the maximum. The stages maximum sensitive to water stress are germination, flowering and seed development.

Plants have adapted various measures to withstand the deleterious effects of water deficit. Root system becomes very much branched and developed to facilities better absorption. The

leaves become smaller in size and develop thick cuticle and hairy covering. The leaf veins become extensively branched and the stomata decrease in number and often become sunken in pits. The leaf anatomy also undergoes changes. The intercellular spaces decrease and the cells become more compact and smaller in size. All such characters helps in reducing the transpiration and increasing absorption.

Water logging

Excess amount of water in soil is also harmful. For optimum growth soil moisture should be 20-30% and 10-20% soil air. At field capacity clayey soil holds more moisture than sandy soil. When water content of soil exceeds its field capacity soil is said to be water logged. Under water logged conditions the capillary pores in between the soil particles are filled with water replacing air. As a result the soil moisture becomes anaerobic. The supply of atmospheric O_2 through water is inhibited. Under anaerobic conditions bacteria reduce nitrate and sulphate into ammonia and hydrogen sulphide which are toxic to the plants. Due to less availability of respiratory energy the membrane become leaky and solutes leach out of root. The osmotic gradient of root cortox cells is lost resulting in inhibition of movement of water from root hair to cortex. Plant also suffers from mineral deficiency due to decrease in rate of active uptake of minerals due to less availability of respiratory energy. Water logging inhibits root and shoot growth. In severe cases leaves turn yellow abscise. Flowering and fruiting also becomes reduced. Crops tolerant to water logging are rice and sugarcane whereas, crops very sensitive to water logging are tobacco and tomato.

Mineral content of soil and soil salinity

The soil solution is usually very dilute with the osmotic potential of -1 bar when sulphates and chlorides of magnesium and sodium or other salts accumulate in soil the osmotic potential of soil decreases to -10 to -20 bars. This inhibits the water absorption even leads to plasmolysis. Soil is said to be saline. The plants which can be grown in saline soils like casuraina are called halophytes (salt concentration may be 20% in soil) whereas, others are called glycophytes. Due to accumulation of salts in soil the osmotic pressure of soil is more as compared to roots which decreases absorption of water. This is known as osmotic stress or physiological drought. Under saline conditions generally chlorides and sulphates of sodium and magnesium are found in abundance. Excess absorption of such elements is toxic to the plants. Excess of Na and chlorides decreases the availability of N, P, K, Ca, Fe, Cu and other elements. Salinity reduces the protein and nucleic acid synthesis in plants. Growth of root and shoot is retarded. Crops sensitive to salinity are beans, radish and pulses while beet root, rice and barely are tolerant.

Chapter 6
PLANT GROWTH SUBSTANCES

Hormones or phytohormones

Organic substances produced by the plants which in minute quantities increase, decrease or modify growth and development of plant. Their site of synthesis is different from site of action. Examples - auxins, gibberellins, cytokinins, ethylene, abscisic acid. Plants also produce florigen and vernalin but these two have not been extracted and purified.

Growth Regulators

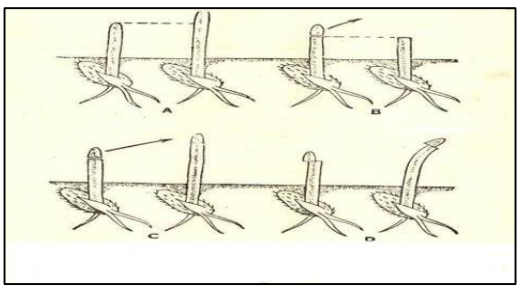
Some artificially synthesized compounds also perform same action as that of hormones. They along with hormones are called regulators. Examples - Indole butyric acid (IBA), Naphthalene acetic acid (NAA), 2, 4 - Dichlorophenoxy acetic acid (2, 4-D), Para chloro phenoxy acetic acid (PCPA).

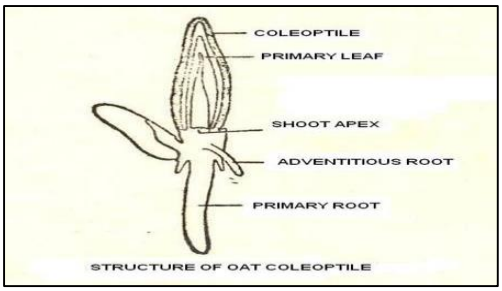
Natural auxins

Plants are known to produce only one auxin i.e. Indole 3 Acetic Acid (IAA). Other compounds are indole pyruvic acid, indole acetonitrile and indole acetaldehyde. These compounds do not act as auxins till these are converted into IAA within plants.

Auxins

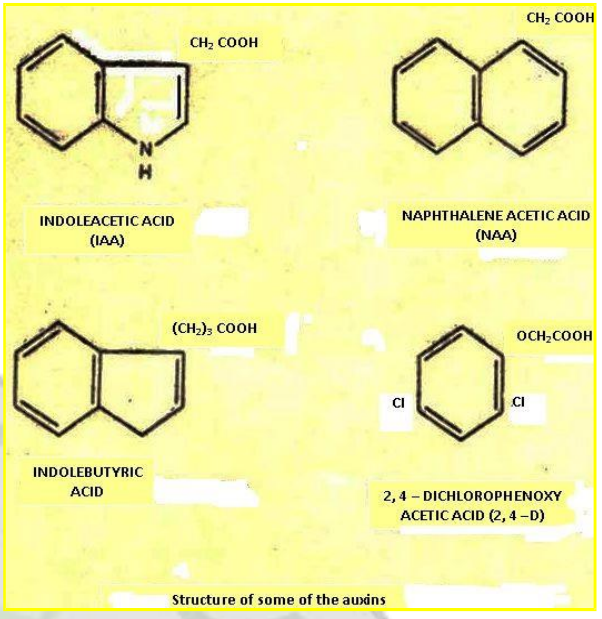
The idea of existence of auxins was first discovered by Charles Darwin (1881). He found that the tip of Canary grass (*Phallaris canariensis*) bends towards light. When tip of coleoptile was removed it does not bend. Therefore, it was thought that some substance found at the tip is transported downward and causes bending. However, auxin was first discovered in coleoptile *of Avena sativa* (oat). When oat seed germinates one green and tubular structure is emerged called coleoptile which covers the primary leaf and stem tip. These structures come out of coleoptile by piercing it. The oat coleoptile grows up to 6-7 cm in dark. The growth of coleoptile is stopped when tip is removed. When tip is again placed over the stump the growth is again resumed. If the tip is not placed exactly in its original place on the coleoptile stump but asymmetrically towards one side, the growth also becomes asymmetric. Therefore, it can be concluded that some type of stimulus is produced in the tip of coleoptile which is necessary for growth of coleoptile. This stimulus travels downward from the tip and causes the growth. The stimulus produced by the coleoptile tip was shown to be hormonal in nature and was named auxin by F. W. Went. The auxin can be collected in thin layer of agar block. Auxin present in the coleoptile tip diffuses and collects in agar block .Kogl (1934) collected Auxin A, Auxin B and Heteroauxin from the human urine.





STRUCTURE OF OAT COLEOPTILE

Chemical nature of auxins

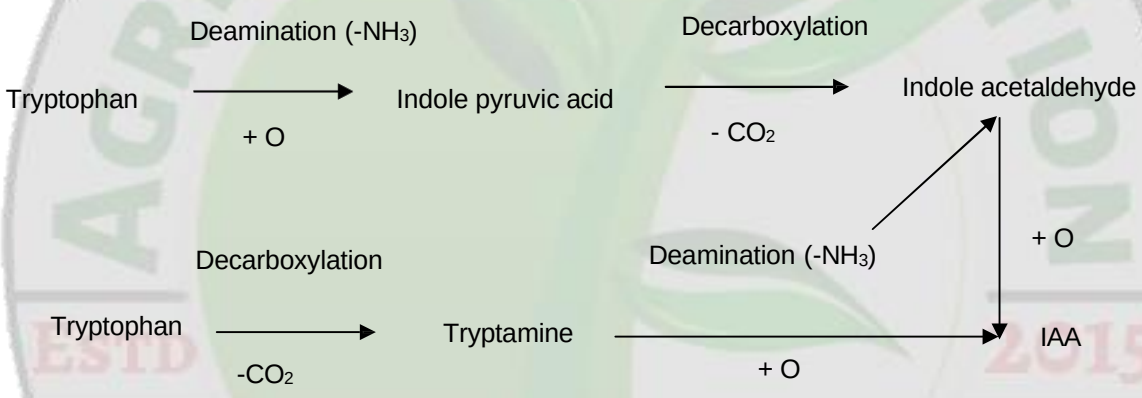


They have an

- Unsaturated ring.
- COOH group.
- At least one carbon atom between ring and COOH group.
- Molecules possess negative as well as positive charges.

Antiauxin -DCA (2, 4 dichloranizole) is antiauxin as it competes with the auxin for same site of action in cell.

Pathways of production: There are two major pathways through which auxins are produced as follows:



Auxin transport

Polar (in single direction), in stem and coleoptile it is basipetal (from tip to the base), whereas in roots it is acropetal (from base to the tip). Transport involves metabolic energy. Inhibitors of transport are cyanide and dinitrophenol.

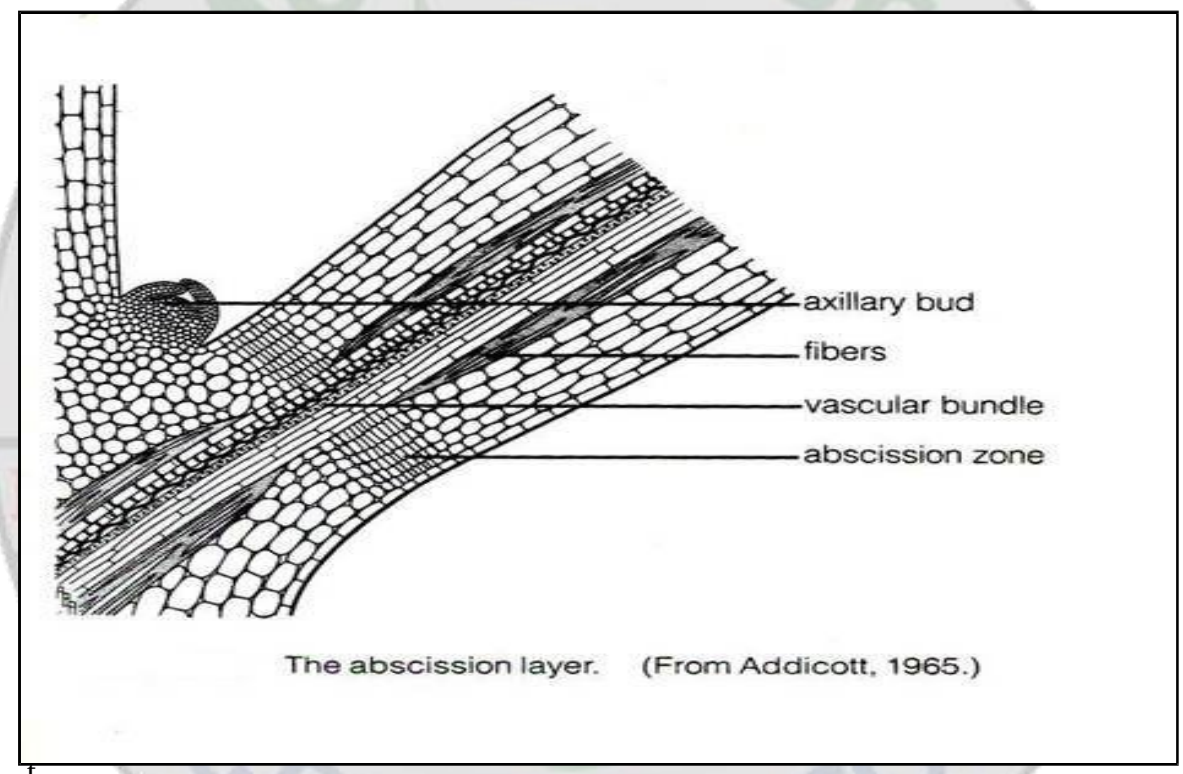
Auxin inactivation

1. Photooxidation 2. Enzyme oxidation by IAA oxidase 3. Binding with glucose or some amino acids to form inactive complex.

Physiological role

- Required for coleoptile and stem growth.
- Useful in ell elongation.
- Useful in secondary growth.
- Required for apical dominance.
- Enhances rooting in cuttings.

- It Increases the number of female flowers and decreases the male flowers in plants. In dioecious species like Cannabis which produce male flowers application starts producing female flowers and reducing the male flowers.
- Generally without fertilization flower drops and fruit formation is inhibited, but in certain plants like tomato and brinjal application of auxin causes development of fruits without pollination and fertilization. Development of fruits from ovary without fertilization is called parthenocarpy. Such fruits are seedless. Certain fruits like Banana and orange are naturally parthenocarpic because in such plants the ovary before pollination possess higher concentration of auxin which enables it to develop into the fruits.
- Auxin prevents leaf and fruit abscission. Cells of abscission layer are compact, thin walled and rectangular in shape and secret hydrolytic enzymes like cellulase and pectinase. This leads to the dissolution of cell wall. The organ detaches at this point and falls. Auxin application prevents action of these enzymes.



The abscission layer. (From Addicott, 1965.)

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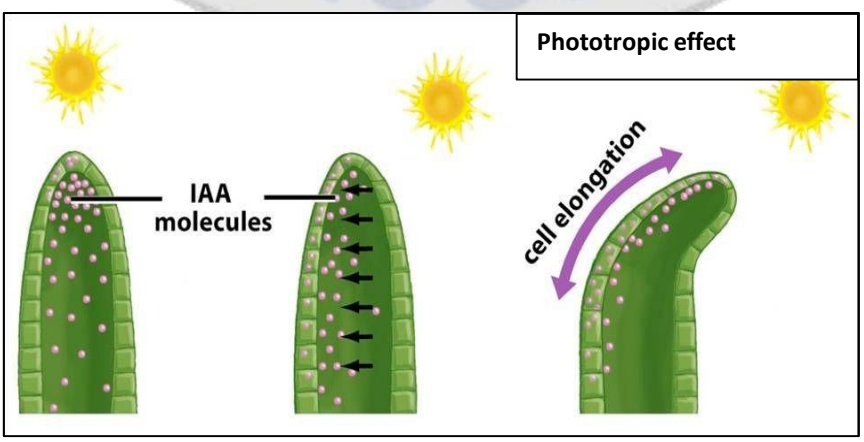
em or coleoptile is shaded it receives 65% of auxin, while portion of light side receives 35% of auxin, therefore cells of dark side develops more. This leads to the bending towards light side. The phenomenon is called phototropism.

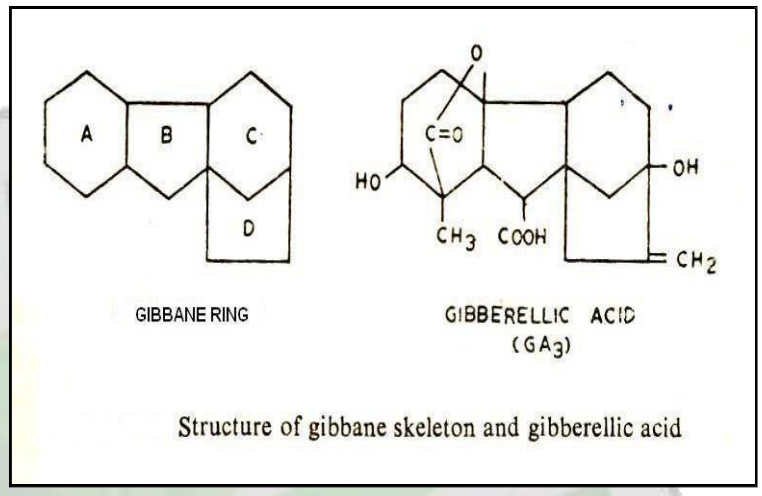


Gibberellins

First discovered by kurosawa a Japanese Plant Pathologist in 1928. He found that rice plant infected with *Gibberella fujikuroi* showed excessive stem elongation which is called Bakanae disease. Substance which caused this effect was named as gibberellin. Found in algae, fungi, liverworts and higher plants. Most common is GA_3.

Structure

- Possess gibbane skeleton consists of 4 rings designated as A, B, C and D.
- Some contains 19 and some 20 carbon atoms.
- Acidic in nature and possess one or more COOH groups.
- Gibberellins differ in presence or absence of OH group and degree of unsaturation of ring A.



Structure of gibbane skeleton and gibberellic acid

Physiological role

- Required for growth of root, stem, leaves and coleoptile.
- Dormancy breaking as it stimulates synthesis of amylase.
- Induces flowering.
- Required for apical dominance.
- It promotes cell division.
- Induces sterility in plants by suppressing growth of androecium.
- Required for parthenocarpy in apple, tomato and cucumber.

Biosynthesis

It starts from mevalonic acid. Kaurene is an intermediate compound.

The first gibberellins formed are GA_4 and GA_{12}. Other gibberellins are formed from these gibberellins.

Transport

Nonpolar, in all directions.

Cytokinins

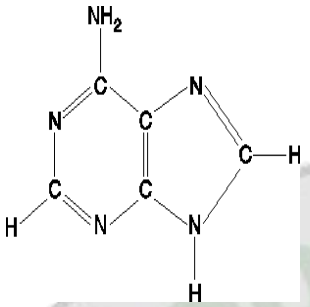
Derivatives of adenine in which the H on the N_6 position of the ring is substituted by either benzyl group forming benzyladenine, furfuryl group forming kinetin or isopentenyl group forming zeatin.

Physiological role

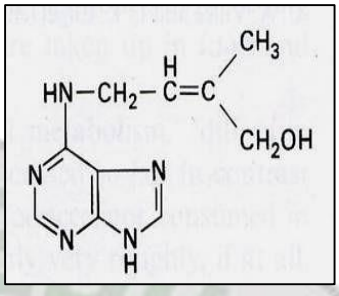
- It causes cell division in combination with auxin in tobacco pith cells. Such cells are called callus. If concentration of auxin to cytokinin is 100:1, then cells of callus form roots. If

ratio of auxin: cytokinin is decreased to 1:1, number of shoot buds are differentiated on the callus. These buds finally develop into tiny tobacco plants.

- Application of cytokinins on leaf delays the senescence. It prevents degradation of metabolites like proteins, nucleic acids and chlorophylls in leaves thus prevent senescence.



Adenine



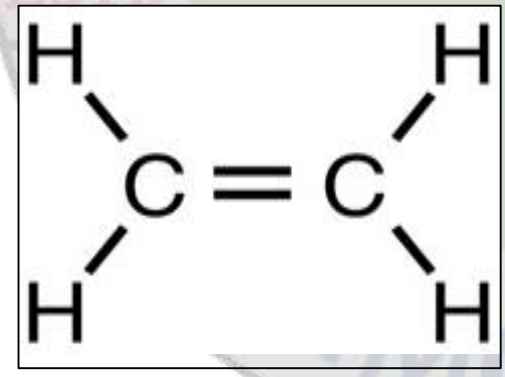
Zeatin

Senescence

Shedding of plant parts in response to ageing, results in degradation of fats, proteins, chlorophylls and nucleic acids into simpler compounds like amino acids, fatty acids, glycerols, nucleotides, nucleosides. These soluble substances are exported out of leaves which become chlorotic, dries and falls down.

Ethylene

It is a gaseous hormone also called pheromones, colorless hydrocarbon gas lighter than air synthesized in every living cells of plant body and moves by diffusion in all directions. Silver nitrate and CO_2 are antiethylene. Main Sites of its synthesis are shoot tip, root tip and nodal regions. Methionine, light, temperature and drought promotes its production. Auxin also promotes its production, therefore it is also called secondary hormone.

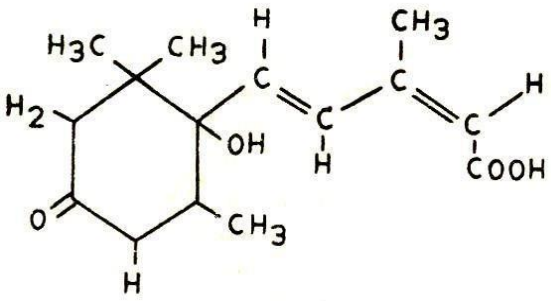


Ethylene

Abscisic Acid (ABA)

sting of 15 carbon atoms. Acidic in nature and possess single COOH group. Two optical isomers of abscisic acid exist but dextro rotatory (+ABA) occurs in plants. The starting material for biosynthesis is mevalonate.

Physiological role



Structure of abscisic acid

- Application inhibits growth of root, stem, leaf and coleoptile. Effect is reversed if gibberellic acid is applied.
- Induces dormancy in many species.
- Under drought conditions ABA accumulates in plants which causes closure of stomata thus prevents further water loss.
- Promotes abscission of leaves, flowers and fruits.

Growth retardants

- The most common are Cycocel, B-Nine and Phosphon D. They inhibit gibberellin production which leads to dwarfness. They are synthetic chemicals and do not occur in plants.

Physiological role

- Used in dormancy breaking in seeds of lettuce.
- Important in germination particularly in wheat, barley etc. It induces synthesis of amylase.
- Induces rooting in cuttings but inhibits formation of adventitious roots on stem cuttings.
- Causes flowering in rosette long day plants and biennials requiring vernalization.

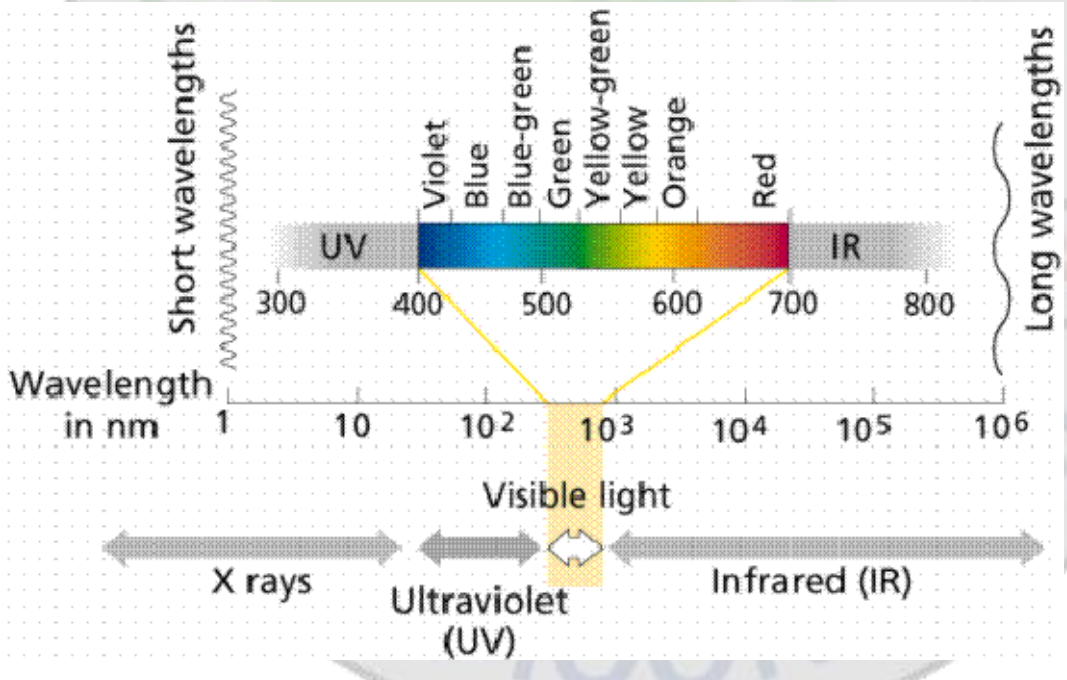
Chapter 7
Photosynthesis

Living things require energy to stay alive. The main energy input to planet earth is from the sun. There are several groups of living things that can harness energy from the sun. These organisms are known as autotrophs – i.e. they produce their own food. There are different ways that they achieve the gathering of sunlight and the conversion of the sun's energy to chemical energy. However, the most common form of photosynthesis occurs in algae, higher plants and certain cyanobacteria. Animals that gain energy by eating other living things are called heterotrophs.

How do photosynthetic organisms capture the sun's energy?

The sun produces a vast amount of energy in many different forms. The main form of energy from the sun is in the form of electromagnetic radiation, although it also produces vast quantities of subatomic charged particles into the space around it.

The electromagnetic radiation from the sun can be shown in a diagram:



Visible light passes readily through the atmosphere, and it is these wavelengths (between 400nm and 700nm) that photosynthetic organisms use. Photosynthetic organisms contain a variety of coloured pigments, normally tightly organised on membranes within chloroplasts. Of these, chlorophyll is the most important. When white light hits these pigments, selective wavelengths of light are absorbed. When radiation is absorbed, it is typically converted into and lost as heat.

For photosynthetic organisms, losing the sunlight they absorb as heat is mostly useless. They must convert the energy into a form that they can store and use later to drive cellular reactions. Photosynthetic organisms convert light energy into chemical energy. This chemical energy can be stored or used to drive cellular reactions. Little of the absorbed sunlight is lost as heat.

Through photosynthesis, light energy is transformed to chemical-bond energy in the form of ATP. ATP is then used to produce complex organic molecules, such as glucose. It is from these organic molecules that organisms obtain energy through the process of cellular respiration. In algae and the leaves of green plants, photosynthesis occurs in cells that contain organelles called chloroplasts. Chloroplasts have two distinct regions within them: the grana and the stroma. Grana consist of stacks of individual membranous sacs, called thylakoids, that contain chlorophyll. The stroma are the spaces between membranes. The following equation summarizes the chemical reactions photosynthetic organisms use to make ATP and organic molecules:

light + energy + carbon dioxide + water → glucose + oxygen

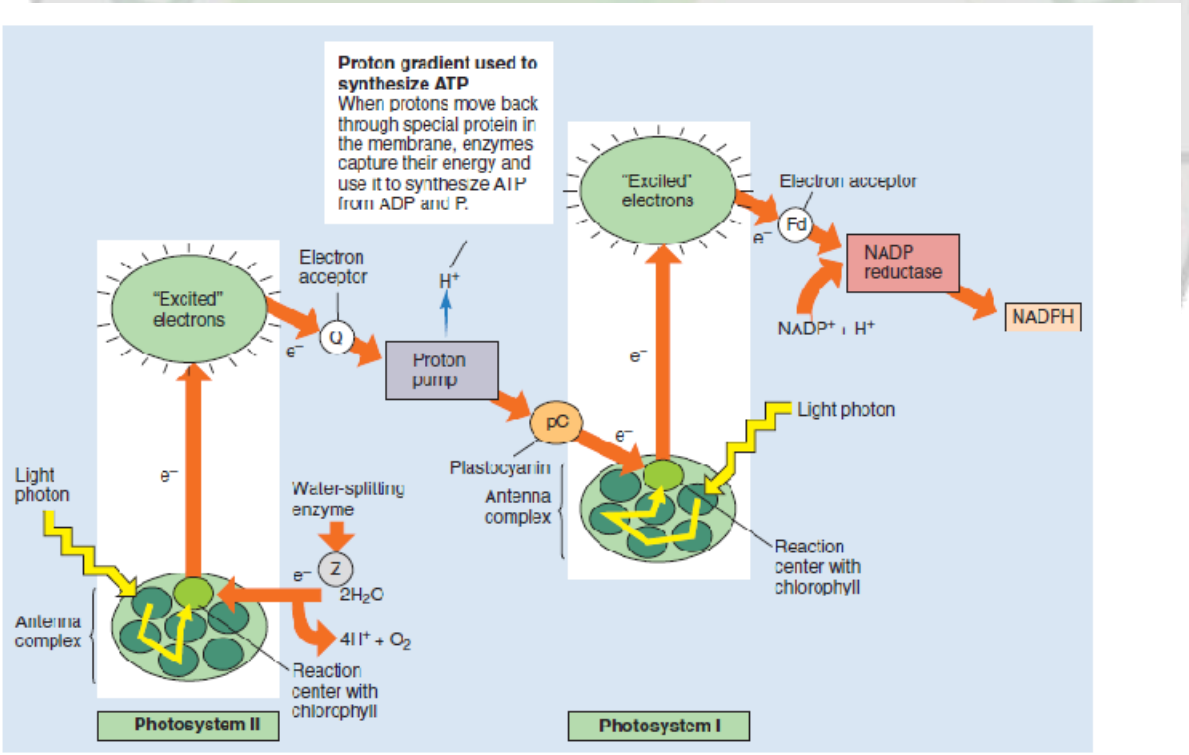
$$\text{light} + \text{energy} + 6\,CO_2 + 6\,H_2O \rightarrow C_6H_{12}O_6 + 6\,O_2$$

There are three distinct events in the photosynthetic pathway:

1. Light-capturing events. In eukaryotic cells, photosynthesis takes place within chloroplasts. Each chloroplast is surrounded by membranes and contains chlorophyll, along with other photosynthetic pigments. Chlorophyll and the other pigments absorb specific wavelengths of light. When specific amounts of light are absorbed by the photosynthetic pigments, electrons become "excited." With this added energy, these excited electrons can enter into the chemical reactions responsible for the production of ATP. These reactions take place within the grana of the chloroplast.

2. Light-dependent reactions. Light-dependent reactions use the excited electrons produced by the light-capturing events. Light-dependent reactions are also known as light reactions. During these reactions, excited electrons from the light-capturing events are used to produce ATP. As a by-product, hydrogen and oxygen are also produced. The oxygen from the water is released to the environment as O_2 molecules. The hydrogens are transferred to the electron carrier coenzyme $NADP^+$ to produce NADPH. ($NADP^+$ is similar to NAD^+. These reactions also take place in the grana of the chloroplast. However, the NADPH and ATP leave the grana and enter the stroma, where the light independent reactions take place.

3. Light-independent reactions. These reactions are also known as dark reactions, because light is not needed for them to occur. During these reactions, ATP and NADPH from the light-dependent reactions are used to attach CO_2 to a 5-carbon molecule, already present in the cell, to manufacture new, larger organic molecules. Ultimately, glucose (C_6 H_{12} O_6) is produced. These light- independent reactions take place in the stroma in either the light or dark, as long as ATP and NADPH are available from the light- dependent stage. When the ATP and NADPH give up their energy and hydrogens, they turn back into ADP and $NADP^+$. The ADP and the $NADP^+$ are recycled back to the light-dependent reactions to be used over again. The process of photosynthesis can be summarized as follows. During the light capturing events, light energy is captured by chlorophyll and other pigments, resulting in excited electrons. The energy of these excited electrons is used during the light-dependent reactions to disassociate water molecules into hydrogen and oxygen, and the oxygen is released. Also during the light-dependent reactions, ATP is produced and $NADP^+$ picks up hydrogen released from water to form NADPH. During the light-independent reactions, ATP and NADPH are used to help combine carbon dioxide with a 5-carbon molecule, so that ultimately organic molecules, such as glucose, are produced.



Photosystems II and I and How They Interact: Although light energy strikes and is absorbed by both photosystem II and I, what happens and how they interconnect are not the same. Notice that the electrons released from photosystem II end up in the chlorophyll molecules of photosystem I.

The electrons that replace those "excited" out of the reaction center in photosystem II come from water.

Types of Photosynthesis:

There are three distinct biochemical variants or types of photosynthesis based on the mechanism that plants employ by which carbohydrate is formed from CO_2: C_3 photosynthesis, C_4 photosynthesis, and CAM photosynthesis.

C_3 photosynthesis:

1. Plants operate Calvin Cycle only in all green cells.
2. There is only one CO2 acceptor, i.e., RuBP.
3. The first stable product of photo-synthesis is PGA (a C3 acid).
4. "Kranz anatomy" is not found. There is no chloroplast dimorphism. They have well defined grana with both PS-I and PS-II.
5. There is no CO2 concentrating device. Fixation and assimilation of C takes place only through Calvin cycle in the day. So, there is no decarboxylation mechanism.
6. Photorespiratory toss of photo- synthates is very prominent due to dual action of rubisco and lack of PEPcase. Up to 40% of photosynthates may be lost.
7. CO_2 compensation point is 40-100 µl l^{-1}.
8. Intracellular CO_2 concentration in light is 200 µll^{-1}.
9. Stomatal frequency is 2000 – 31000.
10. Water use efficiency is 1-3 g CO2 fixed/kg water transpired.
11. Maximum growth rate is 5-20g m-2 d^{-1}.
12. Maximum productivity 10-30 t $ha^{-1}y^{-1}$.
13. Typical species of economic impor-tance are wheat, barley, rice, potato.
14. 89% world flora (in species number).
15. Widely distributed and dominant in forests.

C_4 photosynthesis:

1. Plants operate C_4 cycle in MC in addition to C_3 cycle operating in BSC.
2. There are two CO_2 acceptors — PEP and RuBP.
3. The first stable product is malate or aspartate (aC_4acid).
4. The leaves show "Kranz anatomy". The chloroplasts are dimorphic. The MC chloroplasts are granal whereas the BSC chloroplasts are agranal lacking PS-II.

5. Plants are specially characterized by CO_2 concentrating mechanism. So, there is initial carboxylation in MC followed by decarboxylation in BSC. Both are occurring in same time (day) but separated in space.

6. Photorespiration cannot be detected due to the high activity of PEP case in MC. The C_4 cycle gears the C_3 cycle by pumping CO_2 in BSC. Rubisco cannot behave as oxygenase.

7. CO_2 compensation point is 0-10 μll^{-1}.

8. Intracellular CO_2 concentration in light is 100 μll^{-1}.

9. Stomatal frequency is 10000-16000.

10. Water use efficiency is 2 – 5 g of CO_2 fixed/kg of water transpired.

11. Maximum growth rate is 40-50g m-2d^{-1}.

12. Maximum productivity is 60 – 80 t ha^{-1} y^{-1}.

13. Typical species of economic importance are maize, millet, sugarcane, sorghum.

14. < 1% world flora (in species number).

15. Warm to hot open sites (grassland).

CAM Photosynthesis:

1. Plants operate only C3 cycle in MC for carbon assimilation.

2. Same as C4.

3. The initial fixation product is malate in dark, which remains stored in vacuole.

4. No “Kranz anatomy” is found. The chloroplasts are not dimorphic.

5. Plants show CO_2 accumulating device as malate during night as they are adapted to arid zone. So, acidification and de-acidification occur in the same space (MC) but separated in time. The former takes place in dark while the latter takes place in light.

6. Photorespiration cannot be detected as the stomata remain closed during day. The photo-respiratory CO2 cannot escape instead is re-fixed by Rubisco.

7. CO_2 compensation point is 0-10 μll^{-1} (in dark).

8. Intracellular CO_2 concentration in light is 10,000 μll^{-1} (in dark).

9. Stomatal frequency is 100-800.

10. Water use efficiency is 10-40g of CO_2 fixed/kg of water-transpired.

11. Maximum growth rate is 0.2g $m^{-2}d^{-1}$.

12. Generally Maximum productivity is <10t $ha^{-1}y^{-1}$.

13. Typical species of economic importance is Pineapple.

14. 10 % world flora.

15. Xeric sites (includes epiphytes)

Chapter 8

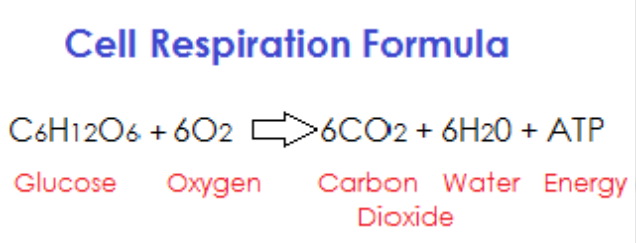
Respiration

What Is Respiration?

When we hear the word 'respire' we probably think of breathing. When you breathe, you are taking in oxygen with each inhale and releasing carbon dioxide with each exhale. This gas exchange is important for respiration, but while breathing is a physical process, respiration can be thought of as more of a chemical process. All organisms, from a single bacterial cell to a coral reef colony to a blue whale, undergo respiration.

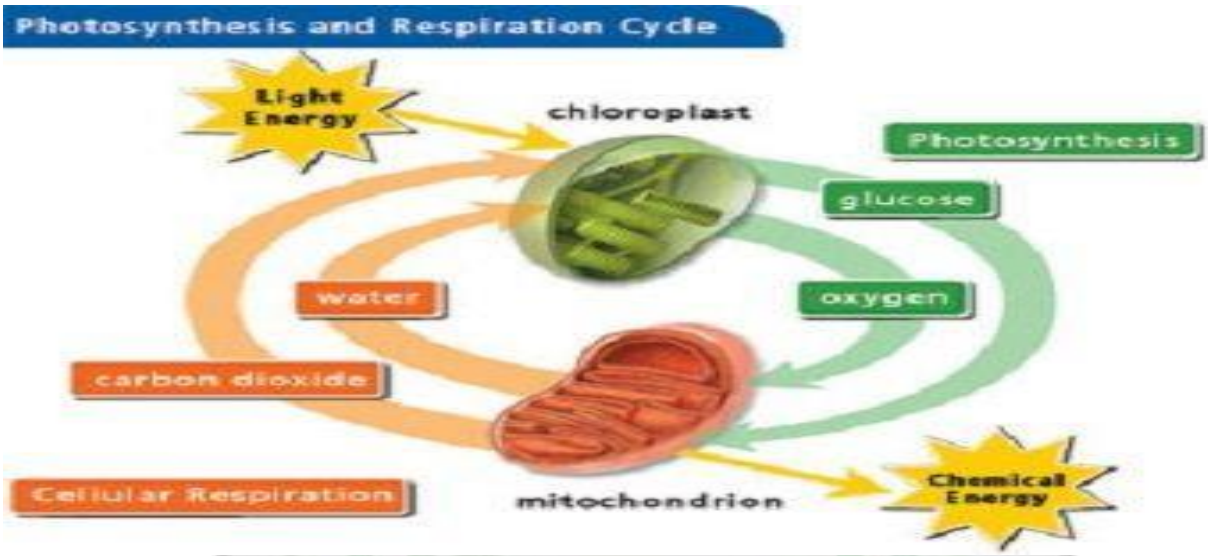
Food molecules absorbed after digestion are taken in, broken down, and the energy freed in the process is used to power the organism's movements and physiological functioning. Respiration is the biochemical process in which the cells of an organism obtain energy by combining oxygen and glucose, resulting in the release of carbon dioxide, water, and ATP (the currency of energy in cells).

When we examine the equation for cellular respiration, we see that the reactants are glucose and oxygen (for aerobic respiration), and the products are carbon dioxide, water, and ATP. Note the number of oxygen, carbon dioxide, and water molecules involved in each 'turn' of the process.



Balance with Photosynthesis

Respiration is the antithesis to the process of photosynthesis, in which carbon dioxide and water are taken in by autotrophs, along with sunlight, to make glucose and oxygen. Autotrophs include any photosynthesizing organisms, such as plants and algae, all of whom also undergo respiration. The products of photosynthesis are taken in by heterotrophs, organisms that cannot make their own energy and rely upon autotrophs for food. The byproducts of their respiration - carbon dioxide and water - are then used for photosynthesis. In a balanced ecosystem, this exchange is an example of a negative feedback loop.

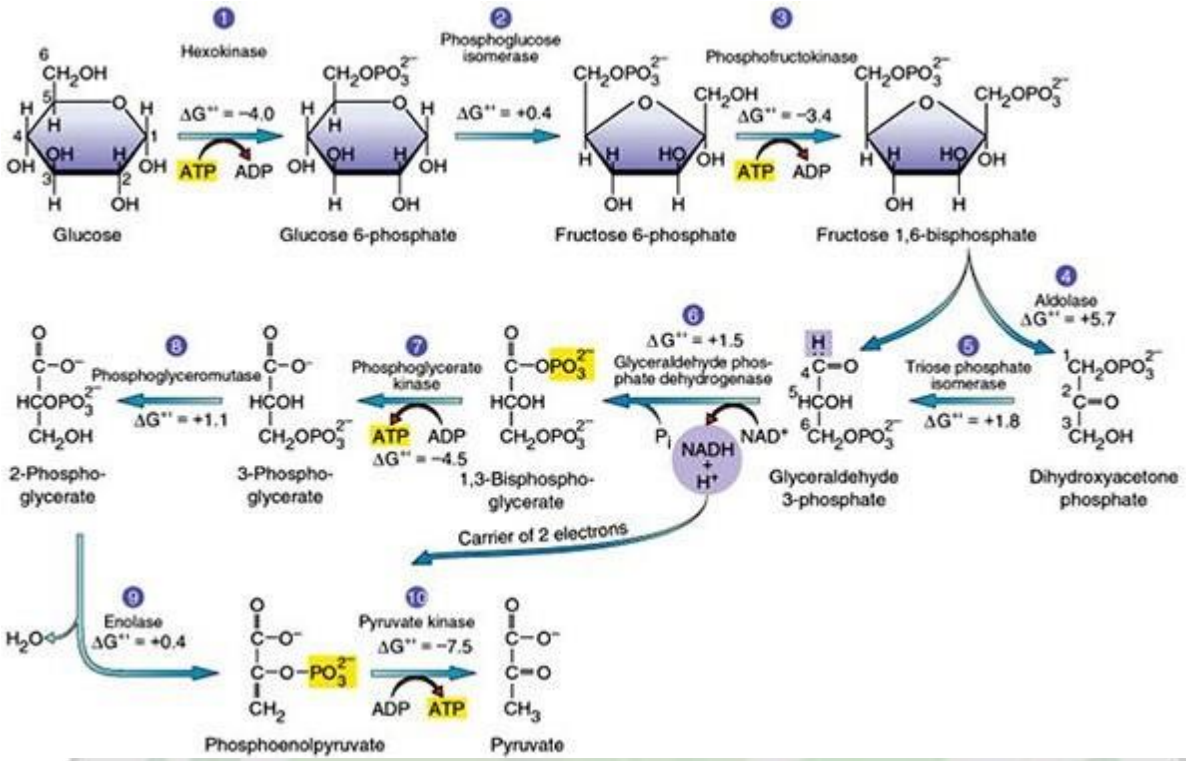


Three Phases of Respiration

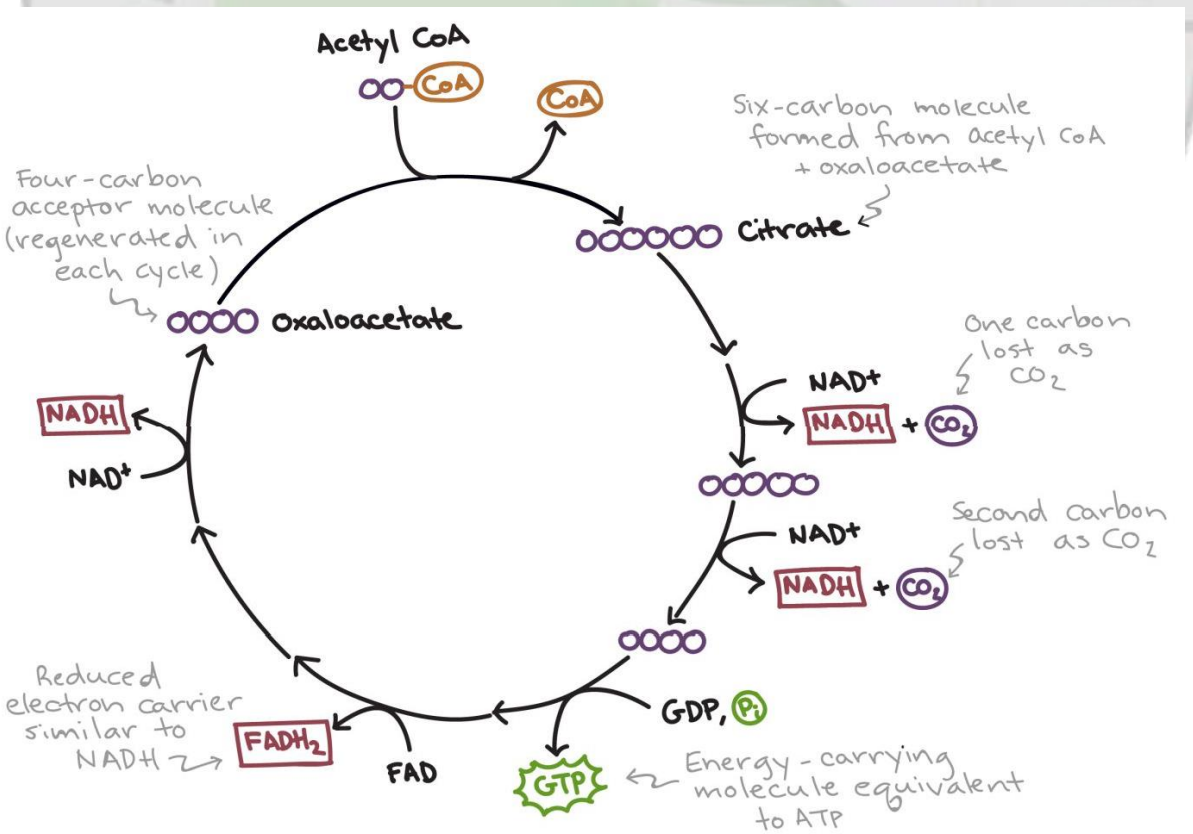
In prokaryotic cells, respiration takes place in the cytosol and across the cell plasma membrane. In eukaryotic cells, it occurs in the cytosol and in the mitochondria. Mitochondria are the powerhouses of eukaryotic cells, and contain high surface areas of membrane folds on which respiration activity can be maximized.

Respiration occurs in a similar way to the internal combustion of your car engine: organic compounds and oxygen go in, carbon dioxide and water come out, and the energy released in the process powers the car or cell. Respiration and combustion are both exergonic processes, in which energy is released from the breaking of molecular bonds. To crank out ATP from the breaking of glucose bonds, respiration occurs in three phases:

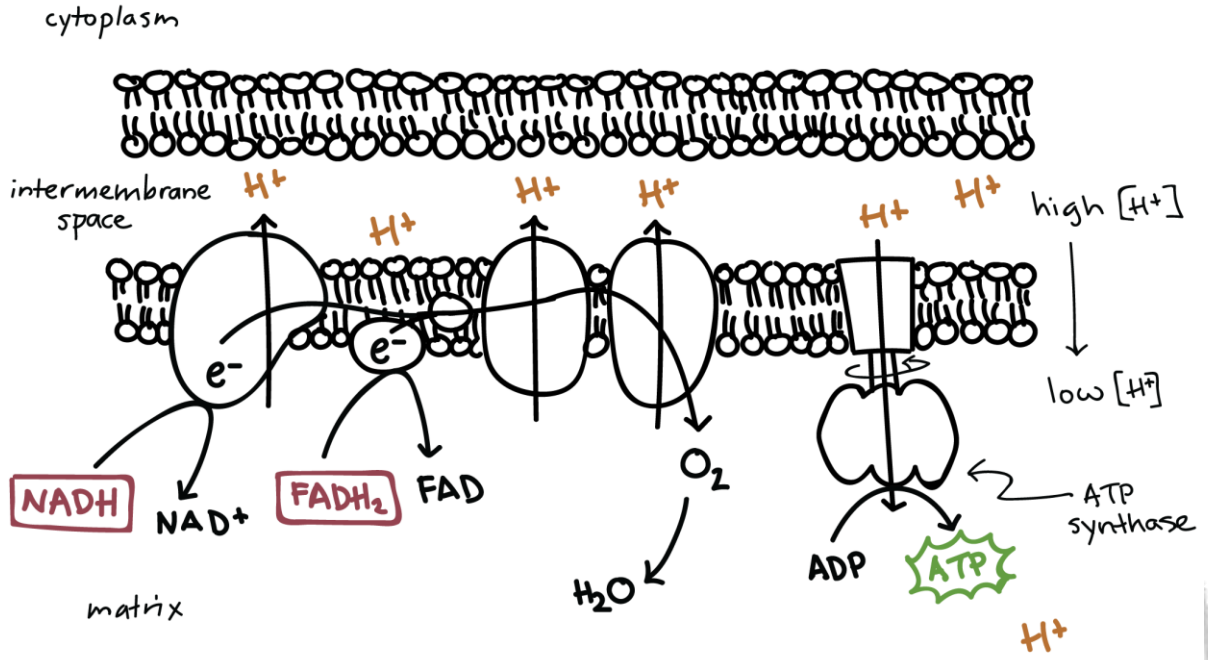
Glycolysis: The original glucose molecule (from food) is broken down to pyruvic acid, which is oxidized into CO2 and water, leaving a two-carbon molecule called acetyl-CoA. Two ATP are generated in this process. This occurs in the cytosol.



The Citric Acid Cycle: The acetyl-CoA from glycolysis is added to an existing carbon chain and sequentially broken down, releasing more CO2 (byproduct) and releasing electrons, which are added to the acceptor molecules. Two ATP are generated for each turn of this cycle. This occurs in the mitochondrial matrix.



Oxidative Phosphorylation: The electron acceptor molecules drop off the electrons, which work to pump H+ ions in high concentration on one side of the plasma membrane, creating a gradient pressure that churns the ATP synthase enzyme, generating about 32 ATP. The remaining electrons are taken by oxygen, which then combines with free hydrogens to create water.



Simple diagram of the electron transport chain. The electron transport chain is a series of proteins embedded in the inner mitochondrial membrane.

The electron transport chain

The electron transport chain is a collection of membrane-embedded proteins and organic molecules, most of them organized into four large complexes. In eukaryotes, many copies of these molecules are found in the inner mitochondrial membrane. In prokaryotes, the electron transport chain components are found in the plasma membrane.

As the electrons travel through the chain, they go from a higher to a lower energy level, moving from less electron-hungry to more electron-hungry molecules. Energy is released in these "downhill" electron transfers, and several of the protein complexes use the released energy to pump protons from the mitochondrial matrix to the inter-membrane space, forming a proton gradient. All the components of the chain are embedded in or attached to the inner mitochondrial membrane. In the matrix, NADH deposits electrons at Complex I, turning into NAD+ and releasing a proton into the matrix. $FADH_2$ in the matrix deposits electrons at Complex II, turning into FAD and releasing $2H^+$. The electrons from Complexes I and II are passed to the small mobile carrier Q. Q transports the electrons to Complex III, which then passes them to

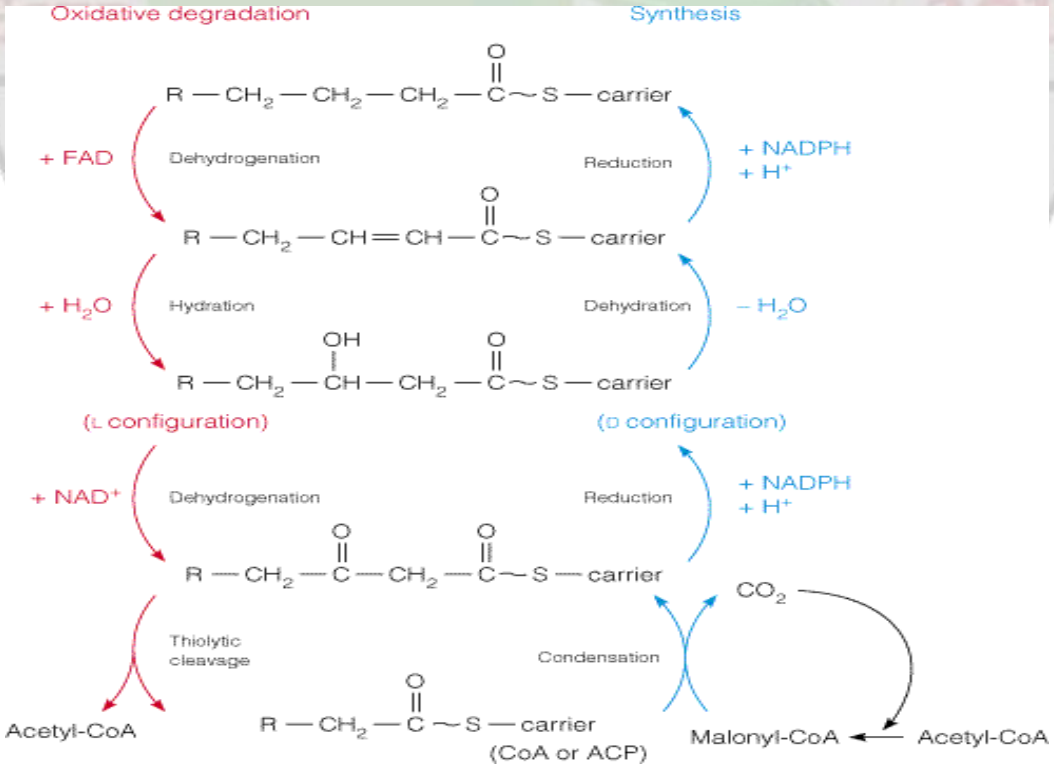
Cytochrome C. Cytochrome C passes the electrons to Complex IV, which then passes them to oxygen in the matrix, forming water. It takes two electrons, 1/2 O_2, and and 2H+ to form one water molecule. Complexes I, III, and IV use energy released as electrons move from a higher to a lower energy level to pump protons out of the matrix and into the inter-membrane space, generating a proton gradient All of the electrons that enter the transport chain come from NADH and $FADH_2$, molecules produced during earlier stages of cellular respiration: glycolysis, pyruvate oxidation, and the citric acid cycle.

• NADH is very good at donating electrons in redox reactions (that is, its electrons are at a high energy level), so it can transfer its electrons directly to complex I, turning back into NAD^+. As electrons move through complex I in a series of redox reactions, energy is released, and the complex uses this energy to pump protons from the matrix into the intermembrane space.

•$FADH_2$ is not as good at donating electrons as NADH (that is, its electrons are at a lower energy level), so it cannot transfer its electrons to complex I. Instead, it feeds them into the transport chain through complex II, which does not pump protons across the membrane.

Fat metabolism

Fatty acid synthesis: Fatty acid biosynthesis occurs similarly to Beta-oxidation - acetyl groups are added to a growing chain, but the mechanism of the pathway is distinctly different from being simply the reverse of Beta-oxidation (exception - elongation of palmitate in the mitochondrion).



Fatty acid biosynthesis occurs in the cytosol (not mitochondria). It uses a moiety called Acyl-carrier protein (ACP) instead of CoA and the reducing agent NADPH (not NAD/FAD). The reaction has a different stereochemisry from Beta-oxidation and the form of the unit added is actually a three carbon unit (malonyl-CoA) which is decarboxylated to incorporated a net 2 carbon unit. Fatty acid biosynthesis can be broken in to three separate pathways shown below:

1. Synthesis of palmitate from acetyl-CoA
2. Elongation of palmitate
3. Desaturation

$$HCO_3^- \text{ Acetyl-CoA} + HCO_3^- + ATP \rightleftharpoons \text{Malonyl-CoA} + ADP + P_i$$

Transport of Mitochondrial Acetyl-CoA into the Cytosol

Acetyl-CoA is produced in two ways in the mitochondria by Beta-oxidation of fatty acids, and by combined action of pyruvate dehydrogenase (to decarboxylate pyruvate, producing acetate) and dihydrolipoyl transacetylase (to add the CoA to the acetate).

Acetyl CoA will accumulate when the ETS/oxidative phosphorylation slows. Under these conditions, acetyl-CoA is transported out of the mitochondrion to the cytosol where it can be used in fatty acid synthesis. This is accomplished using the tricarboxylate transport system in the inner mitochondrial membrane which pumps citrate out. Acetyl-CoA, of course is used in synthesis of citrate when combined with oxaloacetate. Citrate transferred into the cytosol is broken back to oxaloacetate and acetyl-CoA by ATP-citrate lyase (using ATP and CoA). Oxaloacetate can be reduced to malate by malate dehydrogenase and NADH. Malate can be converted to pyruvate by malic enzyme and NADP+. The resulting pyruvate is permeable to the inner mitochondrial membrane and diffuses in. Inside the mitochondrion, pyruvate can be converted to oxaloacetate by pyruvate carboxylase (along with bicarbonate ion, and ATP), completing the cycle. An alternative path is to transport malate across the inner membrane and convert it to oxaloacetate.

Acetyl-CoA Carboxylase

The first committed step of fatty acid biosynthesis is catalyzed by Acetyl-CoA carboxylase. The enzyme contains biotin, and adds a CO2 (resulting in a carboxyl group) to the methyl end of acetyl CoA. This reaction is an energy requiring process (1 ATP per Malonyl-CoA formed). Acetyl-CoA carboxylase is an interesting enzyme. The polymer appears to be the active form of the enzyme. Monomeric units are inactive. Citrate shifts the polymer - monomer equilibrium towards polymer formation. Palmitoyl-CoA shifts the equilibrium towards monomer formation.

Of the two compounds affecting enzyme form, palmitoyl-CoA probably exerts the greater influence.

Another regulation of Acetyl-CoA carboxylase is by hormones. Glucagon, epinephrine, and norepinephrine trigger a cAMP dependent phosphorylation of the enzyme that shifts the equilibrium towards monomer formation. Insulin, conversely, stimulates desphosphorylation, favoring polymerization. The enzymes responsible for phosphorylating Acetyl-CoA carboxylase are cAMP-dependent protein kinase and AMP-dependent protein kinase (AMPK). E. coli's Acetyl-CoA carboxylase is regulated by guanine nucleotides, which are a function of those cells' growth requirements.

Fatty Acid Synthase

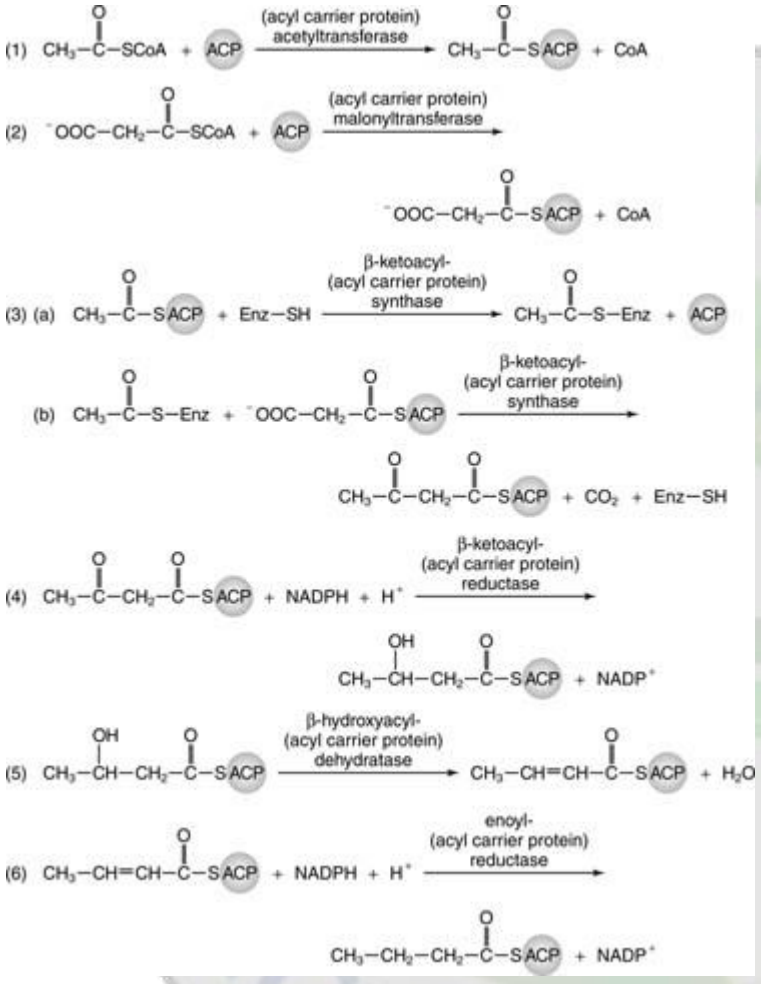
This multifunctional enzyme catalyzes the seven different reactions whereby two carbon units from malonyl-CoA are linked together, ultimately to form palmitoyl-CoA. In some systems, the activities are present on separate enzyme units. In other cells, a single polypeptide chain has multiple activities that can be isolated after protease treatment. The enzyme complex can exist as both a monomer and dimer. The dimeric form is the fully functional form of the enzyme. The overall synthesis of palmitate from acetyl-CoA requires 14 NADPHs, and 7ATPs.

1. Transfer of the malonyl group of malonyl-CoA to ACP Transfer of the acetyl group of Acetyl-CoA to ACP
2. Addition of an acetyl group from malonyl-ACP between the thioester bond of the acetyl-ACP molecule in reaction 1.
3. Reduction of the Beta-keto group to a Beta-hydroxyl group with.
4. Dehydration between the alpha and Beta carbons.
5. Reduction of the trans double bond by NADPH).
6. Repetition of steps 2-6 six more times. The acetyl group of reaction 1 is replaced by the growing acyl-ACP molecule. (That is, new acetyl groups are added at the ACP end of the molecule).
7. The product of this series of reactions, palmitoyl-ACP can be cleaved to palmitate and ACP by the enzyme palmitoyl thioesterase.

The multiple enzymatic activities integrated into Fatty Acid Synthase complex are probably related to the growing fatty acid being "swung" into the appropriate catalytic region of the synthase.

Elongation of Palmitate

The product of fatty acid synthase action, palmitate, is but of course one of many fatty acids synthesized by cells. Elongases are enzymes that act to lengthen palmitate to produce many of the other fatty acids. Elongases are present in mitochondria and the endoplasmic reticulum. Elongation using elongase in the mitochondrion involves a mechanism that is essentially the reverse of Beta-oxidation except substitution of NADPH for FADH2 in the last reaction.



Desaturation of Fatty Acids

Terminal desaturases produce unsaturated fatty acids. One such enzyme is fatty acyl-CoA desaturase. The unusual electron transferring pathway in which electrons from NADH are ultimately passed to oxygen, forming water. The energy released in this process drives oxidation of stearoyl-CoA to oleyl-CoA. From the free methyl end, mammals cannot make double bonds closer to the end than the Delta-9 position (Oleic acid is a Delta-9 fatty acid). Thus, linoleic acid (Delta 9,12 double bonds) and linolenic acid (Delta 9,12,15 double bonds) must be provided in the diet of mammals, and are called essential fatty acids.

Arachidonic acid contains 4 double bonds. Arachidonic acid is a precursor of a group of compounds called eicosanoids to be discussed later in the course.

Control of Fatty Acid Synthesis

Like all metabolic pathways, cells must have appropriate controls on fatty acid metabolism to be able to meet energy needs. Precursors for energy generation - triacylglycerols in chylomicrons and VLDL, fatty acid/albumin complexes, ketone bodies, amino acids, lactate, and glucose - are all carried in the blood as needed for various tissues. One mechanism of regulation involves hormone release.

Signals received in the pancreas (glucose concentration) trigger production of hormones.

Low blood sugar triggers glucagon release.

High blood sugar triggers insulin release.

Both of latter systems control glucose-related metabolism as well. Students should recognize that the regulatory mechanisms of controlling enzymatic reactions we have discussed to date are short-term regulation. They act in minutes (or less). Fatty acid synthesis is controlled partly by short term regulation (mechanisms include substrate availability, allosterism, covalent modification of enzymes) and partly by long term regulatory mechanisms. Long term regulation involves controlling the quantity of enzyme by controlling the rate with which a protein is synthesized and/or degraded. One of the reasons fats do not supply emergency energy is that control of their metabolism is largely by long term regulatory mechanisms whereas control of sugar metabolism is more prominent under short term regulatory mechanisms.

Insulin stimulates increased synthesis of acetyl-CoA carboxylase and fatty acid synthase (two critical enzymes for synthesizing fatty acids). Starvation, conversely decreases synthesis of these enzymes. Fatty acid oxidation is regulated by fatty acid concentration in the blood. This is controlled by the amount of hydrolysis of triacylglycerols in adipose tissue by hormone-sensitive triacylglycerol lipase (HSTL). This enzyme is phosphorylated in the hormonally-controlled cAMP-dependent phosphorylation cascade, which activates the lipase, stimulating release of fatty acids. This cascade is turned on by the cell's binding of glucagon or epinephrine It should also be noted that the cAMP-dependent phosphorylation system also causes inactivation of acetyl-CoA carboxylase, an important control enzyme in fatty acid biosynthesis. Insulin opposes the effects produced by glucagon and epinephrine, stimulating glycogen formation and triacylglycerol synthesis, by favoring dephosphorylation of the enzymes phosphorylated as described above.

Synthesis of Triacylglycerols

Synthesis of fatty acids is only half of the process of making triacylglycerols. In the first part of the process, a fatty acyl-CoA is linked to carbon #1 of dihydroxyacetone phosphate (DHAP) or glycerol-3-phosphate (Gly3P) by either dihydroxyacetone phosphate acyltransferase (for DHAP) or glycerol-3-phosphate acyltransferase (for Gly3P). The product of the reaction for Gly3P is lysophosphatidic acid (LPA)

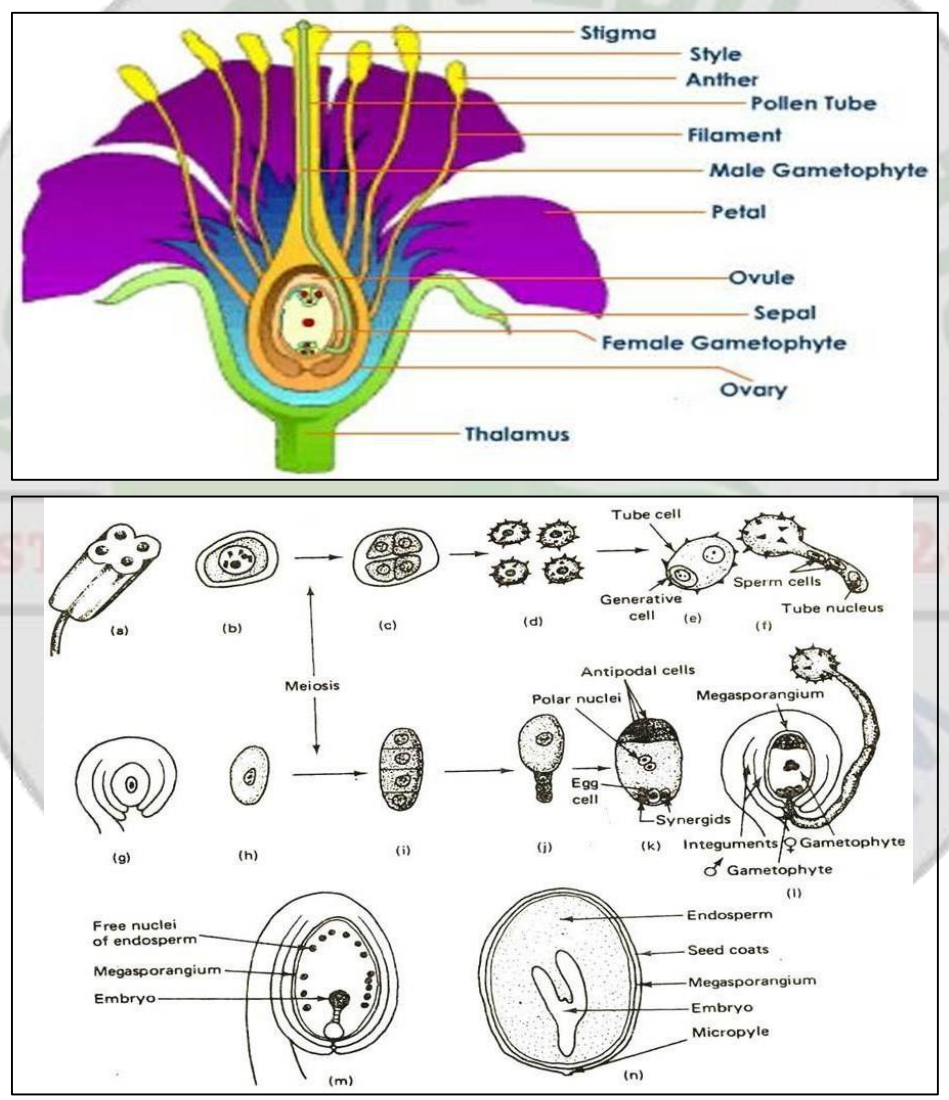
The product of the DHAP reaction (acyl-dihydroxyacetone phosphate) can be converted to the product of the Gly3P reaction (lysophosphatidic acid) by NADPH and acyl-dihydroxyacetone phosphate reductase.

Lysophosphatidic Acid (LPA) is acylated (with an acyl-CoA) at carbon #2 by 1-acylglycerol-3-phosphate acyltransferase to produce phosphatidic acid. This intermediate can be converted to other phospholipids (such as phosphatidyl choline) or converted to triacylglycerols (see below). Phosphatidic acid is thus an important branch point between triacylglyerol biosynthesis and glycerophospholipid biosynthesis.

The phosphate of phosphatidic acid is removed by phosphatidic acid phosphatase, forming diacylglycerol. Diacylglycerol is converted to triacylglycerol (with acyl-CoA, of course) by diacylglycerol acyltransferase.

Chapter 9
SEED DEVELOPMENT

A typical flower mainly consists of four major parts, Viz; Calyx, Corolla, Androecium and Gynoecium. In the early developmental stage the embryo sac is nourished by the cells of surrounding ovulary tissues. These cells are rich in starch, lipids and proteins which are hydrolyzed to form soluble sugars, amino acids, organic acids and other metabolically active materials. The ovule is also connected to the main transport system of the plant by a vascular strand through which water, ions and other solutes are supplied to the developing seeds.



Process of seed formation

Endosperm development

Endosperm is composed of three chromosome sets (3n) two from the maternal and one from the parental parent. However, in gymnosperms such as pine and hemlock the chromosomes are derived from the female gametophyte which is composed of haploid (1n) cells. The main

function of the endosperm is the nourishment to the embryo during early seed formation and maturation and later during seed germination before embryo develops to an independent plant. In angiosperms the development of endosperm generally precedes the development of zygote. The fusion of egg and sperm nuclei to form zygote (2n) and fusion of sperm nucleus with polar nuclei to form endosperm nucleus may occur simultaneously. The endosperm nucleus (3n) usually starts dividing prior to zygote begins to divide. Now endosperm develops without cell wall. Cell wall forms in the later stages. In many dicotyledons the endosperm is absorbed by the cotyledons of developing embryo.

In the first stage of seed development the megaspore mother cell within the ovule undergoes a meiotic division producing four megaspores containing a haploid (1n) chromosome set. Usually only one megaspore survives to produce embryo sac, whereas, the others abort. The nucleus within the embryo sac undergoes three successive divisions to form 8 nuclei (an egg nucleus, two synergid nuclei, three antipodal nuclei and two polar nuclei). In the tissue the microspore mother cell undergoes a meiotic division to form four haploid microspores that develops into pollen grains. The pollen grain contains two cells, a tube cell and one generative cell. At pollination the pollen grains are deposited on the stigma of the ovule, where they germinate and develop pollen tubes that grow through the styler tissue and into the embryo sac. During the growth of the pollen tube the generative cell undergoes a division to form sperm nuclei. After penetration of the embryo sac one of the sperm nuclei unites with the egg nucleus to form zygote, whereas, the other fuses with the two polar nuclei in the embryo sac to form a triploid nucleus (3n) that undergoes division to give rise to the endosperm. Further development of zygote leads to the embryonic development and differentiation of tissues. The zygote undergoes a series of mitosis producing a row of cells called proembryo. The uppermost cells of these divides to form eight cells in two rows. The embryo develops from these eight cells. The lower cells develop into suspensor. The main function of suspensor is to push the developing embryo into endosperm. Very soon the suspensor disintegrates. These developments are accompanied by the formation of endosperm and by changes in certain ovulary tissues that lead to the development of seed coat (after Bold 1961).

Morphological and biochemical changes accompanying seed development and maturation

Phase I

The early stage of seed development involves pollination, fertilization and zygote formation. These stages contribute very little to dry weight increase but metabolic activities takes place at a higher rate. In this stage cell division in zygote starts. The embryo increases in dry weight with the formation of new cells. This is the period of intense metabolic activity with a high demand for low molecular weight precursors such as sucrose, amino acids, fatty acids, nucleosides, organic acids, water and inorganic ions which are mainly supplied by the parent plants through vascular connections, but some also comes from the dissolution of cellular material in the ovule and embryo sac. When embryonic plant is fully differentiated and cell division stops Phase-I ends.

Phase-II is the period of maximum seed dry weight increase. The storage materials are synthesized from small precursor molecules from the parent plant. Sucrose obtained from photosynthesis provides carbon skeletons for starch and fats and also for nitrogenous compounds like amino acids, amides and nucleotides etc. This reserve material is used during seed development due to increased demand for carbon and nitrogenous compounds by seeds. Generally cell division stops with the end of Phase-I, however, in certain dicots nuclear division continues in cotyledonary cells even in Phase-II. The process is known as endoreduplication which leads to polyploid cells (chromosome number more) with high DNA contents. Phase-II comes to an end as seed begins to lose water. The synthesis of storage materials involves the elimination of water molecules. Vascular connections between the developing seeds and parent plant are broken so that no water or solutes can move into seed. Moisture content during seed filling may be in the range of 50 to 60% but after the desiccation process water content may drops to 10 to 15% at maturity. Water loss is not uniform in all parts of the seed.

During Phase III the desiccation process continues till moisture level becomes between 5% and 15%. During this phase polyribosomes (polysomes) break up into single ribosomes. The phase is characterized by a phase of very low metabolic activity. If seed moisture remains low development of embryonic axis into plant may not take place and seed is said to be dormant which can be overcome by supplying water. To obtain seeds of high vitality and vigour it is important that seed moisture level should be 10 - 12% during maturation.

Physiological maturity

Crops are to be harvested at an optimum stage of maturity to get a qualitative and quantitative yield. Seed yield and its quality depends on number of factors. Time of sowing and harvesting stages are among the major considerations in deciding the seed quality and productivity.

(i) Morphological and physiological changes associated with physiological maturity in crops

Determination of physiological maturity in crops is very essential. Certain morphological and physiological changes in different parts of the plants can serve as a guideline to decide the physiological maturity. Effective and practicable guidelines in this regard help the farmers to reap a good harvest.

- Formation of black layer in placenta region near the point of kernel attachment in Maize and Sorghum.
- 35 to 40 days after anthesis in Sorghum.

- Change of internal pod walls in to brown color and attainment of pink colored seed coat in groundnut.
- Change of color of involucre bracts from green to yellow in Sunflower.
- Measuring BRIX reading (Total soluble solids)in sugar cane by 'Brix sugar hydro meter'. If the reading is 17 or more the crop is ready for harvest. Sucrose content of the cane and brix can be estimated with hand refracto meter.

(ii) Harvesting Maturity

Generally, farmers decide the maturity period for harvesting in terms of crop duration from the time of sowing. However, this will change by the variety, soil fertility and climatic conditions. Such harvest, which coincides with the ripeness of seed beyond physiological maturity, is referred to as harvestable maturity. If the seeds are retained on the mother plant beyond physiological maturity, several morphological and physiological changes takes place in seeds and result in the decline of seed quality and yield. For example,

- Rice crop harvested at 15% seed moisture content instead of 21%, results in a yield reduction by 20 per cent.
- Harvesting the seed of Green gram crop after 4 weeks of pod initiation reduces the yield compared to a harvest of 2-3 weeks pod initiation.

Chapter 10

SEED VIABILITY

The viability of the seed is a measure of aliveness of a seed and which could develop into a new and healthy plant when given the appropriate conditions.

Metabolic consequences and causes for loss in seed viability

In non-viable embryo of rice, rye and maize mitochondria appears to be swollen and the internal membrane structure gets distorted, while in viable embryos mitochondrial organization becomes more ordered with advancement of germination. These organelles also become increasingly disorganized after imbibitions eventually leading to death and the activities of cytochrome oxidase, succinic, glutamic, malic and alcohol dehydrogenases, catalases and peroxidases are all lower than in the viable ones. It is not yet known as to what extent there is decline in enzyme activity contributing to failure in respiration or may even be due to deterioration of mitochondria and loss of cell compartmentation. The ATP content of non-viable seed is considerably lower than the viable ones and is insufficient to support metabolic processes essential for germination. ATP measurements cannot be used as a test of seed viability and vigour especially in aged seeds under a variety of conditions The respiratory pattern of deteriorated but still viable seeds (partially) are complex and variable.

The non-viable embryos and embryonic axis fail to conduct protein synthesis on imbibitions. Even in aged but viable embryos there are signs of reduced protein synthesis. Since protein synthesis involves several diverse components as ribosome, mRNA, aminoacylated RNA there is a decline in the activity or content of any one results in decreased transnational capacity.

Factors affecting seed viability

Majority of the seed species retain their viability when these are dried and this is the final phase of maturation for most of the seeds growing in temperate climate. It is a common practice to store seeds in a dry state with low moisture content.

Relationship between temperature and moisture during storage

Those seeds that can be stored in a state of low moisture are called **orthodox seeds** and their viability under certain storage conditions is adjusted to certain rules as mentioned below:-

- For each one-degree decrease in seed moisture content the storage life of the seeds is doubled.
- For each ten degree F (5.6 degree C) decrease in storage temperature the storage life of the seed is doubled.
- The arithmetic sum of storage temperature in degrees F and the percent relative humidity (RH) should not exceed100 with no more than half the sum contributed by temperature.

These thumb rules indicate that temperature and moisture content of seed are the major factors which determine the viability in storage.

Moisture content

The activities of the storage fungi are more influenced by the relative humidity of the inter seed atmosphere than the moisture content of the seeds itself. All cereals and many legumes which are high in starch and low in oil content have a moisture content of about 11% at 45% RH while the oil seeds have a moisture content of only 4-6 % at this RH.

Temperature

Moisture Content	Impact
> 30 % (high)	Non dormant seeds may germinate.
18 – 30 %	Rapid deterioration due to Micro-organism.
> 18-20 %	Seeds will respire and in poor ventilation heat is generated which kills the embryo.
Below 8-9 %	There is little or no insect activity.
Below 4-5 %	Seeds are immune to insect attack and storage fungi, but may deteriorate faster than those maintained at a slightly higher moisture.

Cold storage of seeds is generally desirable but on the contrary it is unadvisable unless these are sealed in the moisture impervious containers or stored in the dehumidified environment. Reason being that the RH of the store may be high and helping the seeds in gaining moisture. Secondly when these seeds are brought to the outer environment for transportation, might deteriorate faster due to high seed moisture. At moisture content below 14 % no ice crystals are formed within the cells on freezing, hence storage of dry seeds at subzero temperature after freezing in a dry atmosphere may improve seed longevity. The batches of seeds are now being placed immersed in the liquid nitrogen in the gene banks.

Cultivar and harvest variability

Cultivars and harvest of a particular species may have varying viability characteristic in the same storage conditions. Initially these differences may be non significant under good storage conditions, but at elevated temperature and humidity these differences can be large. Until recently it was thought that maximum storage potential occurs when seeds are harvested at physiological maturity i.e. end of the seed filling period.

Pre- and post-harvest conditions

Field weathering

Seeds are considered being physiologically and morphologically matured when they attain maximum dry weight. The age between physiological maturity and physical maturity is the major phase when the seeds start deteriorating due to the prevailing environmental conditions. Between these two phases dehydration of the seeds start and continues in the plant. Any fluctuation in the environmental humidity and temperature results in reduced quality of the seeds, thus initiating for loss in viability.

Mechanical damage

Transportation of the produce from the field to the processing unit enhances viability losses especially in leguminous crops. Large seeded legumes are more susceptible to mechanical damage than cereals which are immune to mechanical damage due to their protective outer structures, the lemma and palea. Small seeds escape mechanical damage while spherical seeds suffer less damage than the elongated or irregularly shaped ones. During storage the injured or the bruised areas may serve as centers for infection resulting in accelerated deterioration. Injuries close to the vital parts of embryonic axis or near to the point of attachment of cotyledons to the axis usually bring up more losses in viability.

Oxygen pressure during storage

If seeds are not maintained in hermetic (Closed or airtight) storage at low moisture content then even under conditions of constant moisture content and temperature the gaseous environment may change due to the respiratory activity of seeds and mycoflora. In open storage seeds maintained in an atmosphere of nitrogen may retain their viability considerably longer than those placed in replenished air or oxygen.

Mycoflora infestation

Bacteria

These do not play a significant role in seed deterioration, as germination is rarely reduced unless infection has progressed beyond the point of decay. The bacterial population requires free water to grow hence their population is not increased in stored seeds as they are stored in a relatively low moisture content. In case the conditions are moist enough the fungi growth suppresses the bacterial growth.

Field Fungi

These invade seeds during their development on plants, in the fields or during harvesting and threshing. These need a high moisture content for multiplication i.e. at least 33% for cereals, and are thus infective only where seeds fail to follow their normal pattern of maturation and drying. High rainfall during harvest time may results in extensive loss in viability and is only known during the next growing season. Seeds which are protected from air borne pathogens as pods, fleshy fruits, or some surrounding structures are generally less susceptible than those which are more exposed. The main fungal species associated with Wheat & Barley are, *Alternaria, Fusarium,* and *Helminthosporium sp.*

Storage Fungi

Aspergillus and *Pennicilium* are the common storage fungi which infest the seeds during storage and are not found even in plants standing in the field after harvesting. Each storage fungi have defined moisture content on which it invades. Other factors responsible for its growth are its virulence as ability to penetrate the seeds, conditions of the seeds and nutrient availability. The major deleterious effect of storage fungi is that these decrease seed viability, cause discoloration, produce myco-toxins and develop mustiness and caking. The microorganisms accelerate loss of viability at high moisture content. However these are not uninvolved in loss of viability when seeds are stored in moisture content less than 13% in starchy grains and 9% in oil seeds.

Tetrazolium test for seed viability and vigor

For crop species there is a basic need of minimum environmental conditions and time required for germination test has necessitated the search for quick and effective test to predict seed viability that helps in timely processing and marketing of the seeds. This has become more important with the increase in seed trade in PVP regime. Tetrazolium Test is the outcome of the efforts to search for quick and reliable method of determining seed viability.

Principle involved in tetrazolium viability testing

The principle of TZ testing is based on the response of the living cells of the seed which can reduce a colorless solution of 2,3,5, tri phenyl tetrazolium chloride or bromide into a red coloured compound called Formazan. The reduction of the chemical in the seed takes place by the reaction of a group of enzymes called dehydrogenases. These enzymes are involved in the H transfer during the respiratory activity of the biological systems. Since the reaction takes place within the respiring cells and the Formazan is non-diffusible, a clear topography of the living and non-living tissues within the seed is developed by following the proper procedure. Hence, the test is designated as Topographical Tetrazolium Test.

Equipments

S.No	Devices	Equipment
1	Cutting & Piercing Devices	Blades (Single edged), Dissecting needles.
2	Glassware	Petri dish, Beaker, and watch glass.
3	Magnifying devices	Magnifying lens, Stereoscopic microscope.
4	Conditioning media	Paper towel, Blotter paper, & Filter paper.
5	For Seed Handling	Forceps, Brush, Fine scissors & Scarifiers.
6	Dispensing bottle	Application of Lactophenol.
7	Medicine dropper	For removal of tetrazolium solution after completion of the test.

Preparation of solution

Tetrazolium solution

The concentration of the solution used for seeds that are not bisected is 1.0 % whereas 0.1 % is used for those seeds that are bisected. Even a concentration of 0.5 % can be used for any type of seed.

For preparation of 1.0% solution one gram of tetrazolium is dissolved in 100 ml distilled water. The best staining is achieved when the pH of the solution should be 6.0 to 8.0. In case the pH of the solution is not neutral then the salt should be dissolved in a phosphate buffer solution. The buffer solution is prepared as follows:

Solution 1: dissolve 9.078 g of KH_2PO_4 in 1000 ml of distilled water.

Solution2: Dissolve 11.876 g of $Na_2HPO_4.H_2O$ in 1000ml of distilled water.

Take 400 ml of solution 1 and 600 ml of solution 2 and mix them together. In one liter of buffer solution dissolve 10g of Tetrazolium salt. Thus the pH of the solution is 7.0 and the concentration is 1.0 %. The solution should be stored in dark or in an amber coloured bottle so as to prevent from light. The prepared solution can be kept for several months, but once used should be discarded.

Lactophenol

The lacto phenol solution consists of 20 parts each of Lactic acid, Phenol and water 40 parts of glycerin. This solution is used for clearing of glasses after staining so that the embryo is visible through lemma and palea. The solution may be conveniently dispensed. The Lacto phenol causes the hulls to become transparent making the stained embryo visible for evaluation.

Precautions

Lacto phenol is toxic thus skin contact and inhalation should be avoided. The room should be well ventilated.

Temperature

The temperature range between 20^0C- 45^0C has no effect on accuracy of the tetrazolium test, but staining proceeds faster at higher temperatures, however the test may be performed satisfactorily at room temperatures. Temperatures higher than 45^0C are not desirable.

Preparation of seeds for testing

Conditioning

Before staining the seeds conditioning is required for most of the seeds. The moistening of the living tissues activates the metabolic activities thus activating the enzymes that help in the embryo activity and staining is improved and evaluation of the seeds is easier.

Objectives of staining

- To ensure timely and adequate penetration of the staining solution.
- To accelerate the rate of staining.

Staining procedure

The testing samples should be covered completely with the testing solution and should be kept in dark. A prolonged exposure to light oxidizes the testing solution and results in abnormal tissue colour.

Preparation for evaluation

When the small seeds are to be evaluated immediately the tetrazolium solution is siphoned with the help of medicine dropper. When the seeds are not to be evaluated immediately then these can be placed in water and kept in cool conditions. Tetrazolium solution is removed completely before. For evaluation of small seeds some magnification is helpful.

Seed coats of the legumes are normally removed with the help of needles, forceps etc. Small seeded legumes as berseem may be cleared with the help of lacto phenol and examined under transmitted light without removing the seed coats. Some of the major features for examining the monocot and dicot seeds are mentioned below:

Monocot

Growing tips of the embryonic axis especially plumule, point of attachment of the embryo to scutellum, and region of seminal root emergence.

Dicot

- Radicle and hypocotyls development, cotyledons, and plumule region.
- Improper staining in these areas indicate non-germinable or abnormal seeds.

Accuracy of the results

The differences in the tetrazolium test results and germination may be due to a number of reasons Viz; Sample differences, Improper germination testing, Improper TZ testing, Dormant seeds, Hard seeds, Seed-borne organisms etc.

The chemical injury, fumigation injury over treatment with mercurial seed dressings may not be detected with TZ test. The chemical injury that prevents normal germination may not inhibit the TZ staining process. However when the test is conducted correctly the germination tests are in close agreement. The difference in the results is generally less with high quality seeds.

Advantages

- Quick estimation of viability can be obtained.
- Testing of dormant seeds and those seeds having a slow germination this test is extremely useful.
- Seeds of dicotyledons are not damaged and can further be used for sowing.

Disadvantages

- Distinction between normal and abnormal seeds is difficult.
- There is no difference between dormant and non-dormant seeds.

- The microbes deteriorating the seeds cannot be identified.
- High skill and knowledge of seed structure of the seed under study is essential along with the ability to study the staining pattern and to interpret the results achieved.

Chapter 11

SEED VIGOUR & VIGOUR TESTING

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Seed vigour

- The seed vigour may be defined as some total of all seed attributes which favour stand establishment under favourable conditions **(Isley 1957).**
- All those properties in seeds which upon planting results in rapid and uniform production of healthy seedlings under wide range of environments including both favourable and stress conditions **(AOSA 1975).**

ISTA defines seed vigour as an index of the extent of the physiological deterioration and or mechanical integrity of a high germinating seed lot which governs its ability to perform in a wide range of environments.

The reduction in the ability of the seed to carry out the physiological functions that allow them to perform is called **Physiological deterioration**. This deterioration can begin before harvest and continues during harvest, processing and storage, at a rate greatly influenced by genetic, production and environmental factors. The end point of this deterioration is ultimately death of the seed, but at least initially, the reduced ability of the seed to carry out its physiological functions does not prevent germination. Seeds thus lose vigour before they lose the ability to germinate, so that seed lots that have similar higher germination values can differ in their physiological age i.e. the extent of deterioration and so differ in seed vigour and the ability to perform.

Significance of seed vigour

The significance of seed vigour for sowing depends mostly on seedbed and the environmental conditions. When conditions are benign field emergence percentage will often be close to the germination percentage of the seed lot and seed vigour may not be a factor in seedling performance. Environmental stress may (Low temperature & wet soils) results in varying field performance depending on the vigour status of the seed lot. High vigour seeds will perform (emergence and seedling growth) comparatively better under environmentally stressed seed bed conditions than low vigour seed lots even though the laboratory germination of the lots may not differ. The significance of seed vigour for storage is that the storage potential of the high germinating seeds is related to their vigour status before entering storage. Under both controlled and uncontrolled storage, high vigour seed lots will perform better (germination) after storage than the lower vigour seed lots. Similarly for seeds lots transported within a country or exported high vigour seed lots are better able to withstand the environmental hazards (eg. Fluctuating

Companies use vigour information too

- Identify seed lots which do not meet company standards.
- Rank seed lots for in house quality control.
- Evaluate the potential of carry over seed.
- Assist in seed processing and marketing decisions.
- To answer the customer enquiry about the seed lot performance.

Factors affecting seed and seedling vigour

The major factors responsible for loss in seed and seedling vigour are:

1. Genetic make up

The genetic make up of the plant is an important factor affecting seed vigour. It differs among species, varieties, and within varieties. Examlle seeds of hybrids are found to germinate and grow faster showing higher respiration **ra**tes as compared to the parents. Higher vigour in hybrids is due to efficient mitochondrial activities and extra active system for assimilation. Seed coat colour and permeability influence seed vigor. Grain legumes with white testa have poorer field emergence than those with brown and black testa, which has been attributed to low resistance for water uptake in white testa seeds resulting in imbibitional injury.

2. Seed maturity

As seed reaches maturation it's potential for rapid and vigorous germination increases. A mature seed develops physiologically and physically and expresses maximum vigor. Moisture content present in the seed is often used as an index for seed maturity. Complete seed maturity is sometimes considered as a stage of development producing maximum seedling vigour under adverse germination conditions. Maximum seed quality is attained at physiological maturity and is often characterized as the time of maximum accumulation of seed dry weight. Physiological maturity can be detected morphologically by the formation of black layer in maize and change in pod colour of legumes.

3. Time of harvesting

In plants with determinate flowering seed maturity is rather uniform as compared to those of the indeterminate types. In the indeterminate types it yields seeds of different stages of growth and maturity thus affecting the growth and potential. Hence, the date of harvest is so adjusted to give the greatest amount of germination and high vigour from a population ranging from immature seeds to those which are ready to shatter. Harvesting time and seed position affects seed vigour.

4. Environment during seed germination

Air temperature and moisture availability during seed development affect seed size, germination and seedling vigour thus affecting the subsequent population and ultimately yield. Water availability during seed development may influence seed vigour indirectly through its

influence on chemical composition of mature seeds. Environmental factors regulating seed fill may have a negative impact on seed vigour expression. High temperature during wheat seed development results in decreased seed weight, shriveling and poor seed quality. High temperature during the last 45 days of soybean seed development is associated with poor seedling vigor and this persists throughout the growing period and influences seed yield and is also correlated with oil content during maturity. Water stress during the early stages of soybean development results in the decrease in the number of pods per plant due to the induction of abortion and abscission when the pods fail to expand. Seed viability and vigour may also be adversely affected by low temperature stress during development which results in qualitative and quantitative changes in lipid reserves of some oil seed crops and a non lethal frost during later stages of development.

5. Soil fertility

Fertility of the soils in which the plants grow influences the chemical composition of the seed during its development and consequently its metabolic activities affecting seedling vigour and stand. It appears that nitrogen and phosphorous availability influences seed development and vigour with variations in species and is dependent on the stage of growth of the plant and environmental conditions. The inorganic nutrients stored in greater quantity in the seeds provide valuable reserves during the early stages of development which may be critical for seedling establishment in the soils having low nutrient availability.

6. Mechanical damage

Mechanical damage leading to cracked, broken seeds and other abnormalities are well known. However, its effect on seedlings is subtle since mechanically damaged seeds may appear normal but exhibit less vigour. The physiological basis of mechanically induced vigor loss is yet poorly understood. The physiological deterioration may also be triggered by impaction or it may be purely physical damage as a result of microscopic breaks at crucial locations within the seeds. Mechanically damaged seeds may appear to be normal morphologically but exhibit slower germination, reduced growth rate, delayed maturity and reduction in yield. Each handling process as threshing, cleaning, treating, bagging, transportation and planting may cause impaction of seeds against other seeds or hard surfaces may cause breakage or physiological injury.

7. Age of deterioration

As the seeds undergo gradual changes which lower their vigor potential and their speed of deterioration depends largely on the environment of the store and environmental conditions existing during seed development. Initial changes are physiological in nature and can only be detected by severe stress or biochemical tests. As deterioration proceeds storability and yield potentials are affected resulting in poor germination and seedling establishment under favorable conditions. Hard seededness appears to protect seed from field weathering, seed storage deterioration, and imbibitional damage.

8. Attack by micro-organisms

Infestation by microorganisms causes deterioration of seeds during storage thus reducing seedling vigor. Mostly the species attacking are saprophytes and become parasitic on young seedlings. Substances leached from the germinating seeds may enhance fungal growth. Damping off may be due to the stimulation of *Pythium* and *Rhizoctonia* growth by nutrient leached from the seeds. Low vigor seeds are much susceptible to loss of nutrients through leaching which in turn provides a substrate for fungal growth and the competitiveness of fungi infested seeds is shifted in favour of microbes and this effect may be intensified by other conditions as cold and damp soil and poor seed quality.

9. Vigor loss from chilling injury during imbibitions

A number of warm season species are susceptible to low temperature injury during imbibition and early seedling growth. This injury may have immediate and long term effect. e.g. in soybean at $5^{O}C$ there is reduction in seedling survival. Dry matter accumulation and seedling length depends upon the initial moisture content during chilling. There is severe effect of chilling injury under low oxygen availability. Seeds are more sensitive to chilling injury during the early stages of imbibition in low vigor seeds. Adjusting seed moisture content to safe limits resulting in less exudation of leachates, hence decreasing microbial attack with increased seedling survival and minimizing losses by cold testing seeds of susceptible crop so that low-vigor lots can be eliminated.

Types of seed vigor tests

The standard germination test is conducted under optimum conditions. Consequently, when field conditions at planting are near optimum, the results usually correlate well with field emergence under suboptimal field conditions. However the standard germination results usually overestimate field emergence. Therefore, additional tests are needed for better prediction of seedling emergence under a wide range of field conditions. Many vigor tests have been suggested, however, only a few have attained acceptance by seed analysts and seed testing organizations.

Seedling vigor classification test

This vigor test is an expansion of the routine germination test, requiring the seed analyst to further classify “normal” seedlings into “strong and weak” categories. The test requires no additional equipments and employ concepts and terms familiar to seed analysts, thus it is particularly attractive to seed analysts. Despite its advantages, it has one serious difficulty. To further separate “normal” seedlings into two additional categories is a difficult task and can introduce additional variability.

Strong and weak seedling

Seeds are placed on a moist paper towel at optimum temperature in an incubator. After 5 days the seedlings are categorized into strong and weak seedlings. Seedlings are said to be weak when primary root, cotyledons or primary leaves are missing or short or missing primary leaf, spindly or poorly developed seedlings.

The assessment of seed vigour is carried out through :

- Physical test, Performance test , Stress test , Biochemical tests

Physical test

Seed size

In this test 100/1000 seeds are drawn randomly and weighted in grams. Seed lot having higher seed weight is considered vigorous.

Seed density

Kerosene oil is placed in a graduated measuring cylinder and initial level of the oil is noted. A weighed amount of seed is poured into the cylinders and the level of oil is noted. Difference between the two levels shows the density of the seed. Lot having higher density is considered to be vigorous.

Physical soundness

The seed lots having under sized, undeveloped, discolored, shriveled and insect damaged seeds are considered to be weak.

Performance tests under optimum conditions

First count: The normal seedlings counted at the first count i.e. $4^{th} – 5^{th}$ day represents faster germinating seeds. Maximum percentage of normal seedlings during first count indicates seeds with higher vigor.

Speed of germination

When seeds start geminating their number must be counted daily till maximum germination is obtained. An index is worked out for each seed lot by dividing number of seedlings removed each day by the day on which they were removed after planting.

$$\text{Speed of germination} \quad \frac{\text{No. of seedlings removed daily}}{\text{Days after pla t}}$$

$$\text{Or} \quad \frac{X1}{1} \quad \frac{X2}{2} \quad \frac{X3}{3} \quad \cdots \frac{X\infty}{\infty}$$

Where X represents no. of seeds germinated and y days on which seeds were kept for germination.

$$\text{Coef c oefgt rmination } \sigma \quad \frac{\text{Total number of seeds germinated}}{\text{Total number of days required}}$$

Seedling growth rate

Paper Towel is moistened with distill water. Twenty seeds are placed on it in a straight line and paper towel is folded and kept in a germinator at an angle of 75 and optimum temperature. Only 10 competitive seedlings are selected for observations and the rest are removed. The length of each seedling is measured daily and seedling growth rate is determined as follows.

SL $1/F_1$ (SL1-SL2/F2 +---------- + (SLn - SL (n-1)/Fn

Where

SL1	-	Mean seedling length at first count.
SL2	-	Mean seedling length at second count.
SL1-SL2	-	Mean increase in length at second count
F1	-	Days of first count.
Fn	-	Days to final count of seedling Length

Seedling length

Length of 10 normal seedlings in cm is observed during the final count grown in moist paper towel kept in germinator at optimum temperature. The lot exhibiting maximum seedling length is considered vigorous.

Seedling dry weight

The weight of seedlings excluding the cotyledons is taken on 10th day after drying them in an oven at 100^{0}C temperature for 24 hrs in g. The lot exhibiting maximum dry weight is considered as vigorous.

Vigour index

a) By length

A combination of standard germination test with seedling length provides evaluation for seed vigor (Abdul- Baki and Anderson 1972).

Vigor index = Germination x seedling length at final count

b) By weight

Vigour index by weight is determined by the multiplication of seedling dry weight on the final count with germination percentage.

Seed metabolic efficiency (SME)

It is the amount of dry seed weight respired for producing 1g of dry root and shoots. Thus lighter the value of SME lower the seed efficiency as more amount of seed reserve would be used for producing root and shoot. Amount of food material respired is (RESP) calculated as follows:

$$RESP = SDW (SHW + RTW + RSW)$$

Where,

SDW = Seed dry weight before germination
SHW = Shoot dry weight
RTW = Root dry weight
RSW = Seed dry weight after germination

Mobilization efficiency (ME)

Seed lot having higher mobilization efficiency are considered as vigorous due to its capacity to supply food material to the growing seedlings. Weighed seeds are placed on towel paper and kept in germinator at an optimum temperature on the day of final count. Seedlings and cotyledons are dried separately at 100^{0}C for 24 hrs. ME is calculated as follows:

$$ME \quad \frac{\text{Increase in dry weight of embryonic axis}}{\text{Decrease in dry weight of cotyledons}} \times 100$$

Stress tests

Till date no one vigor test has been considered universally acceptable for all crops. However for some vigor tests research work continues to advance their standardization.

Osmotic stress

When seeds are sown in the field, they are often subjected to drought stress which results in poor emergence. Such drought conditions can be simulated in a laboratory test by use of soil solution and other solution systems. Since standardization of soil conditions is difficult to achieve,

a solution system is preferred. Seeds are germinated in solutions such as sodium chloride, glycerol, sucrose, polyethylene glycol (PEG) and mannitol with specific osmotic potentials. There is evidence, however, that some low molecular weight osmotic substances (sucrose, sodium chloride, glycerol and mannitol) enter germinating seeds and cause toxicity, thus high molecular weight PEG (4000 or more) is a satisfactory compound for simulating true drought without causing toxic side effects. The osmotic potentials of PEG 6000 solutions at various concentrations and temperatures have been determined. The rate of germination under such conditions is markedly reduced and emergence of the plumule is generally more affected than that of the radicle. Since vigorous seeds can tolerate greater osmotic stress, this method has been suggested as a vigor test.

Accelerated ageing test

The accelerated ageing test is the second most popular seed vigor test (Ferguson and Spears 1995). It provides valuable information on both seed storage and field emergence potential. It is applicable to number of crops. Considerable efforts have been made to understand accelerated ageing test variables and standardize the test protocols. Valuable recommendations for these tests have been emphasized and incorporated into the ISTA vigor test. These recommendations are used to assess the vigor test in soybean. For rapid determination of seed vigor and storage potential of the seed lot the seed age is accelerated by weeks or day by subjecting the seeds to high humidity and high temperature conditions. Seed lots showing good germination after ageing are considered to be vigorous and have a good storage potential. The seeds are spread on the wire mesh in a single layer and placed in a desiccator previously filled with water a little below the wire mesh. The desiccator is made airtight by applying grease to its lid and kept in an incubator at 40^0C. Samples are drawn periodically to test the germination till it falls below 50%. The seed lots showing high germination for a longer period are considered to be vigorous.

This test incorporates many of the important traits desired in a vigor test. The accelerated aging test is rapid, inexpensive, simple and useful for all species. It can be used for individual seed evaluation and requires no additional training for correct evaluation.

Controlled deterioration

The controlled deterioration test has been recommended for vegetable crops and is considered in the ISTA Vigor Test Handbook. This test is similar to accelerated ageing as it deteriorates low quality seeds at high temperatures and elevated seed moisture content. It differed from it by using seeds imbibed in water to a precise level allowing the moisture to equilibrate by sealing the seeds in aluminum packets in a refrigerator overnight and then submerging the seeds packets in a water bath at 45^0C for 24 hrs (Powell 1995). The moisture range varies between 19-24%.

Brick grit test

The brick grit test is also known as the Hiltner test. It was originally developed by Hiltner and Ihssen (1911) for detecting seed-borne *Fusarium* infection in cereals. Results of further studies indicated that the test also detected seed weaknesses other than those caused by fungi. In the Hiltner test, seeds are planted on damp brick grit or in a container of sand and covered with 3 cm of damp brick grit, then germinated in darkness at room temperature for a specific time. Seeds weakened by pathogenic fungi, mechanical injury, or storage deterioration are unable to penetrate

the brick grit layers. The percentage of normal seedlings from this test is considered to be an indication of the vigor level.

Paper piercing test

Seeds are placed on 1.25cm of moist sand and are covered with a dry filter paper having basic weight 0f 90 g / mt ,thickness -0.4mm, bulk – 4,dry bursting strength -0.3 kg/cm , breaking length – 1000- 5000 mm , filtering speed – 500 ml / mi , wet bursting strength – 150 mm , ash content – 0.1 % , fibre composition, chemical wood pulp with high alpha percentage. This filter paper is again covered with 3cm of moist sand. The tray is kept at 20-25 ^{0} C up to the days required for final count, seedlings which are able to penetrate the paper, are said to be the seedlings from vigorous lot.

Compact soil test

Seeds are planted in soil at optimum conditions in a tray. Seeds are covered with a uniform and compact layer of the same soil. The seed lots having maximum emergence are considered to be vigorous.

Cold test

The cold test is one of the oldest methods of stressing seeds and is most often employed for evaluating seed vigor in corn and soybean. Seeds are placed in soil or paper towels lined with soil and exposed to cold for a specified period, during which stress from imbibition, temperature, and microorganisms occurs. Following the cold treatment, the seeds are placed under favorable growth conditions and allowed to germinate. The greatest difficulty with the cold test is the lack of uniformity in field soil. Soils different in moisture, pH, particle composition and pathogen levels contribute to divergent results. Use of vermiculite is a more uniform medium. It is widely believed that a cold test requires field soil to be successful.

Seeds are planted on 2cm thick leveled moist soil. The soil is placed on these seeds. Enough cold water (10^{0}C) is added to the soil to make it moist then incubated at 10^{0}C for 7 days. Later they are transferred at optimum required temperature for germination. Another set of the same lot should be kept simultaneously without any check. The lot showing minimum variation in germination when compared may be considered as vigorous.

Cool germination test

Unlike the cold test, the cool germination test is conducted under standard laboratory conditions at low temperatures (18^{0}C) and does not rely on the activity of microorganisms to stress the germinating seeds. It has been demonstrated that low vigor seeds from warm-season crops, such as cotton, have decreased growth rate and lower germination under these conditions. The major advantage of this test is that it is similar to the standard germination test and the same criteria for interpretation of normal seedlings is employed. Its principal limitation is that it is currently limited to use in cotton.

Seed are placed on moist towel paper or sand and incubated at low temperature (10-15^{0}C) up to the day of final count. The lots having maximum germination, seedling length and dry weight are considered to be vigorous.

Biochemical tests

Electrical conductivity test

Low-vigor seeds have been shown to possess decreased membrane integrity as a result of storage deterioration and mechanical injury. During imbibition, seeds having poor membrane structure release cytoplasmic solutes into the imbibing medium. These solutes with electrolytic properties carry an electrical charge that can be detected by a conductivity meter. Measurement of the conductivity of leachates from seeds is a rapid, precise, inexpensive, and simple procedure.

Conductivity test is done to determine seed vigor does not work for all crops which may be due to the presence of semi-permeable membrane that permits the inward entry of water but not the outward diffusion of certain electrolytes. In other cases lack of success has been attributed to genotypes as high sugar sweet corn genotypes that possess thinner pericarps may be more susceptible to damage during harvest and drying. More emphasis has been given to what is leaked from the seed rather than relying on composite conductivity readings. The solutes leaked out from seeds when immersed in water are not available for the respiring and germinating seeds which results in poor emergence. A deteriorated seed lot leaches more water soluble compounds. The electrical conductance of this solution will be higher with higher concentrations of ions in the solution.

Precautions

Initial seed moisture and seed size can affect the rate of solute leakage. Additionally, treatment of seeds with antibiotics may influence conductivity measurements necessitating their removal before determinations are made.

Limitations

One limitation of the present conductivity test is that it expresses result as an average conductivity evaluation for 25 seeds. Such an expression presumes that all seeds are equally deteriorated and will provide the same quantity of electrolyte leakage. A seed lot, however, is composed of a population of individual seed each with its own unique potential to perform in the field. Conductivity test results, therefore, would better reflect the vigor capability of a seed lot if any they are presented on an individual seed basis.

Equipments' and chemicals

Analytical balance, beaker, de- ionized water, incubator and electrical conductivity meter.

Procedure

50 seeds in three replications from each seed lots are weighed to two decimal and placed in a beaker with 250 ml deionised water. These beakers are kept at 20^0C after proper covering to reduce the evaporation and contamination. A beaker containing deionized water with no seed is kept as control. After 24 hours water is poured through a coarse sieve into another beaker. The conductivity of water in control is subtracted from the reading of soak water before calculating the conductance per gram of seed and expressed as (µmhos/cm/g) of seeds.

Interpretation

24 or less	Normal seeds.
25-29	Suitable for early sowing, risk of poor performance under adverse conditions.
30-43	Not suitable under adverse conditions.
44 and more	Not suitable for sowing.

Tetrazolium (TZ) test

The TZ test is one of the most valuable techniques for analyzing seed quality. It relies on the action of the TZ molecule to react with hydrogen atoms released as a result of dehydrogenase enzyme activity in living tissue. These results in the formation of a water-insoluble red pigment called formazan which a trained seed analyst evaluates for staining pattern and color intensity. The analyst then subjectively places the seeds into vigor categories ranging from strong to weak. Though the results of this test correlate well with seed vigor when interpreted by a qualified analyst, it is still subject to certain standardization difficulties, first, the ability of the analyst to ascertain whether a seed is vigorous, second the failure of the test to detect seed treatment phytotoxicity and reveal seed dormancy.

Equipments and chemicals

Petri plates, filter paper, incubator, razor, beaker, spectrophotometer, distilled water, tetrazolium salt and methyl allosolve.

Procedure

Seeds are placed on petriplates lined with moist blotter paper at 25 ± 1^0C for 24 hours. Embryonic axis is excised and kept in 1 ml of tetrazolium solutions for 2 hrs at 30 ± 1^0C in dark. Excess solution is drained out and seeds are washed thoroughly with distilled water. The axises are soaked in 10 ml of methyl allosolve (methoxy cellosol) for 4-6 hours with occasional stirring till the formazan is completely extracted. Axis turns colourless. Extract is decanted and its colour intensity is read at 480 nm in spectrophotometer.

Volatile aldehyde test

200 seeds are placed on eight Whatman No. 1 filter paper of 7 cm diameter and moistened with 125 ml of distilled water and placed in a 150 ml Erlenmeyer flask. A tube containing 10 ml of 0.2% 3 methyl 2 benzothiozolium hydrogen hydrochloride monohydrate (MBTH) is placed in each flask. The flasks are sealed with rubber stoppers and incubated at 25 ± 2^0C for 24 and 48 hours, respectively. 1.0 ml aliquot is pipetted into 2.5 ml freshly prepared 0.23% ferric chloride solution, 6.5 ml acetone is added to each tube after 5 minutes to exhaust the reagent and enhance the colour development. Absorption of the color developed is read on spectrophotometer at 623 nm using fresh MBTH solutions as blank. The values of absorbance are converted to µg Formaldehyde equivalent using the standard curve for formaldehyde.

Respiration test and RQ

The seeds having high respiration rate possess high vigour. Respiration rates during the initial hours of imbibition are correlated with seedling growth rate. During respiration the seeds consume oxygen and inhales carbon dioxide. Hence the ratio of the amount of CO_2 evolved / unit time to the amount of O_2 consumed / unit time is called Respiratory Quotient and it is more related to vigour. The RQ at 1.0 or higher measured as 100% O_2 after 6 hours of planting indicates high vigour.

Equipments

Warburg flask, manometer, water bath, filter paper, cotton plug and pH meter.

Chemicals

Phosphate Buffer (0.1M) of 6.0 pH, distilled water, KOH solution (20% W/V in water), grease etc.

Procedure

For preparation of 0.1M Phosphate buffer (pH 6.8), 35.61g 0.2M $Na_2HPO_4.7H_2O$is dissolved in 1 liter of water (Solution 1), while 31.21g $NaH_2PO_4.2H_2O$ is dissolved in another one liter of water (solution 2). 49 ml of solution A is mixed with 51 ml of solution B and diluted to 200ml. with distilled water.

One gram seeds are placed in the main compartment of the flask. 0.4ml of phosphate buffer with 0.2 ml water is added to the seed material. 0.2 ml of 20% KOH solution is transferred to the central well with a small filter folded in the form of an accordion. All the attachment joints are greased. This flask is attached to the manometer and placed in constant temperature bath (30^0C). The equipment is shaken for 10-15 minutes and the manometer is adjusted at reference point (150 mm) with the help of the stopcock. Observations are recorded after every 10-15 minutes by adjusting the liquid level in the closed arm to the reference point each time.

Glutamic acid decarboxylase activity (GADA) test

Principle

The amount of CO_2 evolved by glutamic acid reflects the level of enzyme glutamic acid decarboxylase which is directly related to vigour and storability of seeds.

Equipments & chemicals

Manometer, water bath, grinder, analytical balance, pH meter, glutamic acid, phosphate buffer and ethyl lactate.

Procedure

30g of finely ground seeds are placed in the manometer jar. 15ml of 0.1M of glutamic acid is in 67M phosphate buffer (pH 6.8) is added to the ground seed and mixed quickly. The lid of the manometer is screwed tightly and the unit is placed at 30 $\pm 2^0$C in water bath. The manometer is adjusted to zero after 10 minutes. The CO_2 evolved results in the displacement of ethyl lactate. Crystal violet colour develops which is measured in mm / 30 minutes at 30^0C.

Chapter 12

SEED GERMINATION

Germination

- Emergence and development of seedling with all those essential structures that for the species described indicates the ability to develop into a normal seedling under favourable conditions of soil (ISTA 1985).
- Emergence of radicle from the seed.

Germination can be divided into two parts as follows:-

Epigeal germination: Epi – upon, geal – earth.

- A mode of germination in which cotyledons are carried above the soil level by the elongation of hypocotyls.
- Germination process in which cotyledons come above soil (Epi-upon, geal – earth). Examples – Cotton, Papaya, Sem, Castor, Sunflower.

Hypogeal germination: Hypo – below, geal – earth.

Mode of germination in which the cotyledons (dicot) or scutellum (monocot) remain in the soil or within the seed. The shoot is carried above the soil level by elongation of epicotyl in dicots and mesocotyl in monocots. e.g. Most monocots; peas, pigeon pea, chickpea etc.

Viviparous germination

- The germination of seeds in the mother plant itself without being dried. e.g. Chow- chow (*Sechium edule*); Mangrove tree (*Rhizophora* sp.).
- The precocious germination of seeds in the fruit while still attached to the plant.

Important events in germination

- Imbibition.
- Hydration of the protoplasm.
- Activation and synthesis of enzymes.
- Increase in respiration.
- Increase in synthesis of nucleic acids and proteins.
- Synthesis and release of hormones from the embryo.
- Increase in cell enlargement and cell division.
- Hydrolysis of reserve food substances present in the endosperm and cotyledons.
- Utilization of soluble organic substances by the developing embryo.
- Growth of the radicle into the root and the plumule into shoot.

The first step in the germination is the adsorption of water by seeds. The process is called imbibition which indicates the adsorption of water by hydrophilic colloids of seeds. Then the adsorbed water molecules are absorbed. Initially the absorption rate is very fast but decreases with time. This is purely physical absorption and continues from 12 to 24 hours till saturation. However, if O_2 is available with proper temperature a second phase of absorption begins which is due to induction of rapid growth in the axis. This phase is called metabolic absorption.

As seed imbibes water all the cells in the embryo, cotyledons and endosperm become hydrated which results in cell division and enlargement. The complete hydration may take 40-60 hours depending on temperature and availability of water. In first 24 to 36 hours no change in dry

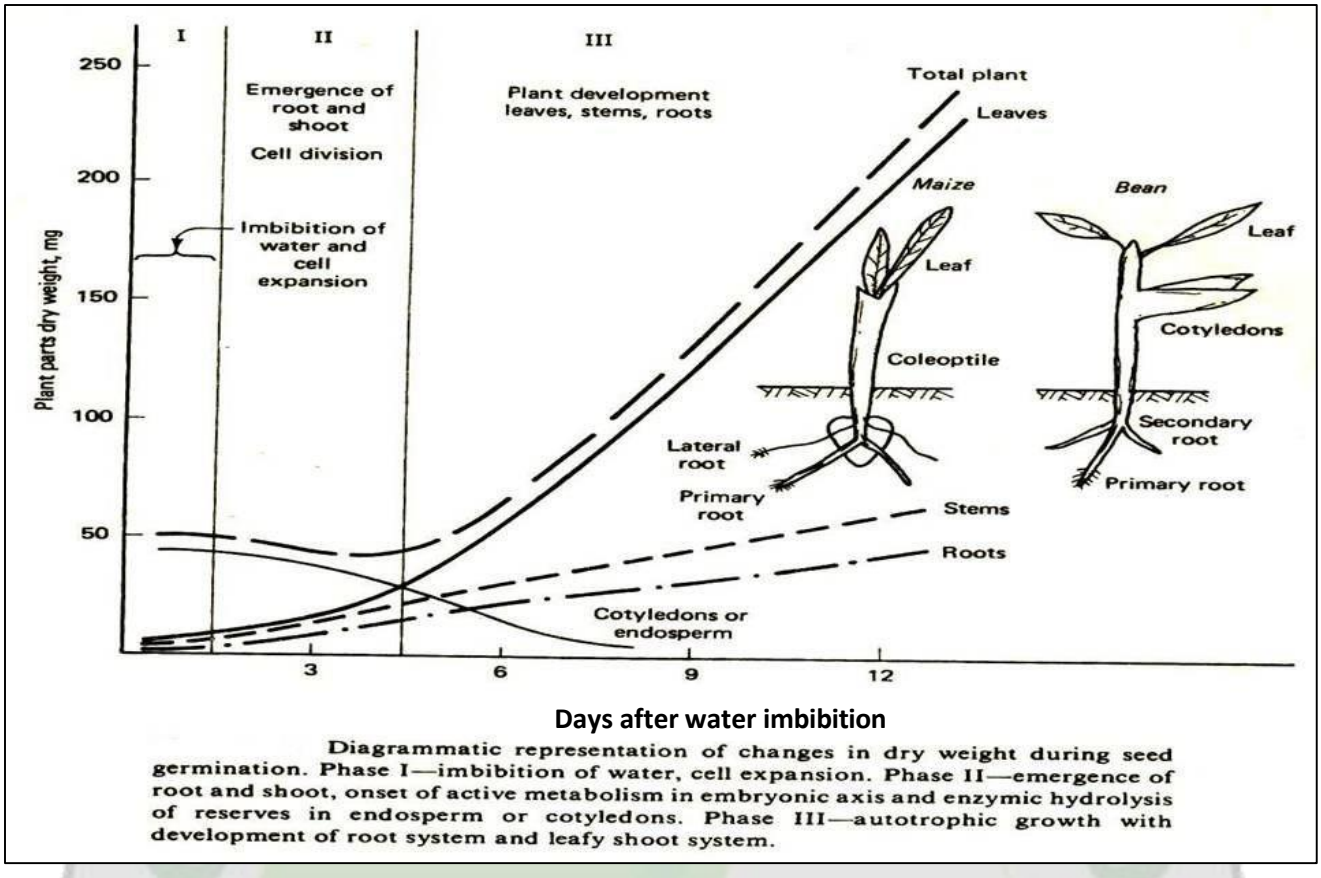
polysaccharides, nucleic acids, phospholipids become hydrated and organelles like ribosomes, mitochondria, endoplasmic reticulum and glyoxysomes are reorganized within 6 to 12 hours period in most of the seeds. Further growth and development of embryonic axis into seedling depend on new cell formation, a process that requires ATP and substrates for anabolic metabolism. About 8 hours of onset of water imbibition the activity of some hydrolytic enzymes can be detected. These enzymes break the macromolecules into micromolecules. Some of the micromolecules or ions may be absorbed directly by the embryonic axis, whereas others are further metabolized before entering the metabolism of embryo. Many enzymes like proteinases, nucleases, lipases and phytases are synthesized during seed maturation but present in inactive form. After hydration they become active, whereas, enzymes like alpha amylase are not present in dry seeds but synthesized de novo following water imbibition. Dure and colleagues found in cotton seeds that two enzymes involved in germination are synthesized de novo following water imbibition. One a carboxypeptidase which catalyses the breakdown of protein to amino acids, whereas, isocitric lyase is a key enzyme in glyoxylic acid cycle. Due to hydration cells of embryonic axis attain full turgor resulting in reorganization of subcellular organelles and cellular membranes. Protein and nucleic acid synthesis have also been reported after imbibition.

In dry seeds respiration rate is very slow but after water absorption it increased rapidly. During first few hours of absorption RQ becomes more than 1. In barley it reached to 4.0 but after 24 hours it declined to 1.0.

The major food reserves like fats, proteins , starch, nucleic acids and phytin starts hydrolyzing and mobilizing to the embryonic axis and provide the embryonic axis with the amino acids, nucleotides, nucleosides, inositol, sucrose, fatty acids and some inorganic ions.

In monocots the hydrated embryo secretes the gibberellic acid which moves to the aleurone layer where it initiates the synthesis and activation of number of hydrolytic enzymes like amylase, protease, nuclease and phytase. It has been observed that IAA- Inositol glycosides are released in the endosperm due to hydration from where they move to the embryonic axis and helps in seedling growth.

The whole germination process can be divided into three phases. Phase-I represents imbibition of water and cell expansion. Phase-II represents emergence of root and shoot and cell division. At the end of Phase II dry weight of the endosperm in monocots decreases followed by increase in dry weight of the root and shoot and plant is capable of an independent mode of metabolism and becomes autotrophic. In dicots cotyledons show a decrease in dry weight followed by increase in dry weight in the parts of embryonic axis. Phase III is represented by autotrophic growth with the development of root and leafy shoot system. Roots start taking water and inorganic solutes from the soil.



Diagrammatic representation of changes in dry weight during seed germination. Phase I—imbibition of water, cell expansion. Phase II—emergence of root and shoot, onset of active metabolism in embryonic axis and enzymic hydrolysis of reserves in endosperm or cotyledons. Phase III—autotrophic growth with development of root system and leafy shoot system.

Phases of germination

Chapter 13

SEED DORMANCY

Dormancy can be of various kinds Viz;

Dormancy (Primary)

Dormancy that exists within the seed at the time of maturity on the plant or immediately afterwards.

Dormancy (Secondary)

Dormancy that develops within the moist seed after it has been removed from the plant if subjected to adverse environmental conditions. Syn. Induced dormancy.

Dormancy (Chemical)

Dormancy caused by chemical inhibitory substances in the fruit or seed. e.g. Coriander. Dormancy may be overcome by leaching with water.

Dormancy (Combined)

Dormancy as a result of two primary factors, such as seed-coat dormancy and embryo dormancy. Both types of dormancy must be broken for germination to proceed. e.g. scarification followed by prechilling. Syn. double dormancy.

Dormancy (Embryo)

Dormancy as a result of conditions within the embryo itself. e.g. inhibiting substances or incompletely developed embryo. syn. Endogenous dormancy.

Dormancy (Exogenous)

Dormancy caused by the outer structures of the seed or fruit. E.g. impermeability of the seed-coat or pericarp.

Dormancy (Mechanical)

Dormancy caused by mechanical resistance of the seed covering (normally the endocarp) to expansion of the embryo. Dormancy is overcome by physically breaking the restricting covering or extracting the seed. e.g. some rice varieties.

Dormancy (Photo)

Secondary dormancy developed in light-sensitive seeds. Dormancy is caused by high level of phytocohrme Pr that must be changed to phytochrome Pfr by exposure to red light (660-760 nm) or exposed to full day light after imbibitions. e.g. lettuce.

Dormancy (Physical)

Dormancy caused by an impermeable seed coat (hard seed). The embryo is quiescent (non-dormant) but is sealed inside the impermeable covering at low moisture content. e.g. Most plants Leguminosae. Scarification can be used to improve germination.

Dormancy (Physiological)

A type of embryo dormancy in which germination is prevented by a physiological inhibiting mechanism. e.g. chemical dormancy or thermo-dormancy.

Dormancy (Seed-coat)

Dormancy as a result of seed coat conditions. This type of dormancy may be overcome by scarification or complete removal of the seed coat. e.g. impermeability (physical dormancy) or phytochrome system (photo-dormancy).

Dormancy (Thermo)

Primary or secondary dormancy in which the seed must be subjected to a low or fluctuating temperature prior to germination. In temperate regions, thermodormancy is overcome by pre-chilling or stratification, in tropical regions by exposing the seeds to normal diurnal fluctuations in temperature prior to germination.

Dormancy due to embryo

Dormant, immature embryo requires exposure to light for germination Examples lettuce, spruce.

Dormancy due to seed coat

Seed coat impermeable to water, O_2 and mechanically resistant seed coat.

After ripening

- A large number of seeds do not germinate immediately after harvesting but if kept for some period of time under favourable conditions they start germinating.
- Many seeds lose dormancy when their moisture content is reduced to a certain level by drying.
- The process in which the seed with immature embryo is made to mature by placing it under warm-moist conditions.

Nikolaeva (1977) termed it as **Organic dormancy** as it is related to the properties of seed itself. Non - germination of seeds due to absence of suitable conditions is termed as **Imposed dormancy** by Nikolaeva.

He divided the dormancy into three groups

- Endogenous
- Exogenous
- Combined.

The factors responsible for causing seed dormancy

a) **Seed coat factor** I - Seed coat impermeable to water II - Seed coat impermeable to oxygen III - Mechanically resistant seed coat

b) **Embryo factor** I- Dormant embryo II - Immature / Rudimentary embryo

c) **Inhibitors** - Presence of germination inhibitors in seeds.

Advantages of dormancy

A wide range of fluctuations ranging from below freezing point in mid - winter to 100^0 F in mid – summer in temperature zones has been observed. Many plants couldn't survive in cold temperature in winter in vegetative or flowering stage. Thus in many plants seed or bud dormancy begins at the onset of cold allowing the plants to pass through the period with a little or no damage.

Role of growth regulators

Dormancy in the seeds due to presence of abscisic acid can be overcome by increasing the level of promoters like gibberellic acid and cytokinins.

Respiratory pathways

Pentose phosphate pathway plays a major role in breaking seed dormancy which is O_2 requiring process. Dormant seeds have been reported to germinate in presence of hydrogen

acceptors like nitrate, nitrite, methylene blue, terminal oxidase inhibitors such as cyanide, azide, hydroxylamine etc. and TCA cycle inhibitors like melonate and monofluro-acetate.

Role of light in breaking dormancy

For some kinds of seeds Ex. Lettuce, spruce etc. exposure to light is essential for germination. Such seeds are called **Positive photoblastic seeds**. The kind of seeds which specifically requires red light to germinate are called **Phytochrome controlled seeds.**

Methods of breaking seed dormancy

(A) Scarification: Any treatment physical or chemical that weakens the seed coat is known as scarification. This method is applied when dormancy is imposed by hard seed coat Ex. Legumes. There are various ways to break dormancy such as:

I. Seeds may be rubbed with sand paper manually. Care should be taken not to damage the axis of the seeds. Ex. Subabool (*Leucaena lenscephalla), Green gram (Vigna radiate)* .
II. Sometimes instead of rubbing the seed coat absorption of moisture is made possible by piercing the seed coat a little with the help of needle or by giving a small incision. Ex. Bitter guard (*Momordica charantia*).
III. When the seed coat is too hard, the seed coat has to be removed completely by breaking it. Ex. Rubber (Hevea spp.).
IV. Placing the hard coated seeds in concentrated or diluted solutions of sulphuric acid for 1 to 60 minutes also removes seed coat impermeability. Seeds should be thoroughly washed in running water after acid treatment to ensure removal of even trace of acid from the seed surface Ex. Cotton (*Gossypium arboreum*).

(B) Temperature treatment

I. When the dormancy is due to embryo factor the seeds are subjected to stratification (i.e. incubating seeds at low temperature 0– 5^0C) for 3 to 10 days before placing them for germination. Ex. Cherry, Mustard. Prolong stratification is required for a number of rosaceae spp. for a period of 2-6 months at 5-10^0C to break the dormancy.
II. Some seeds require a brief period of incubation (from a few hours to five days at 40^0C) before placing them for germination. Care should be taken that moisture contents of the seeds are not more than 15%. Ex. Rice (Agrawal 1981).
III. Hot water treatment is also an effective method of breaking hard seededness in legumes (Anonymous 1984). For this seeds are soaked in water at 80^0C for 1-5 minutes.

(C) Light treatment

Some seeds don't germinate in dark thus providing continuous or periodic exposure to light is essential. Ex. Lettuce (*Lactuca sativa*). Irradiance of lettuce seeds with either red light (660 nm) or white light is essential for germination to occur. In such cases seeds are either placed under red light initially and then germinated in dark or they may be germinated under continuous white light.

(D) Treatment with growth regulators and other chemicals

Since endogenous dormancy may be due to presence of germination inhibitors, application of low levels of growth regulators may break the dormancy. However, most widely used chemicals are GA and Kinetin. Out of several gibberellins gibberellic acid (GA_3) has been found to be the most effective in breaking seed dormancy. 100-1000 ppm concentrations may be used. Seeds are either soaked in solution of 100 ppm GA_3 and 10-50 ppm Kinetin before placing them for germination. Among other chemicals Potassium nitrate (0.2%) and Thiourea (0.5 to 3.0 %) are widely used to break the dormancy. Potassium nitrate breaks dormancy of light requiring seeds in dark. Generally seeds are germinated on a paper, saturated with 0.2% solution of Potassium nitrate. Subsequent moistening is done by water. Potassium nitrate has been found to be effective in breaking dormancy of Oat (*Avena sativa*), Barley and Tomato. Thiourea breaks dormancy of both light and chilling requiring seeds. Ex. Cichorium.

Chemicals which break dormancy

Growth regulators

Gibberellins, Cytokinins.

Plant products

Fusicoccin, Cotylenol, Cotylanin, Strigol.

Respiratory inhibitors

Cyanide, Azide, Carbon - mono - oxide, Sodium fluoride, Melonate, Oxalo - acetate, Iodo-acetate, Dinitro phenol, L& D Chloramophenicol, Hydroxyl-amine.

Oxidants

Hypochloride, Oxygen.

Nitrogenous compounds

Nitrate, Nitrite, Hydroxyl - amine, Thiourea.

Sulphydryl compounds

Dithio- threitol , 2 Mercapto- ethanol , 2,3 Dimercapto- propanol.

Others

Acetone, Ethanol, Methanol, Ethyl ether, Chloroform, CO_2, Phenols.

Germination inhibitors

Abscisic acid, Parascorbic acid, Coumarin, Phenolic compounds like Ferulic acid.

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