جامعة جنوب الوادي قسم النبات والميكروبيولوجي كلية العلوم

Lectures and practical lessons

Plant physiology

2nd year biology students

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Part II

Plant physiology

Chapter 1

Absorption of Water

Water

Life originated in an aqueous environment and in the course of evolution became fully dependent upon water in a number of ways. In general, water is essential for life and its importance to plants may be summarised as follows:

(1) Water is the main constituent of the protoplasm comprising up to about 90 to 95 %of its total weight. In the absence of water, protoplasm becomes inactive and is even killed.

(2) Different organic constituents of plants such as carbohydrates, and proteins, nucleic acid and enzyme etc. , lose their physical and chemical properties in absence of water.

(3) Water participates directly in many metabolic processes. Interconversion of carbohydrates and organic acids depends upon hydrolysis and condensation reactions.

(4) Water increases the rate of respiration. Seeds respire fast in presence of water.

(5) Water is a source of hydrogen atom for the reduction of carbon in the reactions of photosynthesis.

(6) Water acts as a solvent of and carrier of many substances. It forms the medium in which several reactions take place.

(7) Water present in the vacuoles helps in maintaining the turgidity of cells, which is a must for proper activities of life. The turgidity of cells help s in

the elongation of cells resulting in growth. The difference in the amount of available water during summer and winter season for the formation of annual rings in the higher plants. In summer, the turgidity is less and as a result smaller cells are formed.

(8) A network of thin layer of water surrounding each cell plays an important role in the entry and movement of dissolved substances.

(9) Water helps (I) in the transactions of solutes , (II) in the mobility of gametes , (III) in the dissemination of spores , fruits and seeds , and (iv) provides support to aquatic plants .

(10) In tropical plants, water plays a very important role of thermal regulation against high temperatures. Some people think the plants lose about 95 per cent of the absorbed water just to maintain the optimum temperature.

(11) Thousands of characters develop for balancing the water content of planes. Even atmospheric moisture affects plants growth. Different plants absorb water from their general surface while in higher plants roots are the organs concerned with absorption of water.

Soil water

Plants absorb water from the soil by their roots. The water is found in different forms in the soil. The chief source of water to the soil is rain or irrigation. After a rainfall or irrigation some of the water penetrates downwards, under the influence of gravity until it reaches the water table. This is called “the gravitational water” and it is of little benefit for the plants. Moreover, it may be injury to plants. Because it replaces the air between the soil. A major portion of the water is retained by the soil particles against the force of gravity which keep the soil moist. Some of this water is adsorbed by the soil colloids and is held tightly by them in very thin films. This called the “hygroscopic water” and it is non- available for the plants. Another portion of water fills the spaces between the soil particles and called the “capillary water” which is the greatest important for the plants, because it easily absorbed by root hairs. A portion of the gravitational water rises by capillarity and becomes ready available to plants. This portion depends on the structure of the soil, which is generally depend on the size of the soil particle. As soils with relatively small particles held more capillary water than that of relatively large ones.

Movement of water in the plants

1. Absorption of water (root system)

2. Ascent of sap (shoot system)

3. Loosing of water (transpiration) (leaves)

Water absorbing parts of plants

Major portion of water required by plants is absorbed by the roots, but the absorption of water by leaves and stem has also been found in a few plants hydrophytes absorb water by general surface.

The uptake of water by leaves is influenced by:

(i) Structure and permeability of cuticle and epidermis,

(ii) The hairiness of leaf surface,

(iii) The case of wetting surface, and

(iv)The internal environment of deficiency of water in parenchymatous cells closes to the epidermis

Roots play the principle role in absorption of water. Even orchids absorbing water from atmosphere develop modified roots for the purpose.

ROOTS

Roots absorb water mainly from the apical region. Apical organization of root shows three clear demarcations, the zone of elongation and the zone of absorption or differentiation.

The zone of differentiation consist of three different types of tissue system, i.e. dermal, cortical and stellar. Dermal tissue include surface layers of cells. Epidermis in the region has enormous number of unicellular root hairs. Cortical tissue system is complex and consists of pericycle, phloem and xylem etc. Important ones are described here.

Root hair

Root hair is the special modified cell of epidermis meant for the absorption of water. It is specialised not only in appearance but also in its internal structure. The wall of root hair consists of cellulose and pectic substances have great capacity of water absorption.

The cell wall act as permeable layer. Next to cell wall is plasma membrane enclosing cytoplasm, nucleus and vacuole. Vacuole is quite large in size so as to give peripheral arrangement of cytoplasm. The role of vacuole during absorption of water is just like a controller.

MECHANISM OF WATER ABSORPTION

Entry of water in root hair

Root hair maintains contact with soil water and in nature it acts as a soil water –absorbing organ. The water diffuses in to the root hair as a result of diffusion pressure deficit (DPD) gradient. The cell sab contains a more concentrated solute than the water present outside.

Water enters as long as DPD of cell sap is greater distending the cell until the elasticity, of stretched wall is sufficient to balance the osmotic pressure of solutes.

How exactly water enters in the root hair and what is the precise mechanism of water absorption have been explained by two different approaches.

(i) Active absorption.

(ii) Passive absorption.

Active absorption

When we speak about water being absorbed actively, we mean that water is being absorbed through expenditure of metabolic energy. Active absorption occurs as a result of activities in root and does not concern the shoot. Generally it is thought that the active absorption of water may occur in one of two ways, as a result of the active absorption and accumulation of salts or through non-osmotic mechanisms.

a) **Osmotic active absorption:** actually water absorbed by osmosis and this means that it is does not directly require an expenditure of energy. Water is thought to move from the soil to the interior of root along an increasing osmotic pressure gradient. That is water moves through the root epidermis, cortex, and into the xylem ducts because of increasing solute concentrations as it passes from the exterior to the interior cells of the root. The water absorbed by this manner does not directly require an expenditure

of energy. The energy is expended in the absorption and accumulation of salts.

(b) Non-osmotic active absorption: Thimann (1951) and Kramer (1959) suggested that the absorption of water is an active process but occurs due to non-osmotic reason even against diffusion pressure gradient. The process requires an expenditure of energy obtained from respiration. How is the energy utilised is not well explained. It may be used directly.

Following are supporting points of this theory that water is absorbed non-osmotically and there is participation of energy (respiration):

(1) Wilting of roots occurs in non-aerated soils such as flooded areas. It indicates that water is absorbed by living cells under aerobic atmosphere.

(2) Use of respiratory inhibitors such as KCN, reduces the rate of water absorption and exudation from the cut end of stems. Thus, there is some correlation between the processes.

(3) The occurrence of distinctive diurnal variation in water uptake and root pressure. It is faster during day time and slower during night. This fact is also true for respiration.

(4) The water absorption is also influenced by hormones such as auxin. Low auxin concentration increases water uptake and exudation.

(5) The process of absorption occurs in living cells.

Passive absorption

This theory explains that the forces responsible of absorption of water into the roots are governed by other cells. The governing force originates in the cells of transpiring shoots rather than in root itself. This forces develop due to transpiration. With the occurrence of transpiration, the DPD of leaf cells increases, which results in the movement of water from the xylem cells to adjacent mesophyll cells. Due to presence of continuous column of water from leaves to roots through xylem channels, the deficit is transmitted to the xylem of roots and finally to root hairs along which radial movement of water takes place and puts these cells under tension.

Path of Water

We should by now be familiar with the tissues encountered by water moving from the soil to the leaves of plant. In figure 7.8 the path of water through a plant is diagrammatically shown. Water is first absorbed from

the soil by root hairs and other epidermal cells in or near the root hair zone. Water then move through the cortex tissue and across the endodermis and pericycle and finally into the xylem ducts. The xylem tissue of the roots connects directly with the xylem tissue of the stem. The xylem of the stem, thus allowing water to move out of the root and into the stem, the xylem of the stem is divided and subdivided many times to form a complex network of water–conducting tissues, finally ending in the fine veins of the leaf. Water moves from the leaf veins into the mesophyll cells, is evaporated from their surfaces, and finally moves as water vapour through the stomates into the surrounding atmosphere.

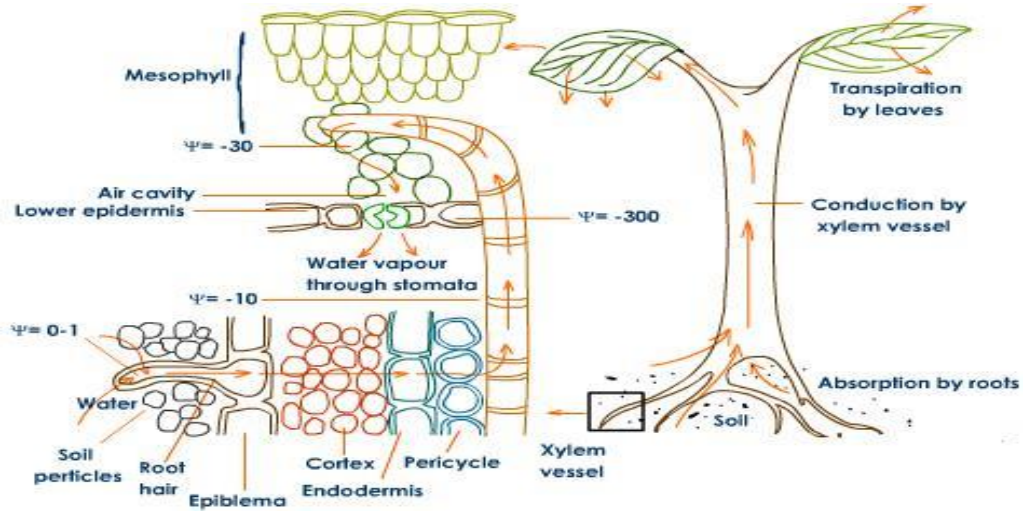


Figure 7.8

Movement of water through plants

Ones the water entered in root hair, it moves first to root stele and then to leaves passing through the different parts of the plant. The movement of water inside the plant shows two different directions. In the first stage, it moves from the root hairs to the stellar region of root via cortex and endodermis, i.e. radial movement of water,

And in the second stage, it moves from the root stele to the top of leaves i.e. upward movement of water. The upward movement is popularly known as “A SCENT OF SAP”

Radial movement of water: (movement of water from root hair to stele):

There are two ways of radial movement of water:

(1) Cell to cell movement of water across the root (osmotic flow).

(2) Movement of water across the cortex along the water filled spaces into the cell walls forming a continuous system from soil water film to endodermis.

Both this mechanisms operate together and help the water to reach up to endodermis.

As a result of the absorption of water from the soil, the root hair cell becomes fully turgid, it is osmotic pressure falls due to dilution and its turgor pressure increased. As a consequence, its suction pressure will fall below that of the adjacent cortical cell **B** as a result water will pass from **A** to **B**.(**Fig. 2).**

The diffusion of water in to **B** likewise reduces its suction pressure which falls below that of the next cortical cell **C**, with the result that water passes from **B** to **C**. in the same manner water pass from the cell **C** to **D**, and from **D** to **E**, from **E** to **F** and from there in to the endodermal cell **N**. from here it is passed on to the pericycle cell **O** which will eventually become turgid. It will then exert no suction pressure and hence, will readily give up water to the xylem vessel with which it is in contact. The walls of the xylem vessel are in elastic so, that there is not turgor pressure and the whole of the osmotic pressure of the xylem sap constitutes its suction pressure. This being higher than the reduced suction pressure of the parenchyme (pericycle) cell **O**, water will draw in to the xylem vessels.

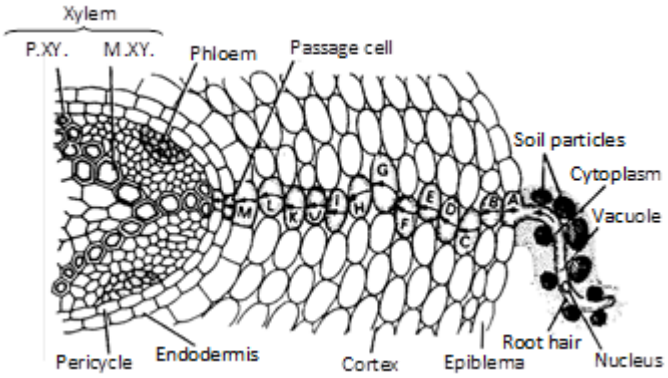


Fig. 2 diagram indicated the movement of water (radial movement) after absorbing from the soil.

FACTOR AFFECTING WATER ABSORPTION RATE

The plant gets two types of environment includes factors such as (a) available soil water , (b) concentration of soil solution , (c) soil temperature and (d) aeration while internal environment includes factors such as (a) transpiration , (b) absorbing root system and (c) metabolism .

External Environmental Factors

(a) Available soil water:

The water in the soil is present in a different forms such as capillary, hygroscopic, gravitational etc, of which capillary water is readily available for absorption. This is in between field capacity and permanent welting percentage where the rate of absorption of water is generally uniform and is not affected. With an increase in water beyond field capacity, aeration of soil is badly affected which reduce s the rate of absorption and under severe conditions wilting results in. The similar wilting is observed in extremely dry soils and a decrease in soil water reduces the rate of absorption.

(b) Concentration of soil solution:

Large number of elements are dissolved in soil water called soil solution. On account of these elements, the concentration of soil solution changes. If the soil solution is highly concentrated, it increases greatly osmotic pressure and when it reaches higher to that of cell sap, water is not absorbed. It is one reason that the plants fail to grow in highly saline fields. This is popularly known as physiological dryness.

(c) Soil temperature

The variation of temperature affect the rate of absorption .20 to 30 °Cis the most suitable temperature for absorption. The low temperature reduces and moderately high temperature increases the rate of absorption. A very high temperature kills the cell. How the low temperature exercises its negative influence in absorption has been explained as follows that it results in:

(I) slower rate of elongation of root thus preventing its contact with areas.

(II) Slower rate of metabolic activities.

(III) Reduce of soil water diffusion into the roots.

(IV) Increased viscosity of water, protoplasts and colloidal gels in the cell wall.

(V) Decreased permeability of cell membrane.

(d) Soil aeration:

Water is absorbed more efficiently in a wall aerated soil than in a poorly aerated soils. Probably the reason may be the respiration as normally roots fail to respire anaerobically and plants shortly die in floated areas. The deficiency of oxygen inhibits the growth and the metabolism and accumulation of CO_2 increases the viscosity of protoplasts and decreases the permeability of cell membrane. Both this factors affect severely and reduce the rate of water absorption. These may be the reasons for plant death in flooded areas. Only few plants such as rice and Typha can grow normally in poorly aerated soils as these are specially adapted to such environments.

Internal Environment Factors

(a) Transpiration.

The rate of absorption of water is nearly directly proportional to that of transpiration. A higher rate of transpiration increases the rate of absorption because of cohesion theory of ascent of sap, i.e. transpiration produces a tension or pull, transmitted to roots through hydrostatic system of plants creating a favourable condition for entrance of water.

(b) Absorbing root system.

The efficiency of water absorption depends upon the absorbing system. The presence of number of root hairs accounts for the rate of absorption. However, the development of root hairs depends upon environment. The maize plants does not produce root hairs in culture solution but produces large number of root hairs when grown in soil. In moist conditions root hairs are well developed and large in quantity. The coniferous plants (gymnosperms) bear few or no root hairs but absorb large amount of water with the help of mycorhizal hyphae. Thus root systems play a major role in absorption of water

(c) Metabolism.

The metabolism and absorption are closely related. Although doubt exist in use of energy during absorption, but factors inhibiting rate of respiration- such as poor aeration application of anaesthetics and KCN reduce the absorption rate . Thus metabolic activities are expected to participate indirectly by forming a constantly elongated root system and always providing newer contacts with soil water.

Chapter 2

Various elements found in plants

Major elements

Serious attempts to determine experimentally the mineral content of plants were made by **Sachs and knop** as far back as 1860. Using liquid cultures, they were able to show that ten elements are essential to the plant. these they listed as carbon (C) hydrogen (H) , oxygen (O) , nitrogen (N) , Phosphorus (P) , potassium (K) , Calcium (Ca) , Sulfur (S) , Magnesium (Mg) , and Iron (Fe) . These ten elements were generally accepted as all that a plant needed for normal growth and development. However, we know today that there are growth of most plants and several additional elements specifically required by certain plants.

Method of detections

Several of the methods used in the early study of plant nutrition are still in use today. The analysis of plant ash and the use of liquid and sand cultures are techniques used for the study of plant nutrition in laboratories throughout the world. However, these methods have been refined and improved upon.

Ash analysis

A reasonably reliable means of detecting the mineral element content of a plant is to subject the plant to high temperatures (about 600 ℃) and then analyse its ash content. Only ten mineral elements are present all of the organic compounds having been decomposed and passed off in the front of gases. These primary elements (carbon, hydrogen, and oxygen) are therefor given off as CO_2, water vapour and oxygen. In addition to carbon, hydrogen, and oxygen, the element nitrogen cannot be detected accurately with this method, since some of it is given off in the form of ammonium or

nitrogen gas. All of the other mineral elements that were absorbed from the soil are present in plant ash.

Although the analysis of plant ash may be thought of as a method of determining the relative quantities of mineral elements in a plant, it is, at best, a crude technique. Too many variables are present to give accurate, reliable results. For example, vaporization or sublimation of some of the elements may be caused by the high temperatures. Generally, elements are not present in pure state in the ash, but are in the form of oxides. finally , the qualitative and quantitative analysis of the ash for the different elements present is depended on different chemical treatments the accumulative error resulting from this facts quantitative data obtained from the ash analysis of plant tissue.

Solution cultures

It did not take scientists long to realize the impracticality of using soil as a medium for growth in any serious study of plant mineral requirements. To render a soil free of the mineral elements used by plants and then control the amounts of nutrients made available to the roots imbedded in the soil is impossible. On the other hand, solution cultures provide an excellent means for controlling the quantity and relative proportions of minerals salts given to a plant in any of one experiment. Two other good reasons for using solution cultures in mineral nutrition studies are the excellent solvent characteristics of water and the relative ease with which water can be freed of most contaminating influences.

Good quantitative studies may be made of the nutritional need of plants using water as a medium. However careful attention to small details is necessary to achieve good results. Due to the fact that satisfactory growth may be achieved with extremely small amounts of trace elements, contamination problems are always present. Some of the sources of the contamination are the rooting medium, reagents used containers, the water, cutting implements, seeds and the dust in the surrounding atmosphere. Obviously, total elimination of these contaminating influences is impossible, but they can be kept to a minimum.

Several studies have shown that the best container for solution cultures are made of borosilicate grass or natural polyethylene **(Hewitt, 1963)**. However, even with the use of these materials, some contamination may be expected, such as the presence of boron in borosilicate glass and, perhaps, molybdenum and cobalt in polyethylene. Water distilled in metal

stills usually is contaminated with trace amounts of copper, zinc, and molybdenum. Redistillation of water in stills made entirely of borosilicate glass is necessary to remove this elements **(Piper, 1942; Ston and Arnon, 1939)**. Another satisfactory method of ridding water of contaminating trace elements is to pass it over cation and anion exchange resins **(Hewitt et al., 1954).**

In early studies of plant nutrients reagents used presented a major source of contamination. These reagents had to be purified by various means before trace elements deficiencies could be demonstrated. Reagents may be purchased today that are pure enough for most studies. But even this contain trace amounts of contaminants.

From the discussion above, one can see that most of difficulties encountered in mineral nutrition studies are associated with trace element contamination. A study of deficiencies caused by major nutrients can be easly accomplished because of the relatively large amounts needed for normal growth. Here, a small amount of contamination is not a serious problem.

With proper attention given to the problems discussed above, the next step is to prepare stock solutions from inorganic salts containing the necessary elements for normal plant growth. Once stock solutions are prepared and the proper containers obtained and filled with deionized water, nutrient solutions may be prepared by simply adding, in the correct proportion, the necessary inorganic salts from the stock solutions. Several satisfactory formulas for nutrient solutions have been prepared.

Sand cultures

Solid media, such as sand or crushed quartz, are generally easier to work with than a liquid medium. On the other hand, purification problems are more difficult to cope with. However, today it is possible to purchase highly purified silica sand or crushed quartz that is very low in available trace elements. The added attraction a solid culture is that the roots are growing in a nature medium and no means of support needs to be provided. Nutrient solutions are added to the solid culture by three different ways: pouring over the surface (slop culture), dripping on the surface (drip culture), and forcing solution up from the bottom of the container (subirrigation). In the all three systems, the nutrient solutions added drain out through an opening in the bottom of the container. In subirrigation, the

system may be attached to a timing mechanism, which may be set to give periodic irrigation to the sand.

Of the three methods, the slop culture is the easiest to manipulate, but offers the least control. The drip culture may be set up so that the amount of solution being added equal to the amount of solution draining off. This method allows for continuous nutrients supply and partial control of the amount of nutrients reaching the root system. The last system, subirrigation, may be set up to work automatically and also gives partial control of the amount of nutrients reaching the plant roots. The subirrigation system is the most desirable of the three systems, but the hardest and most expensive to set up initially.

Chapter 3

Occurrence of the various elements

Because of their relative importance and abundance in the plant carbon, hydrogen, oxygen, and nitrogen will not be covered in this chapter but will receive more extensive attention in separate chapters**.**

Phosphorus

Phosphorus is present in the soil in two general forms, inorganic and organic. In the organic form, phosphorus may be found in nucleic acid, phospholipids, and inositol phosphates, compounds common to the organic fraction of the soil. To the author's knowledge, there have been no reports of plants absorbing organic phosphorus, either from the solid or solution phase of the soil. Therefore, organic phosphorus represents an unusable form of the element with respect to the plant. However, organic compounds are eventually decomposed and phosphorus is released in an inorganic form, which is readily taken up by the plant.

Much of the phosphorus of the soil solution is present in the inorganic form, mainly as the phosphate ions $H_2PO_4^-$ and HPO_4^{2-} (wiklander, 1958). The quantity of either ion present is dependent upon the PH of the soil solution, the lower pH favouring the H_2PO^{4-} ion and the higher pH, HPO_4^{2-}.

Calcium

Generally, calcium is the major exchange cation of fertile soils (**marshall, 1951**) .However, the major portion of calcium in the soil is found in a nonexchangeable form, chemically bound in primary minerals such as anorthite ($CaAl_2Si_2O_8$). Through a weathering, this calcium can be made available. We have already mentioned the presence of calcite ($CaCO_3$) of soils in semiarid and arid regions and the general occurrence of insoluble calcium is available to the plant, depending upon the solubility of the salt and the degree of alkalinity.

Liming

The most effective and economical method of controlling soil pH is the application of the lime. lime to the chemist is calcium oxide (CaO) , but to the farmer , it is any compound containing calcium or magnesium

capable of counteracting the harmful effects of an acid soil **(Millar et al ., 1951) .**

In an acid soil, we have clay micelles with a predominance of exchangeable hydrogen ions absorbed to their surfaces. With the addition of lime compounds, such as calcium carbonate ($CaCO_3$) or calcium oxide (CaO) many of hydrogen ions are replaced by calcium ions. In addition, the released hydrogen ions are tied up in the form of water. The final result is a rise in pH and an increase in the supply of exchangeable calcium ions (figure 13-4).

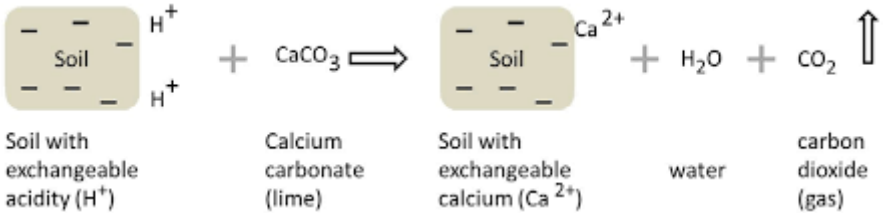


Figure 13-4

One should be cognizant of the harmful effects of liming, as well as the beneficial effects. Over liming a soil may cause the pH of the soil to rise above 7.

Magnesium

Magnesium is present in the soil in water-soluble exchangeable and fixed form and is present in primary minerals (**Bould, 1963**).

Magnesium in minerals such as magnesite ($MgCO_3$) , olivine ($MgCO_3)_2SiO_4$) , and dolomite (MgCO3 .$CaCO_3$)is available to plants in satisfactory amounts for growth .

Potassium

Potassium is present in the soil in a nonexchangeable or fixed form, an exchangeable form, and a soluble form. Although there is a relatively high content of this element in the soil, most of it is nonexchangeable and, therefore unavailable to the plant.

When we speak of an element being unavailable, especially with respect to potassium, we mean that utilization of the element in its present form by the plant is not possible. However, availability of potassium in potassium-bearing minerals, such as biotite, muscovite, and illite , is made possible through normal weathering processes .

An equilibrium exists between the soluble, exchangeable, and fixed forms of potassium

Soluble K == exchangeable K == fixed K

Sulfur

Soil sulfur is found primarily in the organic fraction (**Quastel, 1963**), but may also be found in minerals such as pyrite, cobaltite, gypsum, and epsomite and in the soil solution as the sulfate ions $(SO_4)^{2-}$. Sulfur is the phosphate ion, the sulfat ions is weakly adsorbed, the adsorption increasing with a decrease in soil pH. Adsorption is favoured by the presence of hydrated oxides of iron fate ion is generally thought of as replacing hydroxyl ions in clay minerals , a process known as anion exchange.

Organic sulphur is made available to the plant through biological oxidation. Through the activity of certain microorganisms, sulfur is transformed from the organic form to the sulfataion, the form of sulphur that higher plants absorb. Not only do soil microorganisms oxidize organic sulphur, but also sulphide minerals, such as ferrous sulphide (FeS). Where there is good aeration, moisture and suitable temperature, FeS canbe chemically oxidized to elemental sulfur.

The elemental sulfur is then oxidized sulfate by sulfur bacteria. The two-step oxidation of ferrous sulphide in soil was first demonstrated by **Wiklander et al. (1950).**

And may be written as follows:

$FeS + H_2O + ½\ O_2 \rightarrow Fe\ (OH)_2 + S$

$2S + 2H_2O + 3O_2 \rightarrow 2H_2SO_4$

Biological oxidation in the soil of pyrite (FeS_2) has also been demonstrated, sulphuric acid being the final product **(Wiklander, 1958)**

Another source of soil sulfur is the atmosphere, the sulfur being brought to the soil by rain and snow.

Iron

Soils generally are not deficient in iron, but may be deficient in exchangeable and soluble forms of iron. Appreciable quantities of iron are present in minerals in hydrated oxides such as limonite ($Fe_2O_3.3H_2O$), and

in the sulfide form (**Bould, 1963**). Iron is most available to the plant in the ferrous form, but significant quantities of the ferric ion may also be absorbed.

The availability of iron to the plant is controlled rather sharply by the soil pH. In acid soils, appreciable amounts of iron are dissolved in the soil solution and available to the plant. However, in neutral or alkaline soils, iron is much more insoluble. In fact, one of the dangers of overliming is that the resulting increase in pH will cause symptoms of iron deficiency to appear on plants. However, even in soils poor in soluble iron, this element may be available by the direct contact of plant roots with iron-containing soil particles **(Chapman, 1939).**

Manganese

According to **Leeper (1947)**, the manganese of the soil may exist in the bivalent, trivalent, and / or tetravalent forms. The bivalent ion may be found dissolved in the soil solution or as an exchangeable ion adsorbed to the soil colloids, both of which are available to the plant. The exchangeable bivalent ion is significant in manganese nutrition, since very little of the soil manganese is likely to be found dissolved in the soil water **(Stiles, 1961).**

Copper

The major portion of the copper of primary rock is present as chalcopyrite ($CuFeS_2$), which is the probable source of natural deposits of copper sulfide in the soil **(Bould, 1963).**

The divalent copper cation is adsorbed very strongly to the soil colloids and organic materials of the soil **(Hasler, 1943)** a form of which it is relatively exchangeable. adsorption of copper as a complex monovalent ion ($CuOH^+$, $CuCl^+$) has been demonstrated in organic soils **(Llucas , 1948**) and on clay minerals **(Menzel and Jakson , 1950) .**

Zinc

According to **Bould (1963)**, zinc occurs in the ferromagnesium minerals, magnetite, biotite, and hornblende. Weathering of these minerals releases zinc in the divalent form, which is readily adsorbed onto soil and organic matter in exchangeable form.

As with many other essential elements, one of the factors controlling the availability of zinc is the soil pH. The availability of zinc decrease with

increase in pH, making it very likely that symptoms of zinc deficiency may occur in plants growing in alkaline soils.

Boron

Boron appears in exchangeable , soluble , and nonexchangeable forms in the soil , that is , as boric acid (H_3BO_3) , calcium or manganese borates , and as a constituent of silicates **(Bould , 1963 ;Wiklander , 1958) .** Like zinc, the dissolved boron content in the soil solution is very low.

Molybdenum

According to (**Wiklander, 1958**), molybdenum is present in soils in three forms:

- dissolved in the soil solution as molybdate ions (MoO_4^{2-} or $HMoO^{4-}$),

- adsorbed to soil particles in an exchangeable form,

- In a nonexchangeable form as a constituent of soil minerals and organic matter.

Chapter 4

Phosphorus

Function

- Phosphorous is found in plants as a constituent of nucleic acids and nucleoprotein so, it is found in the meristematic regions with higher concentration.
- It is found as a constituent of phospholipids which form the cell membrane.
- Also, it is found as a constituent of the coenzymes NAD and NADP that are important in oxidation reduction reactions in which hydrogen transfer takes place.
- In addition, Phosphorous is found in the most important constituent ATP which acts as an energy transfer compound.

Deficiency symptoms of phosphorus

1-Falling of the premature leaves.

2- Formation of purple or red anthocyanin pigmentation.

3-Developing of dead or necrotic areas on the leaves, petioles or fruits.

4-With more severe deficiency, leaves turn pale brown and die, and roots may turn dark and discolored in sorghum

5- Stunted growth.

6-Sickle leaf disease is caused in P deficiency, which is characterised by chlorosis adjacent to main veins followed by leaf asymmetry.

Nitrogen

Function

- Nitrogen has an important role as it found in the structure of protein molecule
- It is found in such important molecules as purines, pyrimidines and prophyrines.
- Purines and pyrimidines are found in the nucleic acids, DNA and RNA which are essential for protein synthesis.
- The prophyrines are important for the metabolism of some compounds such as chlorophylls and the cytochrome enzymes.

Deficiency symptoms of nitrogen

1-the chlorophyll content of the plant leaves is reduced which results in pale yellow color.

2-young leaves are pale as the older leaves are yellow and drop early

3- Purple color formed on leaf, petioles and vines of tomato as a result of production of anthocyanin pigment.

4-Reduction in cell size and cell division

5-low of nitrogen availability must cause a decrease in protein synthesis.

6-Reduction in protein results in stunted growth and dormant lateral buds

7- Flowering, fruiting, protein and starch contents are reduced.

8- Shoots are thinner and shorter.

Magnesium

Function

- Magnesium is a constituent of chlorophyll molecule without it photosynthesis would not occur.
- Many of enzymes involved in carbohydrate metabolism require magnesium as an activator
- Magnesium acts as activator for those enzymes involved in the synthesis of nucleic acids (DNA, RNA).
- It has a role in protein synthesis.

Deficiency symptoms of magnesium

1-The first sign of magnesium deficiency is the chlorosis of old leaves which progresses to the young leaves as the deficiency progresses.

2- The low amounts of Mg lead to a decrease in photosynthetic and enzymatic activity within the plants.

3- After prolonged magnesium deficiency, necrosis and dropping of older leaves occurs.

4- Production of smaller fruits.

5- Stem becomes yellowish-green, often hard and woody.

Calcium

Function

- Act as a constituent of cell walls in the form of calcium pectate
- Calcium is important in the formation of cell membranes and lipid structure.
- Calcium has a role in normal mitosis
- It acts as activator of some enzymes.

Deficiency symptoms of calcium

1- Necrosis leading to stunted plant growth

2- Curling of the leaves

3- Reduction of plant height, fewer nodes, and less leaf area.

4- Death of terminal buds and root tips.

5-cell walls become rigid or brittle.

6- The common disease is blossom-end rot of tomato (burning of the end part of tomato fruits.

Potassium

Function

- It has a major role in varies process as respiration, photosynthesis, chlorophyll development and water content of leaves.
- Potassium acts as activator for enzymes involved in protein synthesis.
- Also, it acts as activator for enzymes involved in carbohydrate metabolism.
- It has an essential role in apical dominance in the plants.

Deficiency symptoms of potassium

1-Brown scorching and curling of leaf tips as well as chlorosis (yellowing) between leaf veins.

2- Purple spots may also appear on the leaf.

3- Reduction in plant growth, root development, and seed and fruit development.

4- Potassium deficiency symptoms first appear on older (lower) leaves.

5- Stunted in growth and shortening of internodes.

6- Two common diseases are known **"Rosette** "in beet and carrot, bushy growth or rosette condition develops due to potassium deficiency."**Die back".** In acute deficiency cases, there is a loss of apical dominance and regeneration of lateral buds and bushy of growth. In prolonged cases, die back of lateral buds are also resulted.

Zinc

Function

- It participates in the metabolism of plants as an activator of several enzymes.
- It involved in the biosynthesis of the plant auxin (IAA).
- It plays an important role in protein synthesis.

Deficiency symptoms of zinc

1-An interveinal chlorsis of the older leaves starting in the tips and margins.

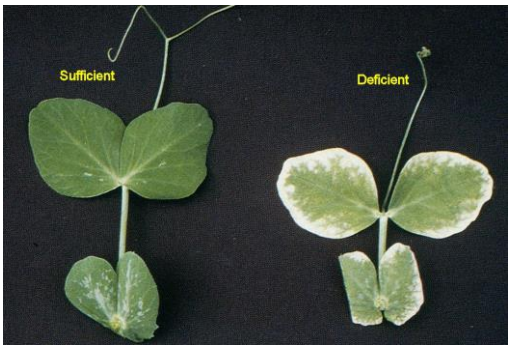
2- White necrotic spots.

3- Seed production and fruits size is greatly reduced.

4- Leaves are often narrower or have wavy margins.

5- Smaller leaves and shortened internodes resulting in stunted growth.

6- The common disease is **little leaf** disease. Yellow mottling of leaves, reduction of leaf size with rosette appearance (due to reduced internodal distance) and die back of the affected branches are symptoms of the disease.



Sulfur

Function

- Sulfur is the constituent of amino acids cysteine, cysteine and methionine
- It also participates in the constituent of vitamin B, co-enzyme A and volatile oils.
- It may be found in sulfhydryl groups, which are present in many enzymes.

Deficiency symptoms of sulfur

1- Sulfur deficiency causes yellowing (Chlorosis) of leaves. Young leaves are affected first.
2- Tips and margins of leaves roll inward.
3- Accumulation of starch, sucrose and soluble nitrogen.

4- Young leaves develop orange, red or purple pigments.

Manganese

Function

- It acts as an activator for enzymes in respiration and nitrogen metabolism.
- It plays an important role in nitrate reduction.
- Manganese thought to be involved in the destruction and oxidation of indol-3-acetic acid (IAA).

Deficiency symptoms of manganese.

1-The leaves start to turn yellow and undergo interveinal chlorosis.

2- The younger leaves near the top of the plant show symptoms first.

3- The chloroplasts of tomato leaves lose chlorophyll and starch grains becoming yellow green in colour.

Copper

Function

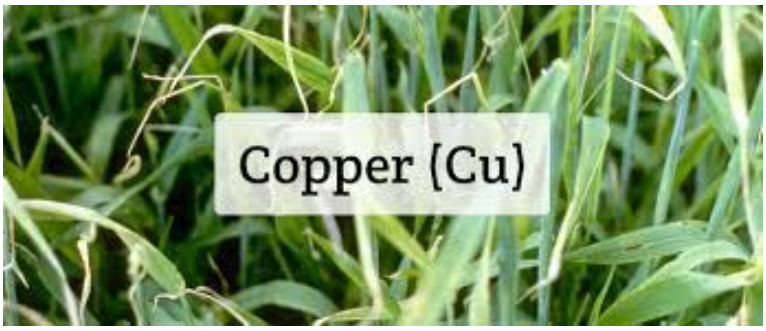
- Copper acts as a component of some enzymes.
- It has a role in photosynthesis process as a constituent of chloroplast.

Deficiency symptoms of Copper.

1-It causes a necrosis of the tip of young leaves that proceeds along the margin of the leaf, giving it a withered appearance.

2- Under sever condition the leaves may be lost, and the whole plant may appear wilted.

3- *Reclamation disease*: It is also called as White Tip disease and is found in legumes, cereals, oats and beet. The tips of the leaves become chlorotic followed by a failure of the plants to set seed.



Iron

Function

- Iron has a number of important functions in the overall metabolism of the plant.
- It appears to be essential for the synthesis of chlorophyll
- It has a major role in the biosynthesis of cytochromes.
- It acts as an activator of some enzymes.

Deficiency symptoms of iron

1- Extensive chlorosis of leaves specially the younger one.

2-The lack of iron may inhibit formation of chloroplasts through inhibition in protein synthesis.

Boron

Function

- Boron has an important role in the transport of carbohydrate within the plant.
- It participates in cellular differentiation, in nitrogen metabolism, fertilization, active salt absorption, hormone metabolism, phosphorous metabolism and photosynthesis.

Deficiency symptoms of boron

1-death of stem and root tips.

2- Abscission of flowers.

3-the leaves may have a coppery texture.

4-leaves sometimes curling and becoming quit brittle.

5- Root growth is stunted.

Molybdenum

Function

- It has an important role in nitrogen fixation and nitrate and phosphorous assimilation.
- It maintain the concentration of ascorbic acid in the plant.

Deficiency symptoms of Molybdenum

1-Chlorotic interveinal mottling of the lower leaves followed by marginal necrosis and infolding of the leaves.

2- Under sever conditions; mottled areas may become necrotic, causing the leaf to wilt.

3-Flower formation is inhibited and if flowers do form, they abscise before setting fruit.

4- A common disease of molybdenum deficiency in cauliflower plants is **whiptail** in which the leaves show an interveinal mottling and leaf margins may become grey and finally brown. The leaf tissues wither, leaving only the midrib and a few small pieces of leaf blade, giving the appearance of a whip or tail.

Chapter 5

Mineral Salt absorption And Translocation

It was thought that osmotically active substances diffused along concentration gradients from soil solution into the plant. The osmotic concentration inside the cell was continuously kept a low point through

utilization of the absorbed substances in metabolism. The osmotic theory sufficiently explained the absorption, but did not account for the rapid translocation of the salts once they were absorbed. Again the transpiration stream was implicated, this time as only aiding in the dispersal of the salts, not their absorption. Thus, early attempts to explain salt absorption and mineral nutrition

Translocation only emphasized physical mechanisms, neglecting almost entirely the participation of metabolic energy.

However, during this time, a statement was made by the brilliant physiologist, **Pfeffer**, which contrasted sharply with prevalent theories on salt absorption and remarkably foreshadowed a popular theory today (**Pfeffer, 1900**). **Pfeffer** claimed:

The nature of the plasma is such as to render it possible that a substance may combine chemically with the plasmatic elements, thus being transmitted internally, and then set free again.

This statement agrees very nicely with the carrier theory in salt absorption generally accepted today.

As is usually the case when one tries to buck the tide of popular thought, this provocative theory in absorption was not taken too seriously, and physical mechanisms and models were continuously produced to explain salt absorption. It was finally recognised, from work done in the 1930, that salt absorption is largely dependent upon metabolic energy, that the uptake of salt is predominately active, not passive as was earlier thought.

Passive absorption

Outer and apparent free space

Salt absorption takes place through the intimate contact of the root system with the soil colloids or soil solution. What are the mechanisms involved in the passage of dissolved inorganic salts from the soil solution into the plant? passive or non-metabolic absorption of ions has been demonstrated by numerous investigators (see review by **Briggs and Robertson , 1957**) it has been found frequently that when a plant cell or tissue is transferred from a medium of low salt concentration to a medium of relatively high

salt concentration there is an initial rapid uptake of ions this is followed by a slow steady uptake that is under metabolic control . The rapid initial uptake is not affected by temperature or metabolic inhibitors; that is, metabolic energy is not involved. If the above tissue is returned to the low salt medium, some of the ions taken up will diffuse out into the external medium. In other words, a part of cell or tissue immersed in the salt solution is opened to free diffusion of ions. since free diffusion implies that ions can move freely in or out of the tissue , the part of the tissue opened to free diffusion will reach an equilibrium with the external medium and the ion concentration of this part will be the same that found in the external medium .

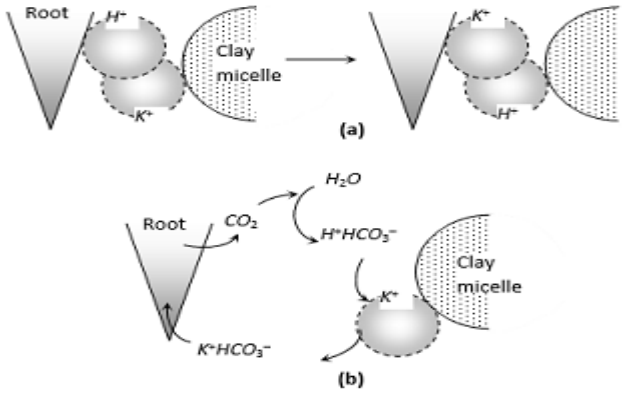
That part of a plant cell or tissue which will allow for free diffusion to take place is referred to as outer space.

With the establishment with the concept of ‘outer space ‘, workers turned to the task of calculating the volume of plant cell or tissue involved. This may be accomplished by immersing a tissue in a solution of known concentration, allowing it to come to equilibrium, and then determining the amount of salt taken up. Assuming that the ion concentration is the same both in outer space and in the external medium and knowing the amount of salt taken up, we can calculate the volume of outer space.

Under the above circumstances, active absorption must be inhibited (e.g., by metabolic inhibitors or by low temperature) or the calculated volume will be greater than the actual volume.

Ion exchange

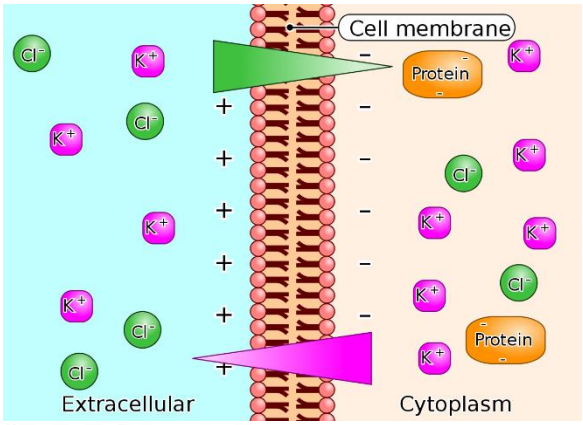
Ions adsorbed to the surfaces of the cell walls or membranes of a tissue may exchange with ions from the external solution in which the tissue is immersed. We have already encountered analogous ion exchange mechanisms between the soil solution and the soil colloids in a previous chapter. Suppose, for example, the cation, K^+, of external solution exchanged with a hydrogen ion, H^+, adsorbed to the surface of the cell membrane. The action would then become adsorbed to the surface of the membrane and rendered osmotically inactive. Anions could exchange with free hydroxyl ion in the same manner. Thus, ion exchange mechanism would allow for a greater absorption of ions from the external medium than could normally be accounted for by free diffusion.



Diagrammatic representation of (a) The contact-exchange theory and (b) The carbonic acid exchange theory

Donnan equilibrium

The Donnan equilibrium theory takes into account the effect of fixed or in diffusible ions. Take, for example, a membrane that is permeable to some ions and not to others and which separates the cell from the external medium. Suppose in the inner side of this membrane there is a concentration of anions to which the membrane is impermeable (fixed anion). Now, if the above membrane is freely permeable to the cations and anions in the external solution equal numbers of cations and anions from the external solution will diffuse across from the membrane until an equilibrium is established. Normally, this equilibrium would also be electrically balanced. However, additional cations are needed to balance the negative charges of (fixed) anions. Therefore, the cation concentration would be greater in the internal solution than in external solution. also , it must be remembered , because , of the excess of negative charges due to fixed anions , the concentration of anions in the internal solution will be less than that of the external solution . As shown in figure 5.3.



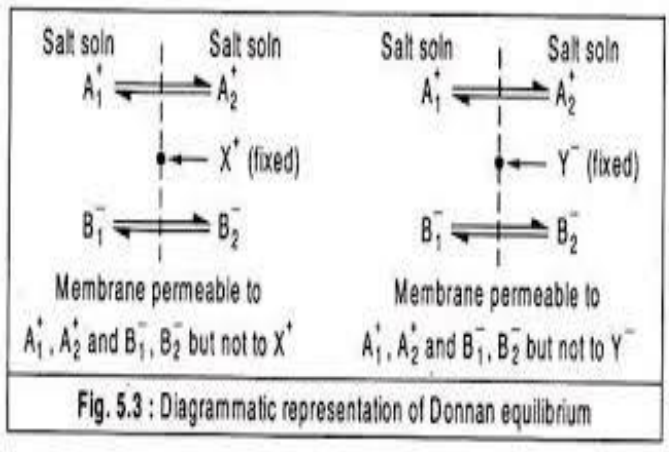


Fig. 5.3 : Diagrammatic representation of Donnan equilibrium

Donnan equilibrium ion diffiusion across membranes. (a) Membrane is impermeable to the cation, X^+ , causing additional anions , B^- , to diffuse across from the outside (accumulation of anions) . (b) membrane is the impermeable to the anion , Y^- , causing additional cations , A^+ , to diffuse across from the outside (accumulation of cations) .

Mass flow

Some of investigators believe that ions can move through roots along with the mass flow of water (**Hylmo, 1953; 1955; Kylin and Hylmo 1957**). According to this theory, an increase in transpiration should cause an increase in absorption of ions. That this is so has been generally accepted (**Russell, and barber, 1960**) but weather the effect of transpiration is direct or indirect is not clear. some authors claim that transpiration in directly effects ion absorption by removing ions after they have been released into the xylem ducts , causing by this dilution an increase in ion absorption activity (**Brauwer , 1956 ; Broyer et al . , 1943 ; Honert , 1955**) .

From this discussion, we have learned that at least part of the total salt taken up by a plant may result from passive absorption. This may be accomplished through free diffusion of ions into the apparent free space of

a tissue. Accumulation of ions against a concentration gradient is possible under the above circumstances due to Donnan equilibrium. Accumulation may also take place against an apparent concentration gradient due to ion exchange mechanisms. Finally, the mass flow of ions through root tissue may be possible with the aid of transpirational 'pull'. All of this mechanisms occur in the absence of metabolic energy. Let us now turn to an analysis of active transport.

Active transport

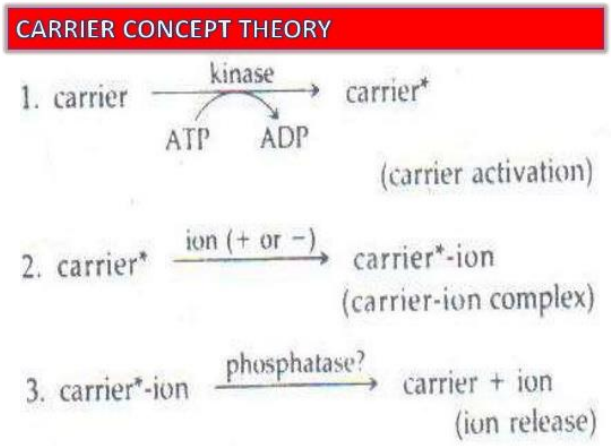
Direct analyses of the vacuolar sap of plants immersed in solution of known salt concentration have demonstrated an equivocally that both anions and cations are accumulated by plants against concentration gradients. Furthermore, the extent of accumulation is such as known physical mechanisms, such as ion exchange and donnan equilibrium, cannot account for the extent of accumulation that occurs, analyses of the ion accumulation in the sap of *Nitella clavata* and *Valonia macrophysa* by **Hoagland (1944**) give an excellent picture both of the accumulation and selective properties of salt absorption mechanisms in plants.

Since ion accumulation is inhibited when the metabolic activity of the plant is inhibited by low temperature, low oxygen tension, metabolic inhibitors, etc., we can only assume that ion accumulation as it occurs in plants requires metabolic energy. The transport of ions with the aid of metabolic energy has been termed active transport. Various mechanisms have been devised to explain active transport, none of which have been universally accepted. All of this suggested mechanisms, however, generally accept the concept that the active transport of an ion across an impermeable membrane is accomplished through the mediation of a carrier compound present in the membrane.

The carrier concept

The space in a tissue or cell to which ions penetrate, through the mediation of metabolic energy, is termed inner space. Where outer space ends and inner space begins has not been clearly established. however , it is thought that this dividing line begins somewhere in the middle of the cytoplasm , since apparent free space volume measures have implied that part of cytoplasm allows for free diffusion of ions . The area between outer and inner space is impermeable to free ions. Passage across this area is thought to require the intercession of specific carriers, which combine with ions in

outer space and release them in inner space. This impermeable barrier is usually spoken of as a membrane and the carriers as existing within it.



The most important feature of the carrier theory is the assumption of an intermediate carrier-ion complex, which is capable of moving across the above mentioned impermeable membrane. The direction of movement of the complex is from outer to inner space only. Ions released into inner space cannot move out and thus are accumulated. A model giving a simplified description of the carrier concept is shown in figure 22.4.

The carrier concept has received impressive support by numerous investigators since its formulation by **Van den honert in 1937**. We will discuss three characteristics of salt absorption and active transport that appear to suggest strongly the validity of the carrier concept.

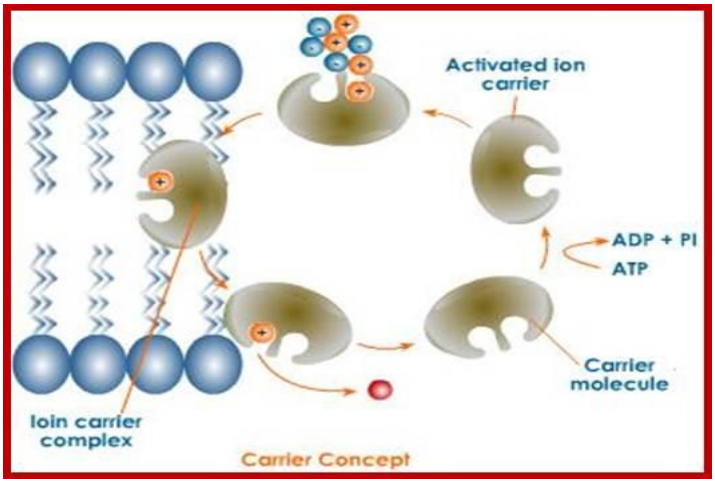


Fig. 22.4

<u>Saturation effects</u>

The fact that a level maximum rate of absorption may be maintained over a relatively long period of time suggests the participation of a finite number of carriers working, so to speak at maximum efficiency. That is, the active sites on the carriers in the above situation are occupied all of the time. As soon as a carrier released an ion to inner space, it is immediately occupied by an ion from the outer space areas in the tissue. Thus, at the saturation point of cycle is kept in continuous motion and cannot be made to proceed faster by increasing the salt concentration.

Specificity.

The carrier concept offers a reasonable explanation of the fact that roots selectively absorb ions. That is, ions are absorbed at different rates and have different levels in accumulation in the root tissue, suggesting the presence of specific carriers. This specificity is rather rigid with ions of dissimilar chemical behaviour, but weak or non-existent with ions of similar behaviour.

Cytochrome pump

Early workers observed that although salt accumulation is depended upon metabolic energy, there appeared to be no quantitative relationship between salty absorption and respiration. However, **lundeggardh and burstrom (1933)** claimed that such a relation exists between anion absorption and what they called ‘anion’ or ‘salt’ respiration. They observed that the rate of respiration increases when a plant is transferred from water to salt solution. The amount by which respiration is increased over normal or ground respiration by the transfer of a plant or tissue from water to a salt solution is known as salt respiration. The original observations of **lundegardh and Burstro** have since been expanded and developed to a workable theory in active salt absorption by **lundegardh (1950, 1954)**.

Lundegardh theory assumes the following:

1. Anion absorption is independent of cation absorption and occurs by a different mechanism.

2. An oxygen concentration gradient exists from the outer surface to the inner surface of a membrane, thus favouring oxidation at the outer surface and reduction at the inner surface

3. The actual transport of the anion occurs through a cytochrome system.

Since there is a quantitative correlation between anion absorption and salt respiration and since this correlation does not exist with cation absorption it was assumed that only anions are actively transported. The inhibition of salt respiration and consequent inhibition of anion absorption by cyanide or carbon monoxide led lundegardh to propose that transport of anions is mediated through cytochrome oxidase and that cytochromes may be anion carriers. A diagrammatic representation of lundegardh cytochrome theory is shown in figure 7.3. According to lundegardh theory, dehydrogenase reactions on the inner surface produce protons (H^+) and electrons (e^-). The electrons produced move outward via a cytochrome chain, while anions move inward. At the outer surface of the membrane the reduced iron of the cytochrome in oxidized, losing an electron and picking up an anion. The released electron unites with a proton and oxygen to form water.

At the inner surface the oxidized iron of the cytochrome becomes reduced by the addition of an electron released in a dehydrogenase reaction. The anion is released on the inside in this last reaction. Cations are absorbed passively to balance the potential difference caused by the accumulation of anions on the inner surface.

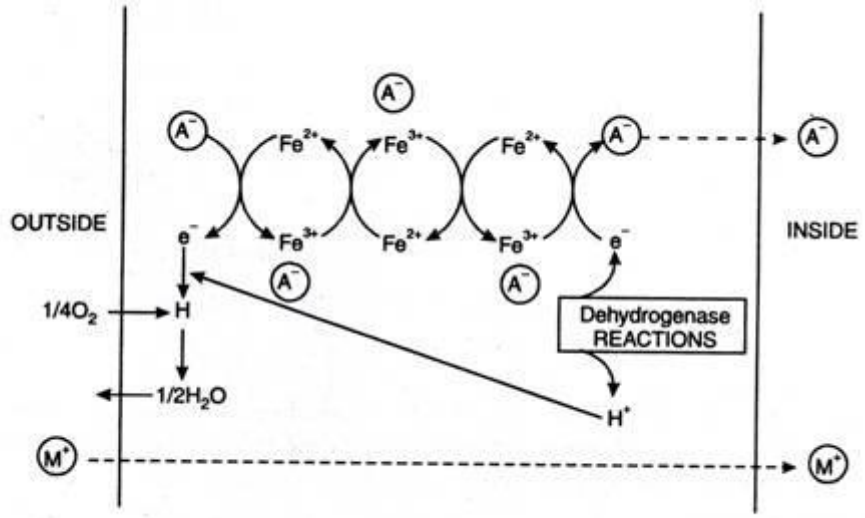


Fig. 7.3 Diagrammatic representation of the Lundegardh's cytochrome pump theory.

Although the cytochrome transport theory des give a clear picture of how metabolic energy might participate in ion absorption, it has not been universally accepted and has been criticized by a number of investigators. For example, **Robertson et al. (1951)** found that 2, 4- dinitrophenol (DNP), an inhibitor of oxidative

Phosphorylation, increases respiration but decreases salt absorption. This implies that phosphorylation should be included in any theory of ion

accumulation. The original proposal that only anions are capable of stimulating respiration has come under considerable attack. For example, **Handley and overstreet (1955)** found that both potassium and sodium ions simulated respiration. Finally, if there is only one carrier for all anions, then competition for binding sites among anions should be apparent.

On the contrary, as pointed out in an earlier discussion, the anion sulfate, nitrate, and phosphate do not compete with one another.

Carrier mechanism involving ATP

A mechanism for active salt absorption that utilizes ATP has been proposed by **Bennet – clarck (1956).** This investigator has suggested that phospholipids may be important in the transport of ions across membranes otherwise impermeable. In this transport **lecithin**, a phospholipid, is synthesized and hydrolysed in a cyclic manner, picking up ions on the outer surface and releasing them on hydrolysis into inner space. The synthesis of at least one of the components of this phosphatide cycle requires ATP. A diagram showing the 'phosphatide cycle' and how it might proceed in ion transport is given in figure 7.4.

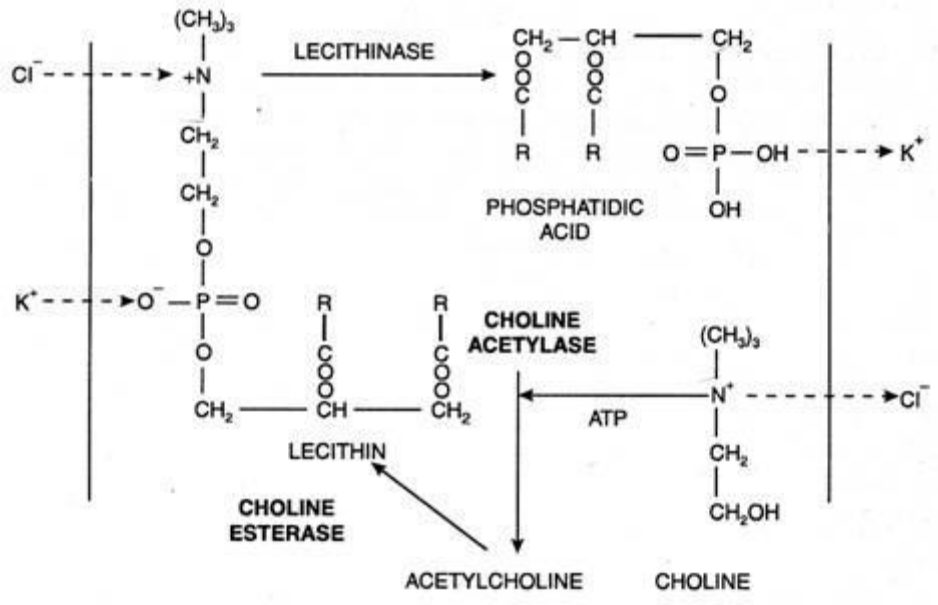


Fig. 7.4. Diagrammatic representation of the Bennet-Clark's Protein-Lecithin theory.

Factors affecting salt absorption

The physical and biochemical activities of living organisms are subject to the influences of their external and internal environments. Salt absorption is not an exception, being speeded up, slowed down, or kept in dynamic equilibrium by a complex of ever changing factors. The scientist has

learned to study the influence of individual factors by controlling the environment and studying the effect of the one factor in question. This has been done with the process of salt absorption, and we now have an extensive, if incomplete, picture of how this process might proceed in nature's ever changing environment. We will discuss the effects of temperature, pH, light, oxygen tension, interaction, and growth on salt absorption.

Temperature

In general, an increase in temperature results in an acceleration of salt absorption.

However, the influence of temperature in salt absorption is confined to a relatively narrow range. In addition to accelerating salt absorption, increase in temperature past a maximum point will inhibit and eventually terminate the process. Most likely, the inhibitory effects of high temperature are because of the denaturation of enzymes involved either directly in salt absorption or in the synthesis of some necessary component of salt absorption.

Boss passive and active absorption process are affected by temperature changes. The rate of free diffusion, for example, depends upon the kinetic energy of the diffusing molecules or ion which is, in turn, dependent on temperature. Therefore, lowering of temperature will slow down any process dependent upon free diffusion. Low temperature will, of course, slow down the biochemical reactions found in active transport.

Hydrogen ion concentration

The availability of ions in the soil solution, discussed in a previous chapter, is profoundly affected by the hydrogen ion concentration. Ionization of electrolytes or the

Valence numbers of different ion species are influenced by changes in pH. For example, the monovalent phosphate ion, H_2PO^{4-} , is the form of phosphorus most readily taken up by plants. However, as a medium approaches a more alkaline pH, production of first the bivalent phosphate (HPO_4^{2-}) and then the trivalent phosphate (PO_4^{3-}) is favoured. The bivalent ion is only sparingly available to the plant, while the trivalent ion is not available at all. Since the monovalent ion is absorbed more readily than the bivalent ion, absorption of phosphate is accelerated at an acid pH. **Robertson (1958)** has pointed out that since boron is taken up as the

undissociated acid, H_3BO_3, or as the $H2BO^{3-}$ ion, it too must be absorbed more readily at a lower pH. In contrast to the above observations, with anions increase in pH will favour the absorption of cations.

There have been numerous experiments showing little pH effect, as judged by growth (**Robertson, 1958**). Marked pH effects most likely occur when ion availability is inhibited. However, if the concentration of ion is high enough, it will be difficult to show a deficiency for that ion in the plant over a physiological range of pH values. Of course, at pH values outside the physiological range, damage to plant tissues and carriers will inhibit salt absorption.

Light

The effects of the light in the opening and closing of stomates and on photosynthesis indirectly affect salt uptake. Opened stomates increase the mass flow of water in the transpiration stream and thus may indirectly influence salt absorption. The energy derived from the photosynthetic process provides energy for salt uptake and the oxygen given off improves conditions for the active absorption of ions

Oxygen tension

The active phase of salt absorption is inhibited by the absence of oxygen. Indeed, it was this observation that most strongly supported early theories active transport.

Chapter 6

PLANT GROWTH REGULATORS

Plant growth regulators or phytohormones are organic substances produced naturally in higher plants, controlling growth or other physiological functions at a site remote from its place of production and active in minute amounts. **Thimmann (1948)** proposed the term *Phyto hormone* as these hormones are synthesized in plants. *Plant growth regulators* include auxins, gibberellins, cytokinins, abscisic acid and ethylene.

Plant growth regulators

An endogenous compound, which is synthesized at one site and transported to another site where it exerts a physiological effect in very low concentration. But ethylene (gaseous nature), exert a physiological effect only at a near a site where it is synthesized.

• Defined as organic compounds other than nutrients, that affects the physiological processes of growth and development in plants when applied in low concentrations.

• Defined as either natural or synthetic compounds that are applied directly to a target plant to alter its life processes or its structure to improve quality, increase yields, or facilitate harvesting.

Five major classes of plant hormones are known in plants. With progressing research, more active molecules are being found and new families of regulators are emerging.

(1) Auxin

(2) Gibberellin

(3) Cytokinin

(4) Abscisic acid

(5) Ethylene

Auxins

The term auxin is derived from the Greek word ‘auxein’ which means to grow. They are a class of plant hormones which has a cardinal role in coordination of many growth and behavioral processes in the plant's life cycle essential for development of plant. Auxin is the first plant hormone to be identified. They have the ability to induce cell elongation in stems and resemble indole acetic acid (the first auxin to be isolated) in physiological activity.

Discovery of auxin: **Darwin (1881)** was the first person who discovered the existence of auxin in plants, the first phytohormone known. He noted that the first leaf (coleoptile) of canary grass (Phalaris canariensis) was very sensitive and responsive to light and he demonstrated the bending of the grass coleoptiles towards unilateral source of light. This bending occurred only when the coleoptile was also illuminated. When the tip of the coleoptiles was covered with a black cap, it resulted in loss of sensitivity of the plant towards the light as shown in Figure 2. Darwin concluded that some influence that causes curvature is transmitted from the coleoptile tip to the rest of the shoot. Boysen – Jensen (1913) also made similar observations on oat (Avena) coleoptiles as shown in Figure 2. Paal (1918) demonstrated that when the decapitated coleoptiles tip was replaced on the cut end eccentrically, more growth resulted on the side which causes bending even when this is done in complete darkness.

Sites of biosynthesis of auxin: IAA is synthesized primarily in actively growing tissue in leaf primordia and young leaves, fruits, shoot apex and in developing seeds. It is made in the cytosol of cells.

Classification of auxins: Auxins are classified into two types based on its occurrence, if they occur naturally or are synthesized artificially.

1. Natural auxins

2. Synthetic auxins

Natural auxins: The four naturally occurring (endogenous) auxins are Indole-3-acetic acid, 4-chloroindole-3-acetic acid, phenylacetic acid and indole-3-butyric acid; only these four are synthesized by plants.

Synthetic auxins: Synthetic auxin analogs include 1-naphthaleneacetic acid, 2,4dichlorophenoxyacetic acid (2,4-D) and many others. Some synthetic auxins, such as 2,4D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), are used also as herbicides. Broad-leaf plants (dicots), such as dandelions, are much more susceptible to auxins than narrow-leaf plants (monocots) such as grasses and cereal crops, so these synthetic auxins are useful as synthetic herbicides.

Distribution of auxin in plants

In plants, auxin (IAA) is synthesized in growing tips or meristematic regions from where; it is transported to other plant parts. Hence, the highest concentration of IAA is found in growing shoot tips, young leaves and developing auxiliary shoots.

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

Some physiological effects of auxin

1-Cell division and elongation

2- Apical dominance

3- Root initiation

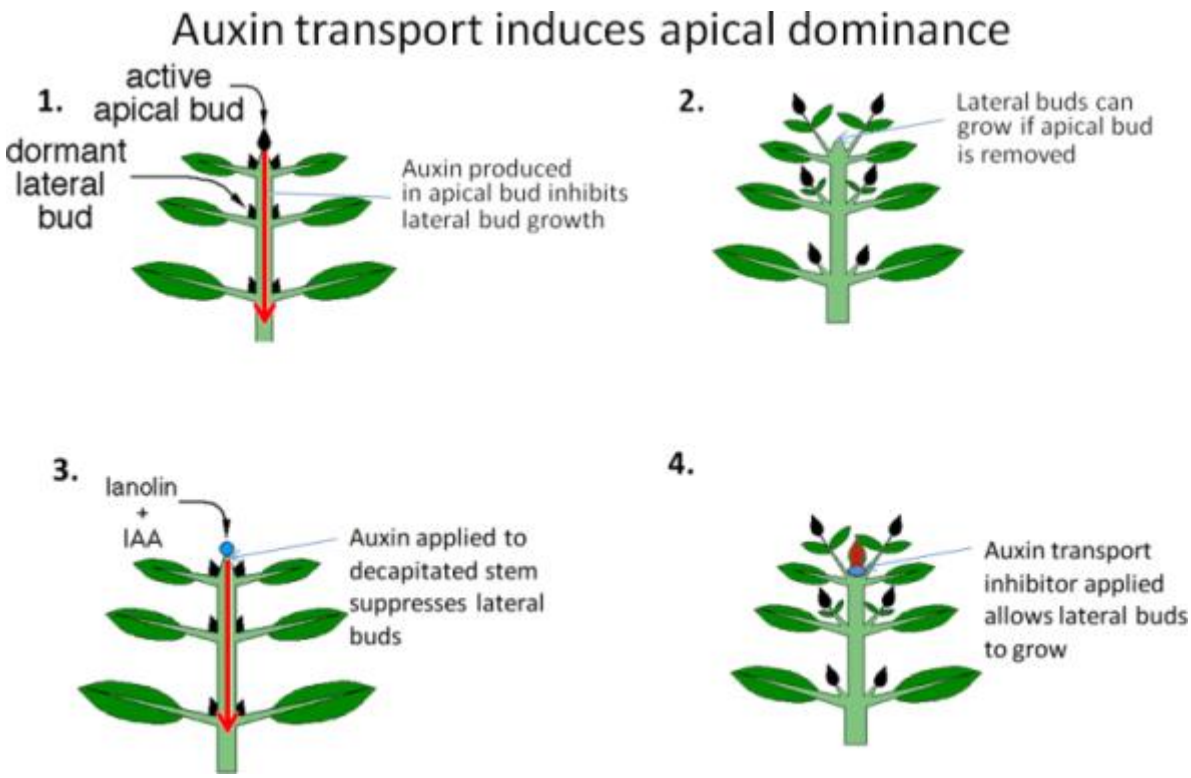
1. Cell division and elongation

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

2. Apical dominance

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

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

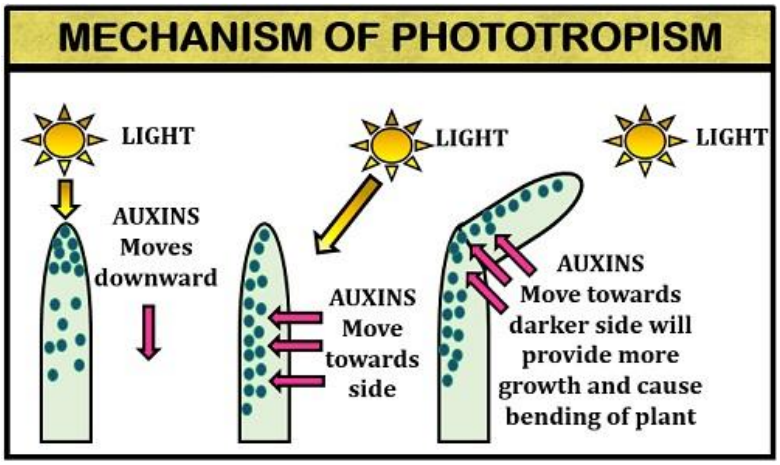


3. Root initiation

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

4. Phototropism

Photo means "**Light**" and tropism means "**Turning**". Therefore phototropism merely refers as the bending of plants towards the light for its growth by absorbing solar energy.

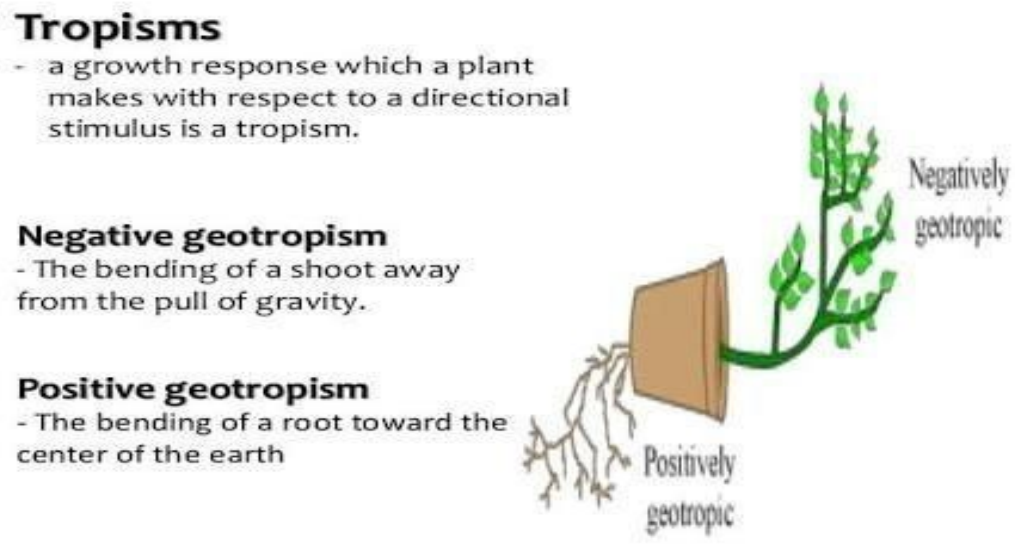


When a growing plant is illuminated by unilateral light, it responds by bending toward the light. The bending of the plant is caused by cells elongating on the shaded side at a much greater rate than cells on the illuminated side. This differential of growth response of the plant to light, called phototropism, is caused by an unequal distribution of auxin, the higher concentration of the growth hormone being on the shaded side. Many attempts have been made to explain why there is a higher concentration of auxin on the shaded side of unilaterally illuminated coleoptile. There is a large evidence supporting the theory that unilateral illumination is capable of inducing lateral transport of auxin.

5. Geotropism

If an intact seedling is placed in a horizontal position it will respond to the earth's gravitational field with a particular pattern of growth. Growth of the stem under these circumstances will cause it to curve upward until it is vertical again and the root system will curve downward until it too is vertical again. Like phototropism, the geotropic response is controlled by an unequal distribution of auxin but, unlike phototropism, gravitational pull instead of light is the influencing factor in geotropic auxin distribution. The **colony-went** theory provides an explanation for geotropism as well as phototropism. They proposed that the differential growth exhibited by a

horizontally placed organ is due to the accumulation of auxin on the lower side. They suggested that auxin is laterally transported from the upper to the lower side under the influence of gravity. The accumulation of auxin on the lower side of a horizontally placed stem causes an accelerated growth to occur on the lower side causing the stem to curve upward. The horizontally placed root, however, will exhibit a positive geotropic response even though auxin concentrates on the lower side. Roots are much more sensitive to IAA than stems and the concentrations of IAA which stimulate cell elongation in stems are actually inhibitory to cell elongation in roots. The accumulation of auxin on the lower side of a horizontally placed root would, therefore, retard cell elongation on that side.



Gibberellin

Gibberellin was first discovered in Japan by **Kurusowa**. He observed from his field that some of the rice seedlings had grown much taller than the others. On further observation, he found that such taller rice plants had shown unusual internodal elongation. This internodal elongation is known as the 'bakanae' or 'foolish seedling' disease of rice. Later, it was discovered that the elongation was due to the action of a substance produced by a fungus, Gibberella fujikuroi. This substance was successfully isolated from the fungus and it was named as gibberellic acid.

There are over 90 different gibberellins isolated from fungi and from higher plants. Gibberellins occur in various plant organs.

They are named as GA1, GA2, GA3, etc. These phytohormones occur in all groups of plants.

Physiological effects of gibberellin

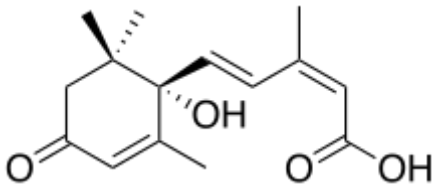
1. Gibberellins produce extraordinary elongation of stem. The elongation of stem is caused by the cell division and cell elongation induced by gibberellic acid.
2. One of the most striking effects of the gibberellins is the reversal of dwarfism in many genetically dwarf plants. For e.g. 'Rosette' plant of sugar beet, when treated with GA undergoes marked longitudinal growth of axis attaining the normal size.
3. Rosette plants usually show reduced internodal growth. These plants exhibit excessive internodal growth when they are treated with gibberellin. This sudden elongation of stem followed by flowering is called bolting.
4. Many biennials usually flower during the second year of their growth. For flowering to take place, these plants should be exposed to cold season. Such plants could be made to flower without exposure to cold season in the first year itself, when they are treated with gibberellins.

5. Formation of seedless fruits without fertilization can also be induced by gibberellin treatment in many plants. e.g. Tomatoes, apples, cucumbers, etc.,
6. Some of the light sensitive seeds can germinate by the treatment of gibberellic acid even in complete darkness. e.g. barley, gibberellin breaks dormancy in potato tubers.

Cytokinins

The effect of cytokinins was first reported when it was found that adding the liquid endosperm of coconuts to developing plant embryos in culture stimulated their growth. The stimulating growth factor was found to be cytokinin, a hormone that promotes cytokinesis (cell division). Almost 200 naturally-occurring or synthetic cytokinins are known, to date. Cytokinins are most abundant in growing tissues, such as roots, embryos, and fruits, where cell division is occurring. Cytokinins are known to delay senescence in leaf tissues, promote mitosis, and stimulate differentiation of the meristem in shoots and roots. Many effects on plant development are under the influence of cytokinins, either in conjunction with auxin or another hormone. For example, apical dominance seems to result from a balance between auxins that inhibit lateral buds and cytokinins that promote bushier growth.

Abscisic acid



Abscisic acid

Abscisic acid (also called ABA) is one of the most important plant growth inhibitor. It was discovered and researched under two different names before its chemical properties were fully known, it was called *dormin* and *abscicin II*. Once it was determined that the two compounds are the same, it was named abscisic acid. The name "abscisic acid" was given because it

was found in high concentrations in newly abscissed or freshly fallen leaves.

This class of PGR is composed of one chemical compound normally produced in the leaves of plants, originating from chlorplasts, especially when plants are under stress. In general, it acts as an inhibitory chemical compound that affects bud growth, and seed and bud dormancy. It mediates changes within the apical meristem, causing bud dormancy and the alteration of the last set of leaves into protective bud covers. Since it was found in freshly abscissed leaves, it was thought to play a role in the processes of natural leaf drop, but further research has disproven this. In plant species from temperate parts of the world, it plays a role in leaf and seed dormancy by inhibiting growth, but, as it is dissipated from seeds or buds, growth begins. In other plants, as ABA levels decrease, growth then commences as gibberellin levels increase. Without ABA, buds and seeds would start to grow during warm periods in winter and be killed when it froze again. Since ABA dissipates slowly from the tissues and its effects take time to be offset by other plant hormones, there is a delay in physiological pathways that provide some protection from premature growth. It accumulates within seeds during fruit maturation, preventing seed germination within the fruit, or seed germination before winter. Abscisic acid's effects are degraded within plant tissues during cold temperatures or by its removal by water washing in out of the tissues, releasing the seeds and buds from dormancy.

In plants under water stress, ABA plays a role in closing the stomata. Soon after plants are water-stressed and the roots are deficient in water, a signal moves up to the leaves, causing the formation of ABA precursors there, which then move to the roots. The roots then release ABA, which is translocated to the foliage through the vascular system and modulates the potassium and sodium uptake within the guard cell, which then lose turgidity, closing the stomata. ABA exists in all parts of the plant and its concentration within any tissue seems to mediate its effects and function as a hormone; its degradation, or more properly catabolism, within the plant affects metabolic reactions and cellular growth and production of other hormones. Plants start life as a seed with high ABA levels. Just before the seed germinates, ABA levels decrease; during germination and early

growth of the seedling, ABA levels decrease even more. As plants begin to produce shoots with fully functional leaves, ABA levels begin to increase, slowing down cellular growth in more "mature" areas of the plant. Stress from water or predation affects ABA production and catabolism rates, mediating another cascade of effects that trigger specific responses from targeted cells. Scientists are still piecing together the complex interactions and effects of this and other phytohormones.

Ethylene

Ethylene is a gas that forms through the breakdown of methionine, which is in all cells. Ethylene has very limited solubility in water and does not accumulate within the cell but diffuses out of the cell and escapes out of the plant. Its effectiveness as a plant hormone is dependent on its rate of production versus its rate of escaping into the atmosphere. Ethylene is produced at a faster rate in rapidly growing and dividing cells, especially in darkness. New growth and newly germinated seedlings produce more ethylene than can escape the plant, which leads to elevated amounts of ethylene, inhibiting leaf expansion. As the new shoot is exposed to light, reactions by phytochrome in the plant's cells produce a signal for ethylene production to decrease, allowing leaf expansion. Ethylene affects cell growth and cell shape; when a growing shoot hits an obstacle while underground, ethylene production greatly increases, preventing cell elongation and causing the stem to swell. The resulting thicker stem can exert more pressure against the object impeding its path to the surface. If the shoot does not reach the surface and the ethylene stimulus becomes prolonged, it affects the stem's natural geotropic response, which is to grow upright, allowing it to grow around an object. Studies seem to indicate that ethylene affects stem diameter and height: When stems of trees are subjected to wind, causing lateral stress, greater ethylene production occurs, resulting in thicker, more sturdy tree trunks and branches. Ethylene affects fruit-ripening: Normally, when the seeds are mature, ethylene production increases and builds-up within the fruit, resulting in a climacteric event just before seed dispersal. The nuclear protein Ethylene

Insensitive2 (EIN2) is regulated by ethylene production, and, in turn, regulates other hormones including ABA and stress hormones.

Seed dormancy

Plant hormones affect seed germination and dormancy by acting on different parts of the seed.

Embryo dormancy is characterized by a high ABA: GA ratio, whereas the seed has a high ABA sensitivity and low GA sensitivity. In order to release the seed from this type of dormancy and initiate seed germination, an alteration in hormone biosynthesis and degradation toward a low ABA/GA ratio, along with a decrease in ABA sensitivity and an increase in GA sensitivity, must occur.

ABA controls embryo dormancy, and GA embryo germination. Seed coat dormancy involves the mechanical restriction of the seed coat. This, along with a low embryo growth potential, effectively produces seed dormancy. GA releases this dormancy by increasing the embryo growth potential, and/or weakening the seed coat so the radical of the seedling can break through the seed coat. Different types of seed coats can be made up of living or dead cells, and both types can be influenced by hormones; those composed of living cells are acted upon after seed formation, whereas the seed coats composed of dead cells can be influenced by hormones during the formation of the seed coat. ABA affects seed coat growth characteristics, including thickness, and effects the GA-mediated embryo growth potential. These conditions and effects occur during the formation of the seed, often in response to environmental conditions. Hormones also mediate endosperm dormancy: Endosperm in most seeds is composed of living tissue that can actively respond to hormones generated by the embryo. The endosperm often acts as a barrier to seed germination, playing a part in seed coat dormancy or in the germination process. Living cells respond to and also affect the ABA:GA ratio, and mediate cellular sensitivity; GA thus increases the embryo growth potential and can promote endosperm weakening. GA also affects both ABA-independent and ABA-inhibiting processes within the endosperm.

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Practical physiology

Determination the osmotic suction force by curvature the Ricinus petioles

Tools:

Ricinus petioles- knife or scalpel, NaCl solution (concentrated), distilled water, petri-dishes, different concentrations.

Procedures:

1- Cut the Ricinus petioles with knife
2- Put some of these petioles in 3 petri dishes
3- One of the petri dish contain distilled water , the second contain concentrated solution of NaCl
4- Put different concentrations of NaCl in petri dishes
5- Observe the curvature of Ricinus petioles in each petri dish.

Observation

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Determination the osmotic suction force by weighing method using potato tubers

Tools:

Potato tuber, knife or scalpel, NaCl solution, distilled water, petri-dishes.

Procedures:

1-Cut potatoes into six groups of small, uniform cubes measuring 1/2 cm by 1/2 cm.

2-Make four different solutions of NaCl: 0.0N,0.2 N, 0.4N ,0.6 N,0.8N and 1N

3-Weigh each group, on a mass balance, before immersing it in the appropriate NaCl solution for half an hour.

4-After immersion, weigh each group again and calculate the changes in the potato masses.

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Recognition some organic compounds in the plant cell

1- Carbohydrates

a- Detecting monosaccharide

Tools:

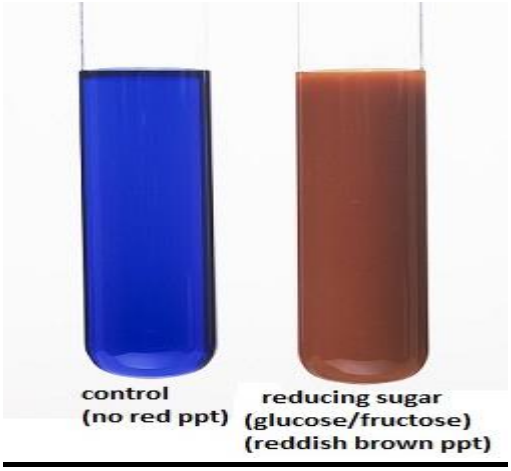
Glucose soln.- Fehling reagent – test tubes – water bath 100℃

Procedures:

1- Take 5ml of Glucose soln.in clean test tube.

2-Add 5ml of Fehling reagent (blue color)

3-Put the tube in water bath at 100℃ for 15 min.



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b- <u>Detecting disaccharide</u>

Tools:

Sucrose soln.- Fehling reagent – test tubes – water bath 100℃ , 40℃- invertase

Procedures:

1- Take 5ml of sucrose soln.in two clean test tubes.

2-Add 2ml of invertase in one tube and let the other tube without addition

3-Put the tubes in water bath at 40℃ for 30 min.

4-Add 5ml of Fehling reagent in the two test tubes.

5- Put the tubes in water bath at 100℃ for 15 min.

Observation

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c- Detecting of polysaccharides (Starch)

Tools:

Starch soln.- Fehling reagent – chines plate – water bath 100℃ , 40℃- diastase- iodine soln. -

Procedures:

1- Take 3 test tubes and add 5ml of starch soln.in three test tubes.

2-Add 2ml of diastase in two tubes and let the third one without addition

3-Put the tubes in water bath at 40℃ for 30 min.

4- After 15 min. take drops of the mix. In the chines plate which contain iodine soln. to detect the complete conversion of starch to simple sugar.

4-Add 5ml of Fehling reagent in the two test tubes (the tube contain diastase and the tube without diastase).

5- Put the tubes in water bath at 100℃ for 15 min.

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2-Proteins

a- **<u>Yellow protein test (Xanthoproteic Test)</u>**

Tools:

Egg albumin (protein sample)- concentrated nitric acid – test tube – NaOH

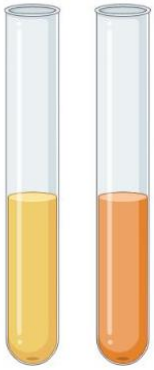
Procedures:

2- Take 5ml of egg albumin in clean test tube.

2-Add 1ml of concentrated nitric acid (white ppt.)

3-Heat in water bath

4- Add 2ml of NaOH (40%) and cooling under tap water.



Xanthoproteic Test Positive

Presence of aromatic amino acids (tyrosine and tryptophan)

Presense of the dark yellow or orange color

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b- Biuret test

Biuret test is a general test for compounds having a peptide bond.

Tools:

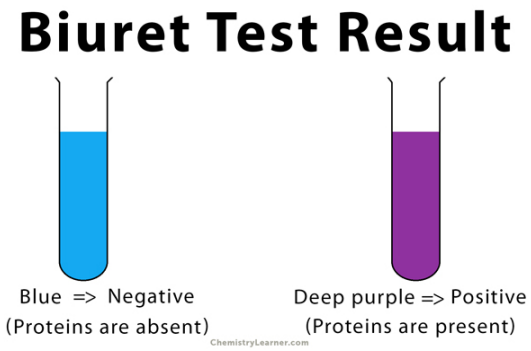
Egg albumin- NaOH – test tube – $CuSO_4$

Procedures:

3- Take 5ml of egg albumin in clean test tube.

4- Add 1ml of NaOH (40%)

5- Add 1ml of $CuSO_4$(10%)



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3-Pigments

Tools:

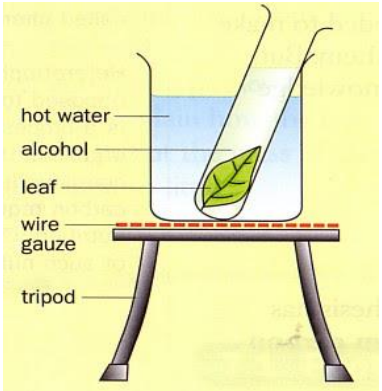
Green leaves- ethyl alcohol or ethyl alcohol (conc.) – test tubes - water bath

Procedures:

1- Take the green leaves in clean test tube

2- Add 5 ml of concentrated ethyle or methyle alcohol

3- Put the test tube in water bath at 60C.



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4-Fats and oils

Tools:

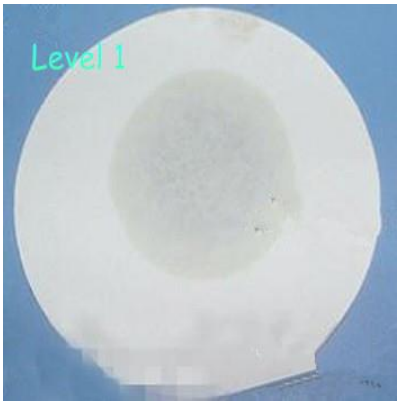
Filter paper- oil or butter– distilled water –

Procedures:

1- Take the filter paper

2- Add drops of oil or butter on the filter paper.

3- Add drops of water on another filter paper .



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Factors affecting on diffusion in the plant cell

1- pH

Tools:

Beet roots – test tubes– solutions of different pH(2,4,6,8) –

Procedures:

1-Cut the beet roots to small pieces

2-Put an equal amount in each test tube

3-Add 5 ml of different pH(2,4,6,8)solutions in each tube.

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2- Temperature

Tools:

Beet roots – test tubes–distilled water – water bath at(0,20,50,70C)

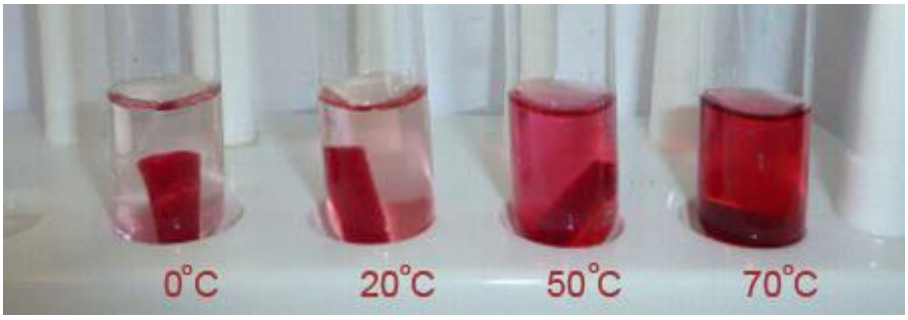
Procedures:

1-Cut the beet roots to small pieces

2-Put an equal amount in each test tube

3-Add 5 ml of distilled water in each tube.

4-Put each tube in different water bath



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3-Ethyl alcohol

Tools:

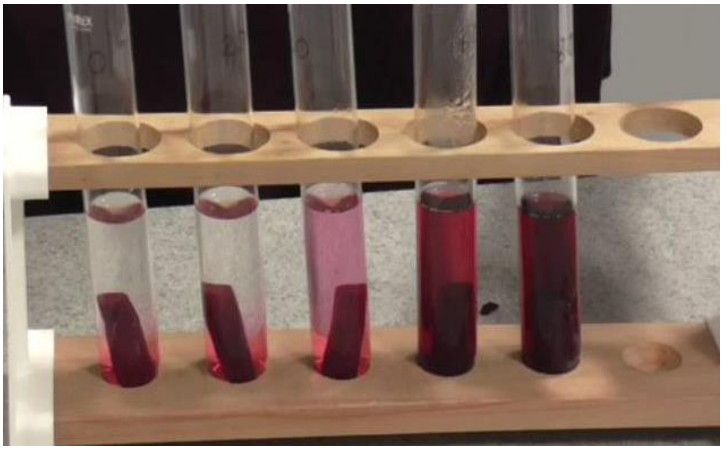
Beet roots – test tubes–different concentration of ethyl alcohol –

Procedures:

1-Cut the beet roots to small pieces

2-Put an equal amount in each test tube

3-Add 5 ml of different concentration of ethyl alcohol.



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Determination of some plant minerals in plant extract

- **Determination of calcium (Ca^{2+}) and magnesium (Mg^{2+})**

Tools:

Plant extract – Muroxide indicator – versine – distilled water- conical flask – burette – E.C.BT indicator.

Procedures:

1- Put known amount of plant extract in the conical flask
2- Add drops of E.C.BT indicator in the conical flask (the color become purple red).
3- Fill the burette with versine (0.1N)
4- Titrate the mix in the conical flask against versine untile the color turned to blue.
5- Calculate the volume consumed from versine
6- Calculate the concentration of calcium and magnesium together (V1)

For determination the calcium (Ca^{2+}) only

7- Put known amount of plant extract in the conical flask
8- Add trace amount of Muroxide indicator in the conical flask (the color become red).
9- Fill the burette with versine (0.1N)
10- Titrate the mix in the conical flask against versine untile the color turned to purple.

11- Calculate the volume consumed from versine

12- Calculate the concentration of calcium only (V2)

13- Subtract V2 from V1 to calculate the concentration of magnesium Mg^{2+}

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- **Determination of Chlorine (Cl^-) in plant extract**
- **Tools:**
- Plant extract –$AgNO_3$ – distilled water- conical flask – burette – $K_2Cr_2O_4$ indicator
- **Procedures**

1- Put known amount of plant extract in the conical flask

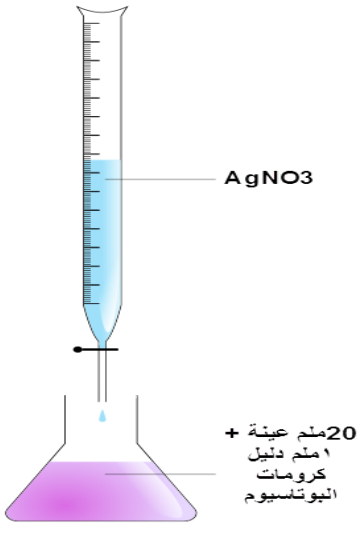
- 2-Add drops of $K_2Cr_2O_4$ indicator in the conical flask (the color become yellow).

3-Fill the burette with $AgNO_3$ (0.01N)

4-Titrate the mix in the conical flask against AgNO3 untile the color turned to red.

5-Calculate the volume consumed from $AgNO_3$

6- Calculate the concentration of Cl^-



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Transpiration

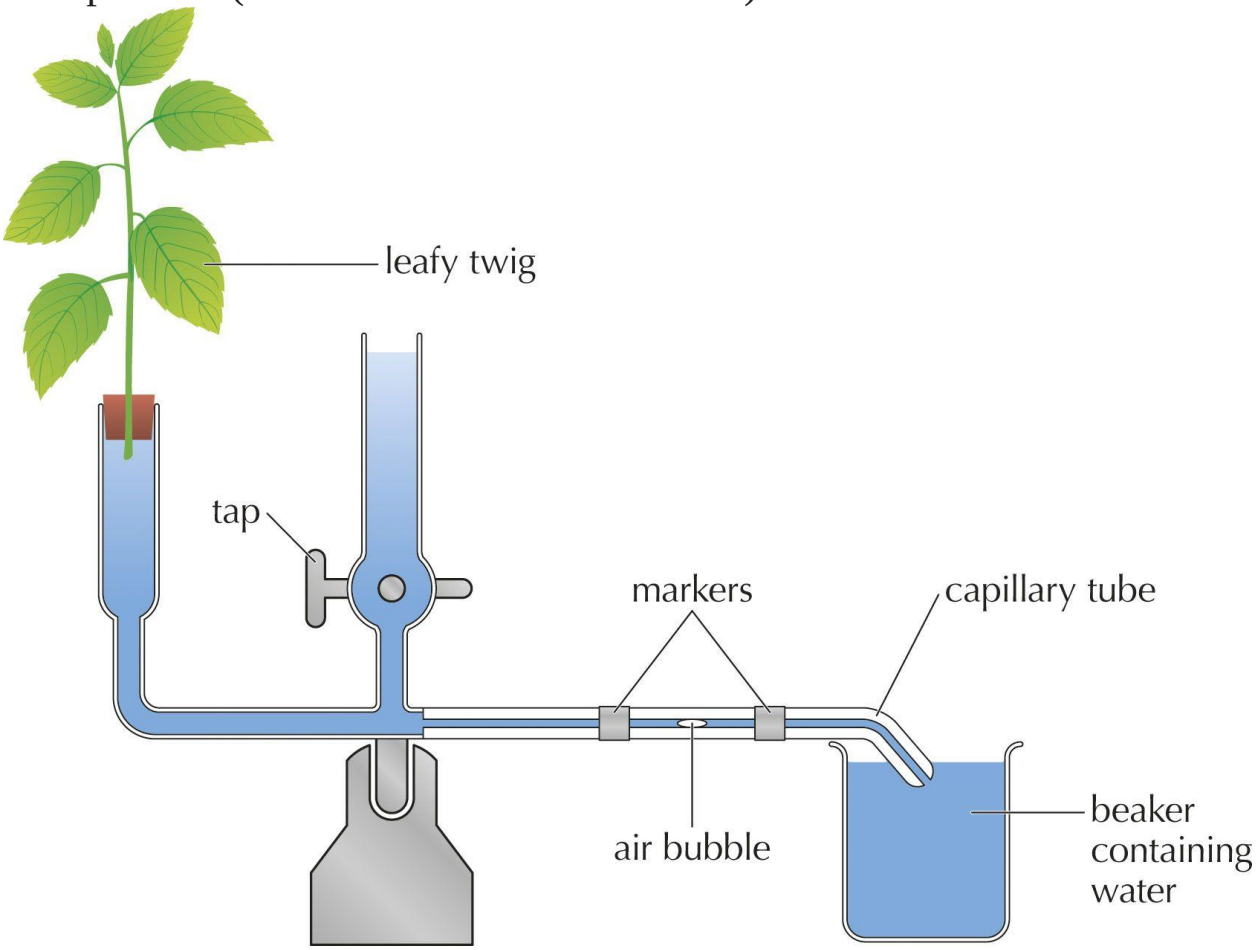
3-Potometer

- **Uses**

It is used to measure the rate of transpiration

- **The idea of its work**

It is based on the idea that the rate of absorption (the rate at which water plants acquire) almost equals the rate of transpiration (the rate at which water is lost)



Mechanism of work and the law

The plant that we want to measure transpiration rate for it is fixed in part designated for it inside the device, and the leaf surface area for this plant is estimated

There are two ways to measure the rate of transpiration

1-The gravimetric method for estimating the rate of transpiration

The apparatus is filled with water and branch of plant is fixed in its appropriate place, then the apparatus is weighted before beginning the experiment and weighted after the end of the experiment, the amount of lost water is calculated as the difference between the two weights

W1 = weight before beginning the experiment

W 2 = weight of the apparatus after the end of the experiment

$$\text{Transpiration rate} = \frac{\text{Weight of water lost (W1-W2)}}{\text{Leaf area (cm) x time (hours)}}$$

g / cm2 / hour

Methods for determining leaf area

1-By graphing

The paper is placed on a graph paper, then its dimensions are drawn with bullets and the number of squares taken is calculated, where each square represents 1 cm through the number of squares The area of the paper is approximately calculated

1- Weighted method

Draws a square with known dimensions, length and width, on a filter paper, and let its area be (A 1), then cut it and weight it

(W1) .on the same type of filter paper draw the leave that you want to find its area and let its area A2 and weight it lets its weight W2

W1/ A1= W 2 /A 2

A2 = A1 x W2/W1

Leaf area = number of leaves x area of one leaves

If the stomata are found on two surfaces, the area of the leaves *2

Determining the effect of environmental conditions on transpiration rate

Aim

To determine the effect of environmental conditions on transpiration rate using a simple potometer.

1-Wind velocity

Tools:

Soft green leafy shoot – conical flasks – distilled water-

Procedures:

1-fill the two conical flask with 250ml distilled water

2-fix the Soft green leafy shoot in the conical flask.

3-weight the two conical flask (W1, W2)

4-leave one conical flask expose to wind for 48 hr. and leave the other without exposure.

5-after 48 hr. weigth the frist conical flask exposed to wind W3 and the second (does not exposed to wind) W4.

6-Calculate the rate of transpiration in the two conical flasks.

Observation

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2-Effect of salts (NaCl)

Tools:

Soft green leafy shoot – conical flasks – distilled water- NaCl soln.

Procedures:

1-fill one of the conical flask with 250ml distilled water and the other with 250 ml NaCl soln.

2-fix the Soft green leafy shoot in the conical flask.

3-weight the two conical flask (W1, W2)

4-after 48 hr. weigth the frist conical flask (contain dist.water)= W3 and the second (contain NaCl soln.) =W4.

6-Calculate the rate of transpiration in the two conical flasks.

Observation

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