

VOL

1

PLANT PHYSIOLOGY AND METABOLISM

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WATER AND MINERAL NUTRITION

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**PHOTOSYNTHESIS AND TRANSLOCATION OF
PHOTOSYNTHATES**

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PLANT PHYSIOLOGY AND METABOLISM

Plant Physiology Course has two volumes. Volume 1 contains Block 1 and Block 2 while Volume 2 contains Block 3 and Block 4.

Physiology is the study of the functions of living organisms and their constituent parts. You will study the physical and chemical terms, the mechanisms that operate in living organisms at all levels, ranging from the subcellular to the integrated whole organism.

Although there is striking similarity between the molecular mechanisms of plants and animals in terms of biosynthesis and catabolic pathways, and many cellular functions among the lower levels of organization, the differences are more pronounced between the higher plants and higher animals. For example plants have evolved unique machinery for synthesizing their food by photosynthesis. Although plants do not have an active pump like a heart, they possess an intricate transport system to carry water, dissolved minerals, photosynthates and hormones to all parts of the body. The respiratory mechanism is also similar at the cellular level, though there are distinctly different systems of gaseous exchange in both the groups. Plants also respond to the external stimuli, but the manifestation of their response differs from those in animals. Growth and development in plants is regulated by various external (environmental) and internal (hormonal) factors. Plants also respond to these environmental factors.

The basic theme of Plant Physiology is divided into **four** blocks. The first block deals with the plant water relations at the cellular and whole plant level. In addition, the inorganic nutrients required by the plants, their occurrence, uptake and transport is also described.

The second block comprises the energy conservation by plants through photosynthesis and translocation of the photosynthate by phloem.

The third block deals with the biocatalysts, their kinetics and mechanism of action. Mechanisms operative for the oxidation of photoassimilates and other reserve food materials have been described in units on respiration.

The final (fourth) block (Units 13 and 14) is devoted to a study of nitrogen assimilation by plants including the biological nitrogen fixation carried out by a few 'gifted' prokaryotes. Unit 15 deals with a study of plants growth regulators, their discovery, structure, mode of action and commercial application. The final Unit 16 gives a comprehensive account of the responses of plants to environmental stress highlighting various physiological adaptations for acclimation.

Objectives

After studying this course, you will be able to:

- ❖ appreciate the water relations of the plants, mechanism of absorption of water, and the different pathways of water transport; and describe the role of essential nutrients.
- ❖ enlighten, describe and discuss about photosynthesis and its mechanism of action;
- ❖ explain and discuss about mechanisms of enzyme action and respiration in plants;
- ❖ discuss nitrogen fixation and various hormones and their action; and
- ❖ appreciate how plants deal with stress and adapt themselves.

General Study Guide

To get maximum benefit out of this study material please take note of the following points:

- i) Make a notebook with one side rule and the other side plain, keep a pen and some coloured pens/pencils with you.
- ii) Read the material slowly and attentively. Spend enough time on figures and flow charts. Try to draw the figures/flow charts and label them properly. This will help you in better understanding of the text.
- iii) While studying the text, underline the important points with a distinct colour (red, green, blue etc.) in the block itself. Write down salient points in the space provided on each page, or in your notebook, if necessary.
- iv) After finishing a section or subsection; ask yourself – what have I learnt? Try, to list the important points in you note book and compare them with the text and see if you have missed any.
- v) Attempt all the self-assessment questions (SAQs), wherever they appear. Don't skip any of them as they are designed to assess your understanding of the subject. If you cannot answer, read the text again.
- vi) The answer to the SAQs and Terminal Questions are given at the end of each unit. Don't get tempted to see the answers, before you try them.
- vii) If you don't understand any word in the text, consult a dictionary. For scientific and technical words consult the glossary given at the end of each block or a scientific dictionary, if necessary.
- viii) For exploring the topics further, we have provided a list of suggested readings at the end of each block.

Block

1

WATER AND MINERAL NUTRITON

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BLOCK 1 : WATER AND MINERAL NUTRITION

Block I deals with the plant water relations, transport of water in a plant along with the nutrition of plants. Since plants have different modes of life than animals, there exist many differences in their physiology. This block primarily concerns with nutrition of plants, particularly the higher plants. A major difference between plants and animals, which influences the water relations of both, is the presence of cell wall in plants and its absence in animals. Plants need enormous amounts of water in order to live and grow. The unique properties of water make it an ideal elixir of life. The transport of water across the plant involves the existence of water potential and its components.

Unit 1 and 2 The water content and its availability and rate of involvement in soils depends upon the soil type and structure. The physicochemical properties of water make it an ideal medium for various biochemical reactions. Components of water potential operate for the uptake of water from soil to the specialised areas in the root like the root hairs. You will also study the movement of water through the apoplast, the symplast and the transmembrane pathway in the roots prior to its transport through the xylem conduits.

In addition, the ascent of sap in a plant is governed by the forces created by the transpiration pull which helps water transport to great heights. You will also study different mechanisms involved in the opening and closing of stomata. Although minerals are transported along with water, the uptake of minerals into root cells involves special transport mechanisms, which are dealt in **Units 3 and 4**. In addition to gaining knowledge of essential elements in plants, you will also study about the criteria for essentiality, along with role of macro and micronutrients, and the techniques of studying mineral deficiencies. Also, the association between roots and the mycorrhizal fungi are vital for the uptake of certain elements like phosphorus, which are otherwise immobile.

Unit 4 deals exclusively with the uptake of mineral nutrients into the roots via the Donnan free space and thereafter transport of ions across the cell membranes involving the membrane transport proteins, ionic pumps and *ATPases*. The radial movement of ions in roots as well as the mechanism of long distance transport involving xylem loading and unloading is also discussed in detail.

Objectives

After studying the block, you will be able to :

- ❖ describe the importance of water to plants; enumerate the unique physico-chemical properties of water explain the various components of water potential and their relationship to osmosis and osmotic pressure;
- ❖ describe the mechanism of absorption of water, and the different pathways of water transport; and different aspects of ascent of sap and transpiration; and
- ❖ describe the role of essential nutrients; explain the uptake of nutrients by roots and the role of mycorrhizal associations.

UNIT 1

PLANT –WATER RELATIONS

Structure

1.1	Introduction	Osmotic Pressure
	Objectives	Diffusion Pressure Deficit (DPD)
1.2	Life Supporting Characteristics and Biological Importance of Water to Plants	Chemical Potential
		Components of Water Potential
1.3	Water Transport	Gradients of Water Potential
	Diffusion	1.4 Summary
	Imbibition	1.5 Terminal Questions
	Osmosis	1.6 Answers
	Membrane Permeability	

1.1 INTRODUCTION

Water is the most important single component which supports all living organisms on earth. All animals and plants, whether terrestrial or aquatic, are dependent on water for their survival. We could survive for some days without food, but it is impossible to live without water.

Our daily weight fluctuates on account of excess or loss of water. Water is the most abundant component in any living system. It constitutes nearly 70% of the total body weight.

In plant cells most of the water is localized in the vacuoles (upto 95%) and the rest resides in the peripheral cytoplasm. The amount of water in plant varies among different species depending upon their structure. Aquatic plants such as *Chlamydomonas*, *Spirogyra*, *Chara*, red algae, and others contain 97-98% of their weight as water. This is true also for *Azolla*, *Eichhornia* and other freshwater plants. However, among terrestrial plants, whether they are crops such as wheat, rice, maize, or trees such as sheesham, mango, neem and

many others, the amount of water varies in different plant parts. It would be as high as 95% or more in young roots and as low as 30-40% in wood of a tree trunk. Young leaves often contain 85-90% water whereas mature leaves contain 60-80%. This means that the amount of water changes during the growth of the plant as well as during the growth of an organ such as leaf or seed. In this unit we will discuss the properties and importance of water for plants.

Objectives

After studying this unit, you should be able to:

- ❖ explain the unique properties of water which make it the elixir of life;
- ❖ differentiate between the processes of diffusion, osmosis and imbibition;
- ❖ explain the various components of water potential and the relationship between them; and
- ❖ describe and relate the movement of water in a single cell to a tissue and to the whole plant.

1.2 LIFE SUPPORTING CHARACTERISTICS AND BIOLOGICAL IMPORTANCE OF WATER TO PLANTS

The life-supporting characteristics of water are due to its unique physical and chemical properties. Nearly 75% of our planet is covered with water in the form of oceans and other water bodies. The dominating presence of water in most cells must be seen in terms of its much larger number of molecules as compared to others. This is because water has a low molecular mass (18 Daltons).

Water as a Chemical Reactant

Water in a liquid state is an ideal medium facilitating the mobility of different macro/micro molecules within and across the cells. Water also influences the structure of different chemical components of cells. Water participates in many biochemical reactions either as a reactant itself (e.g., photosynthesis) or a product in others (e.g., respiration). During photosynthesis water splits in light reaction (photolysis) into oxygen and hydrogen. The latter is used in carbon fixation through CO_2 for making glucose and constitutes the basic reaction of the autotrophic food chain. Various life characterizing biochemical reactions like condensation, hydrolysis, oxidation, and reduction occur in an aqueous medium.

Water for Seed Germination

Seeds do not germinate unless they get enough water to hydrate their protoplasm. Hydration triggers various metabolic reactions, and the metabolites are mobilized in an aqueous medium.

Water as a Solvent

Water is a polar molecule and has exceptionally high hydrogen bonding ability. This unique property allows it to dissolve a variety of molecules like sugars, amino acids, carrier proteins like hemoglobin and myoglobin. Sugars and proteins which contain polar -OH or -NH_2 groups easily dissolve in water. Thus, an aqueous medium would be extremely/ highly suitable for uptake of dissolved mineral nutrients-so vital for growth and development of plants. It was precisely this property of water which made possible a variety of reactions in the “**hot primeval soup**” of the oceans during the origin of life.

Thermal Properties of Water

Water is unique as it remains in liquid form over a wide range of temperatures where life exists comfortably. If one considers the molecular size alone, water must be expected to exist in a gaseous state in most of the areas on earth. However, this is not true. The melting and boiling points of water are much higher than what would be theoretically expected. Presence of oxygen has introduced polarity and the tendency to form hydrogen bonds. It is this extensive hydrogen bonding which gives water its unique thermal capacity i.e., high specific heat, thermal conductivity, latent heat of vaporization and heat of fusion.

Adhesion and Cohesion

Adhesion and cohesion essentially refer to the “stickiness” that water molecules have for each other and for other substances. These attributes are due to the extensive hydrogen bonding. Water drop remains a drop on account of cohesion i.e., mutual attraction between molecules. For this to occur, the area of an air-water interface needs to be increased. This would require breaking of the hydrogen bonds and energy input. This energy needed to increase the surface area of a gas-liquid interface is called **surface tension**. High surface tension makes the water drops spherical and help movement of capillary water through the micro spaces. Another related characteristic is adhesion, which is the attraction of water to a solid phase. In plants, water “sticks” to the cell wall of the tracheary elements, i.e., xylem. This again is on account of extensive hydrogen bonding. Both adhesion and cohesion contribute to another related property, i.e., **tensile strength**. Tensile strength can be defined as maximum force per unit area that a continuous water column can withstand before breaking. This property is characteristic of metals. Water is an exception and exhibits a high tensile strength of 30 megapascals (MPa).

The trio of adhesion, cohesion and surface tension gives water another unique property of **capillarity**, i.e., the ability of a water column to rise as a continuous column in plants.

Providing Turgidity to the Cells

Uptake of water results in turgor pressure which inflates the semi-elastic cellulosic cell wall resulting in initial volume expansion. Proteins, carbohydrates along with other metabolites are deposited only later during growth.

SAQ 1

Match the terms in Column A with those of Column B

Column A	Column B
a) Temperature regulation	1. Dipoles
b) Mutual attraction	2. 0.239 calorie
c) Hydrogen bond	3. Heat of vaporization
d) Orientation towards (+ve) and (– ve) ions	4. 10 picoseconds
e) Hydration	5. Germination
f) Joule (J)	6. Cohesion

1.3 WATER TRANSPORT

How does water flow in and out of the cells? How is water taken up from the soil by the roots? How does water escape from the plant into the atmosphere? To answer these questions, you would need to understand the dynamics of water flow with reference to the movement of substances and the different forces which are operative in this movement. Particles also exert forces by themselves. Before you study about them in detail, you must know that they are diffusion, imbibition, mass flow, and osmosis. Translocation is the term which broadly defines the movement of substances from one region to the other. The process of translocation may be passive or active, depending upon the need for expenditure of energy.

The movement of water occurs through cell to cell by two principal pathways:

- Symplastic** pathway where water moves from cell to cell via plasmodesmata. Since cells are joined via intercellular connections, the entire living portion of the plant forms a continuous single entity and is called **symplasm**.
- Apoplastic** pathway is another route for the transport of water through cell walls, intercellular spaces, and non-living cells of the xylem. The non-living portion of plants is called **apoplasm**.

1.3.1 Diffusion

You can smell from a distance the aroma of food being cooked in the kitchen. A bottle of perfume opened in one corner can be smelt in another corner. A crystal of copper sulfate dissolves in a beaker of water turning the latter blue. So does a drop of blue ink dropped in water. All the above phenomenon highlight a common point i.e., the random movement of particles is caused by the inherent kinetic energy they possess.

The particles strike one another repeatedly thus, resulting in their dispersal in different directions. Also, these particles would always tend to move (perpetually move) in the direction where there is minimum obstruction offered by the other particles. It would mean, therefore, that the net movement would be favored from the area of higher concentration to the area of low concentration (region of higher free energy to a region of lower free energy). Thus, the spontaneous process or the tendency of particles (ions, atoms, or molecules) of gases, liquids and solids to get evenly distributed through the available space due to their random kinetic motion is called Diffusion (Latin *diffuses* = spread out). In other words, diffusion is a spontaneous process that leads to directed net movement of a substance from a region of higher concentration to a region of lower concentration, or simply as the net movement of molecules from a region of high free energy to a region of low free energy (see Fig 1.1). The term was coined by R. Brown in 1928.

After sometime the particles/molecules will evenly spread throughout the available space. This would mean an equilibrium where the movement of particles will be equal in all directions, and thus net movement will be zero. The process of diffusion can be explained based on Fick's First Law (a quantitative description of the process formulated by Adolf Fick in 1855), which in its oversimplified way suggests that the rate of diffusion is directly proportional to the cross-sectional area of the diffusion path and to the concentration vapor pressure gradient. At the same time rate of diffusion is inversely proportional to the length of the diffusion path.

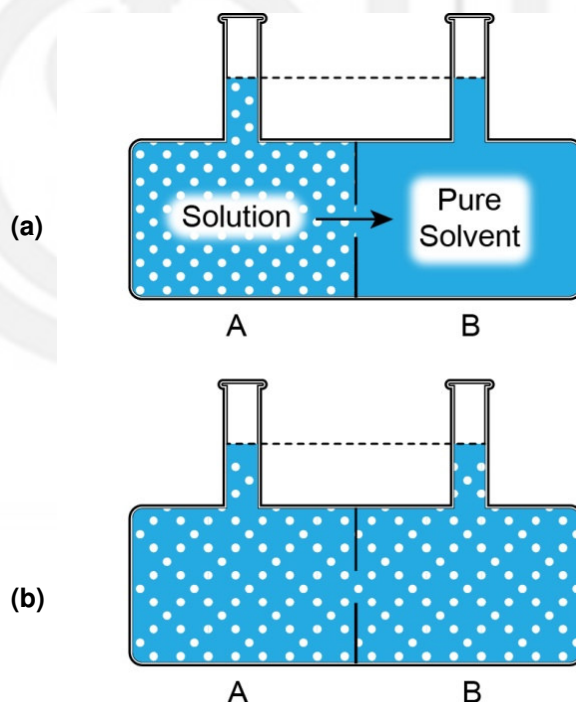


Fig.1.1: a) Diffusion of solute molecules from a region of high concentration (A) to a region of low concentration (B) till the molecules are uniformly distributed; b) At the time of uniform distribution, there is no net diffusion.

It is believed that diffusion is most effective over shorter distances, as the average time required for a substance to diffuse for a given distance increases as the square of that distance. By this analogy, it is easy for glucose to diffuse in water, and with a diffusion coefficient of about $10^{-9} \text{ m}^2 \text{ s}^{-1}$, a glucose molecule can diffuse across a cell of about $50 \mu\text{m}$ diameter in just 2.5

seconds. Interestingly, the time taken by the same glucose molecule to diffuse to a distance of 1m in water will be nearly 32 years !!! Thus, diffusion is not much effective for translocation over long distances (see Unit 8).

An example of solid to solid diffusion is when aluminum diffuses into silver when both are placed in contact.

The ions particles/molecules which diffuse through the medium are the **diffusate** while the medium into which these particles disperse is the **diffusant**. For example, perfume is the diffusate and air is the diffusant; sugar is diffusate and water is the diffusant. Diffusion can take place between gas-gas, gas-liquid, liquid-liquid, solid-liquid and solid-gas and solid to solid phases. Rate of diffusion depends upon several factors.

- a) **Temperature:** Diffusion is directly proportional to temperature as it increases the kinetic energy of diffusate particles. Diffusion show a Q_{10} of 1.2-1.3. (The Q_{10} denotes temperature coefficient, which is a measure of the rate of change of any biological or chemical system because of increasing the temperature by 10°C).
- b) **Density of diffusate:** Diffusion rate is inversely related to the square root of the density of the diffusate (d).

$$D \propto \frac{1}{\sqrt{d}}$$

D = Diffusion rate,

d = density of diffusate.

Thus, gases diffuse more readily than do the liquids while the solids diffuse the slowest.

- c) The pressure exerted due to the tendency of the diffusate to diffuse is called its **diffusion pressure**. Because of their inherent kinetic energy, the movement of molecules will take place from the region of higher diffusion pressure to region of their lower diffusion pressure.
- d) Density and concentration of diffusant also determines/influences the rate of diffusion. More concentrated and dense the diffusant, lesser/slower will be the rate of diffusion.

Importance of Diffusion

The principle of diffusion is of great importance in plants as it is involved in the movement of almost all substances. Processes such as uptake of water and minerals, osmosis, transpiration, exchange of gases during photosynthesis and respiration, short distance translocation in the symplast, spreading of ions and other substances throughout the protoplast and wetting of cell walls, and release of odor for pollination depend in part on diffusion.

1.3.2 Imbibition

Imbibition (Latin *imbibere* : to drink) is a process that involves absorption of water or any liquid by the solid particles of an absorbent substance without forming any solution. The liquid or water which is imbibed is called an **imbibate** while the insoluble substance that imbibes water/substance is the

imbibant. The imbibants are usually in the form of colloidal particles which hold liquid water adsorbed on their surface as well as inside the minute spaces between the particles themselves. Although the liquid involved in imbibition in plants is water, other substance may also act as imbibants. For example, rubber imbibes ether/kerosene oil rather than water. Most important among the plant imbibants are the hydrophilic colloids like proteins, pectins, starches and cellulose. These substances differ in their imbibition capacities. The phycocolloid agar-agar has the maximum imbibition capacity and can imbibe almost 99 times its weight of water. Proteins also have a high imbibition capacity of imbibing nearly 15 times water their own volume. Cellulose and starch are poor imbibants and lignin does not imbibe water.

Agar agar > oily seeds > proteinaceous seeds > starchy seeds.

You must have observed at home that gram seeds when soaked, swell much more than those of rice or wheat.

Since imbibition holds water in between the solid particles, the immobilized water loses its kinetic energy. The lost kinetic energy is released in the form of heat, often termed as **heat of wetting**. Kneading of wheat flour results in an increase in temperature. Moreover, imbibition results in increase in the volume (swelling) of the imbibant. However, this increase in volume is less than the volume of water imbibed.

The process of imbibition develops a high pressure called **imbibition pressure**. It can be defined as the maximum pressure which can develop in an imbibant when brought in contact with water without allowing the imbibant to swell up.

You will read in the following subsections that imbibition pressure is also referred to as **matric potential**. Dry seeds show a matric potential value of nearly –100 bars. Dry seeds like pea on encountering water can develop high imbibition pressure of up to 1000 atm (other equivalents used are bars and kg/cm²). 1 bar=0.987 atm=10⁶ dynes/cm²=1.019 kg/cm²=14.5 lb./in².

This property has been exploited since ancient times to break rocks by filling dry wood in natural grooves of rocks and watering them. The wood swells up and cracks the rocks.

Various factors influence imbibition:

- a) Rise in temperature enhances the rate of imbibitions.
- b) Texture of the imbibant, i.e., cohesion of molecules is inversely proportional to imbibition capacity. So, gelatin, with loosely packed particles will imbibe much more than hard wood.
- c) Since the imbibants are predominantly colloidal, imbibition is influenced directly by pH of the medium. For example, a negatively charged colloid like cellulose will imbibe maximum at alkaline pH. Since proteins are amphoteric, their absorption is maximum in other alkaline or acidic medium, but least in neutral medium.

- d) Electrolytes are imbibed at slower rates as they tend to naturalize the charges of the imbibants.
- e) Seed germination primarily depends on imbibition of water.
- f) Imbibition results in the breakage of seed coat.
- g) Many dehiscent fruits like pods of legumes, cotton bolls dehisce through imbibition in dry conditions by xerochastic, hygroscopic movements. Elaters of bryophytes also show hygroscopic movements.

1.3.3 Osmosis

The term osmosis (Gr *Osmos* = impulse) was coined by Nollet in 1748 who first observed that a cylinder full of wine whose mouth was covered with animal bladder started swelling up and even burst when placed in water.

Osmosis can be defined as the diffusion of water (or solvent) from a dilute solution into a stronger solution when separated by a semipermeable membrane. Thus, during the process of osmosis the direction and rate of flow depends on a semi permeable membrane, concentration difference and pressure difference in the two compartments.

Osmosis can be demonstrated by a simple thistle funnel experiment which you must have performed in your schools.

A solution which can cause entry of water into it is called an **osmotically active solution**. The osmotic entry of water into a system, organ or a cell from outside is called **endosmosis**, while **exosmosis** is the withdrawal of water from a cell or an osmotic system in response to a hypertonic solution. Swelling of water soaked dry raisins with intact stalks is an example of endosmosis while the fresh grapes shrink in size and become wrinkled due to exosmosis, when placed in 15% salt or sugar solution.

Three types of solutions can be classified, based on the cell sap.

- a) **Hypertonic solution** : A solution having more concentration than that of the cell sap. If a cell is placed in such a hypertonic solution, water will diffuse out due to exosmosis resulting in shrinkage of the protoplasm (plasmolysis).
- b) **Hypotonic solution** : In this case, the concentration of the external solution is less than that of the cell sap. When a cell is immersed in a hypotonic solution, water diffuses into the cell (endosmosis) resulting in an increase in cell volume.
- c) **Isotonic solution** : When the cell sap and the external solution have the same concentration. There is no change in the cell volume as there is no net movement.

1.3.4 Membrane Permeability

The tendency or ability of a membrane to allow or restrict the passage of substances through it is referred to as **Membrane Permeability**. This is a characteristic feature of all membranes. Rate at which the passage of solute

particles is allowed through the membranes varies with the permeability property of membranes. For example, a **permeable** membrane allows the passage of both the solute as well as the solvent particles through it. Majority of cellulosic cell walls behave as permeable membranes. On the other hand, the suberized walls of cork cells, or cutinized walls do not allow the passage of any substance and are, therefore, **impermeable**. **Semipermeable** are those membranes which allow only the solvent but not the solute particles to pass through e.g., parchment, copper ferrocyanide membrane or collodion membrane. Plasma membrane, tonoplast and membranes of organelles have unique permeability characteristics. These **differentially permeable** or **selectively permeable** membranes allow the passage of solvent (water) as well as selected (specific) solutes by a variety of mechanisms, operative within their lipoprotein complexes. For example, special protein-lined channels called **aquaporins** are responsible for water absorption and its further packaging. In addition, ions of solutes are transported through special **ion channels** (approximately 30 kDa).

1.3.5 Osmotic Pressure

It is the hydrostatic pressure developed in a solution which is just necessary to prevent the flow of water or a pure solvent to a solution (osmosis) when the two are separated by a semi-permeable membrane. In other words, it is the maximum pressure which can develop by an osmotically active solution when it is separated from its pure solvent by a semi permeable membrane.

Osmotic pressure (OP) can be measured by an **osmometer**, which involves a manometer. Pfeffer's osmometer and Hartley's osmometer are the two most used instruments for this purpose. The former employs very dilute solutions whereas the latter set up uses an external pressure source to counter the osmotic pressure. Since both the entities are equal, OP can also be alternately defined as the pressure that is required to be applied to a solution in order to prevent an increase in its volume due to the inherent tendency of water (on solvent) to enter it, when the two are separated by a semi-permeable membrane.

The value of OP is directly proportional to the concentration of the solution. The highest value of OP is observed in halophytes (>200 atm). Osmotic pressure is denoted by the following equation given by van't Hoff (1887).

$$OP = m RT$$

or

$$OP = c RT$$

Where OP = osmotic pressure

m/c = molar concentration of solution (concentration of solute/litre)

R = Universal gas constant (0.082 atm/mol)

T = Absolute temperature in Kelvin (K) = (t + 273)

t = temperature in °C

Theoretically, OP of one mole glucose solution at 0°C will be

$$OP = 1 \times 0.082 \times 273$$

$$22.38 = \# 22.4 \text{ atm}, = 22.69 \text{ bars} \quad (1)$$

$$= 2.269 \text{ MPa} = 22.69 \times 10^5 \text{ Pa},$$

and at 20°C will be

$$= 1 \times 0.082 \times (273+20)$$

$$= 1 \times 0.082 \times 293 \quad (2)$$

$$= 24 \text{ atm} = 24.31 \text{ bars} = 2.431 \text{ MPa}$$

$$= 24.31 \times 10^5 \text{ Pa}$$

The above-mentioned formula for OP is applicable to non-electrolytes e.g., glucose and sucrose. For electrolytes like NaCl or KCl, the degree of dissociation will also have to be considered.

For electrolytes, the formula becomes $OP = c/RT$

Where i is the van 't Hoff's ionization constant. Ionization increases the number of particles in a solution. Thus, it is natural that the same volume, of electrolyte would exert more OP than its equimolar non-electrolyte counterpart at the same temperature and pressure. To give you an example, 0.01 M glucose (non-electrolyte) will have an OP of 0.24 atm whereas equimolar sodium chloride (electrolyte) will have an OP of about 0.47 atm.

Thus, the OP of an electrolyte is greater than that of the equimolar non-electrolyte. Therefore, water moves from lower OP towards the higher OP.

Turgor Pressure (Hydrostatic Pressure/Pressure Potential)

The pressure developed by the turgidity in a cell is called **turgor pressure** (TP). Turgor pressure is applicable to living cell and not to a free solution. TP is called as **hydrostatic pressure** or **pressure potential**. When a cell is immersed in water, water enters the cell as cell sap has a higher osmotic pressure. It causes swelling of the osmotic system. As a result, the hydrated cytoplasm presses against the cell wall.

The outward force applied by the turgid protoplast on the cell wall is called **turgor pressure** (Fig.1.2). Since the cell wall is semi-elastic, it expands to a certain extent. The cell wall prevents a plant cell from bursting, when placed in water (hypertonic solution). An animal cell in a similar situation will burst. The cell wall exerts an equal and opposite pressure to counter the turgor pressure and it is called the **wall pressure** (WP). Thus, the TP and WP will be equal but exerted in opposite directions Fig. 1.2).

$$TP = WP$$

Turgor pressure keeps the protoplast hydrated and appressed to the cell wall. A fully (turgid) hydrated cell will have maximum hydrostatic pressure which will be equal to its OP. On the other hand, TP = 0 in a flaccid cell. The value of TP would, therefore, lie between zero and the value of OP. Under exceptional conditions of plasmolysis, TP may even become negative.

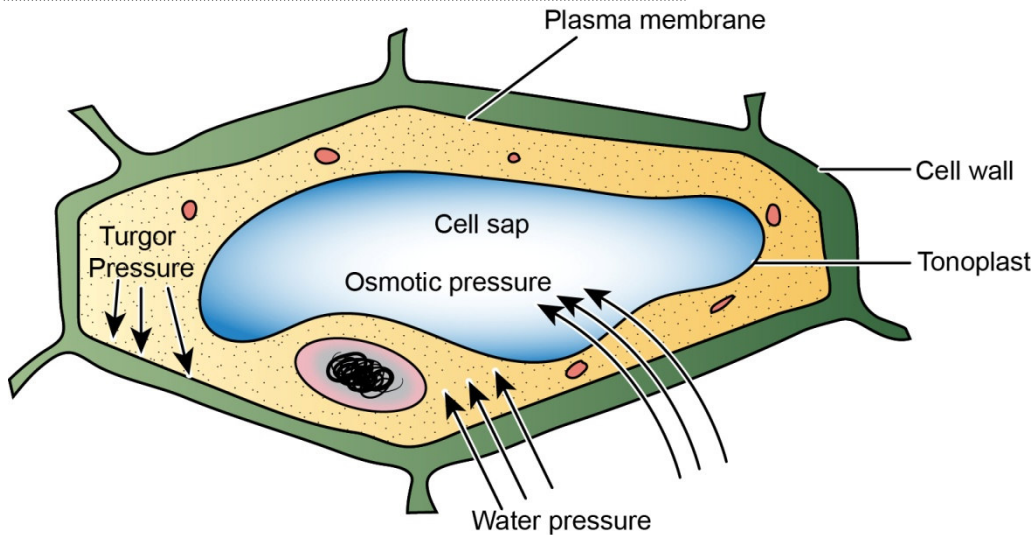


Fig.1.2: Osmotic relations exhibited by a plant cell.

Turgor pressure helps in keeping the protoplasm in a hydrated state which is important to maintain the 3-D structure of the organelles for their proper functioning. In addition, turgor pressure helps in cell elongation, germination and brings about a variety of plant movements including the seismonastic movements as well as opening and closing of stomata.

1.3.6 Diffusion Pressure Deficit (DPD)

Each liquid possesses a definite diffusion pressure with which it can move. Pure water has maximum diffusion pressure. Addition of solute to it reduces its diffusion pressure. So, the diffusion pressure of a solution is always less than its pure solvent. This reduction in the diffusion pressure of water in a solution or system over its pure state due to presence of solutes is called **Diffusion Pressure Deficit (DPD)**. In other words, DPD is the difference between the diffusion pressure of a solution and its pure solvent when present at the same temperature and pressure. The term DPD was coined by Meyer (1938). This term is preferred over the earlier term **Suction Pressure (SP)** proposed by Renner (1915). DPD constitutes the power of absorption by the cell and determines the direction of osmosis.

Seismonastic movements are paratonic movements brought about by external mechanical stimuli like vibrations, strong winds, rain drops, or contact with a foreign body.

The earlier term SP was used to denote the amount of pressure by which water can be sucked into the cell or likewise, expelled out of the cell. Another way to define DPD will be that it is the amount of water needed by a cell to make it fully turgid, as it is a measure of the water absorbing capacity of a cell. A solution will absorb more water or solvent molecules to equalize its deficit of diffusion pressure. So, water will always move from low DPD to high DPD.

Water Movement
Lower DPD —————→ **Higher DPD**

The value of DPD is always positive for a cell and is equal to osmotic pressure of the system minus wall pressure.

$$\text{DPD (SP)} = \text{OP} - \text{WP (=TP)}$$

When a cell is placed in pure water or in a less concentrated solution (hypotonic) than that of the cell sap, water enters the cell, resulting in an

increase in TP. Simultaneously the OP of the cell goes on decreasing as there is a continuous inflow of water and the concentration of the cell sap goes down. The value of TP goes on increasing and eventually becomes equal to OP. At this stage, the cell becomes fully turgid

$$\text{DPD} = \text{OP} - \text{TP}$$

when $\text{OP} = \text{TP}$; then $\text{OP} - \text{TP} = 0$

$$\text{DPD} = 0.$$

Thus, a fully turgid cell does not have any capacity to suck water further.

On the other hand, when a cell is placed in a hypertonic solution, water from the cell goes out by the process of **exosmosis**. As a result, the OP increases and there is a corresponding decrease in TP. The cytoplasm contracts and eventually the protoplast leaves the cell wall. This flaccid cell is said to be plasmolyzed. At the time of complete plasmolysis, value of TP becomes zero.

So, at complete flaccidity, since $\text{TP} = 0$

and $\text{DPD (SP)} = \text{OP} - \text{TP}$

$\text{DPD} = \text{OP}$ Such a cell will have maximum absorptive capacity.

If a cell was placed in some hypotonic solution other than water, then its

$\text{DPD (SP)} = (\text{OP} - \text{OP}_1) - \text{TP}$ (OP_1 , is osmotic pressure of the hypotonic solution)

Sometimes the value of TP becomes negative when the cell wall of the plasmolyzed cell pulls water in the opposite direction

Since TP is -ve

$$\text{DPD} = \text{OP} - (-\text{TP})$$

$$\text{DPD} = \text{OP} + \text{TP}$$

Thus, the DPD of the plasmolyzed cell is greater than the value of OP. It is clear from the above that the DPD of plant cell is not directly proportional to osmotic concentration of the cell. It depends on OP as well as TP of the osmotic system. So, it is DPD which is the basic determining force with which a cell or an osmotic system will lose or absorb water, rather than the OP and TP individually.

SAQ 2

In the following statements fill in the blank spaces with appropriate words:

- Cells undergo plasmolysis when kept in solution.
- Osmosis is diffusion of water through from a region of high concentration of water to a low concentration of water.
- In a hydrated cell, the hydrostatic pressure will be, and equal to its

1.3.7 Chemical Potential

The chemical potential of a substance in a system is a measure of its free energy. The free energy denotes the potential for performing useful work or the energy available to do work, which is force x distance.

Here the system refers to the thermodynamic concept wherein studies are of system rather than individuals or bodies. Thus, when we study the properties of a solution in a container, we are studying a system wherein each individual component interacts internally. For example, suppose we have pure water in a system, be it in a beaker or soil, all the molecules of water can do work, so their free energy is maximum. When a small amount of sugar is added a few molecules of water get associated with each molecule of sugar, the free energy of water decreases. Hence, the free energy of pure water is maximum, and addition of any solute lowers the free energy or chemical potential of water. This shows that the chemical potential is a relative quantity, which represents the difference between the free energies (potential) of the substance in each state to that substance in a standard state.

The unit of chemical potential is energy per mole of substance (J mol^{-1}).

The chemical potential of water in a solution is related to its vapour pressure and is given by the equation

$$\mu_w - \mu_{w^\circ} = RT \ln \frac{e}{e^\circ}$$

$$\Delta_\mu = RT \ln \frac{e}{e^\circ}$$

Where:

μ_w = chemical potential of water in question (joules/mole)

μ_{w° = chemical potential of pure water under STP

Δ_μ = change in free energy

R = gas constant

T = Absolute temperature in Kelvin

e = vapor pressure of water in question

Note that

$$\text{Relative Humidity} = \frac{e}{e^\circ} \times 100$$

If it is pure water, then $\ln \frac{e}{e^\circ} = 0$ and Δ_μ also becomes 0.

So, the chemical potential of water is set to zero. If chemical potential is less than that of pure water then $\ln \frac{e}{e^\circ}$ will be negative number, and therefore, Δ_μ will be less than zero, a negative number. So, the maximum value of chemical potential is 0, and addition of the solute lowers it to negative values. Water will spontaneously flow from regions of higher chemical potential to those possessing lower water potential.

Partial Molal Volume (J) of a solution is the change in volume of a solution when one mole of a substance is added to it.

1.3.8 Components of Water Potential

Water potential is expressed in pressure units. Energy per unit volume of water is expressed in joules per cm³. 106 dynes=1 bar. The present unit used for pressure is pascal (Pa). It is a pressure equal to the force of one Newton acting uniformly over one square metre.

1 bar = 10⁵Pa

10³ Pa= 1 KPa
(1 kilo pascal)

10³KPa = 1MPa
(mega pascal)

A related term **Water potential** (Stalyer and Taylor, 1960) has been used historically by the plant physiologists, which is the difference between chemical potential of water at any point in a system and that of pure water at STP (standard temperature and pressure). By conversion, the chemical potential of water is referred to as water potential. It is denoted by the Greek letter Ψ (-pronounced as *psi*).

Ψ_w is expressed in pressure units. It is the free energy per unit volume of water (Jcm⁻³). Ψ_w is equivalent to the chemical potential of water ($\Delta\mu$) divided by the partial molal volume of water, i.e., the volume of 1 mol of water, which is $18 \times 10^{-6} \text{ m}^3 \text{ mol}^{-1}$.

If water potential of the source (the region supplying the water) is higher than the water potential of the sink (the receiving region), then there is a spontaneous transfer of water from the source to sink ($\Psi_{\text{source}} > \Psi_{\text{sink}}$).

By convention, the water potential of pure water at atmospheric pressure is taken as zero. Therefore, water potentials other than that of pure water will generally be negative. Thus, lower potential means a more negative value, and higher potential is less negative value.

Since the water potential of a system is related to the difference in the vapor pressures of water in a solution and pure water state, it is taken as equivalent to the diffusion pressure deficit (DPD).

As explained earlier, Ψ_w of pure water is 0, and so is the value of DPD. Addition of solute (now a solution) will have a water potential as negative, whereas the DPD would increase (greater than 0). You will read in the next sections that the direction of movement of water is from lower DPD to higher DPD. On the other hand, water moves from a region higher (less negative) water potential to lower (more negative) water potential.

Water potential in plants is affected by three major factors: solute concentration, hydrostatic pressure, and matric forces. In addition, gravity also plays some role. Water potential (Ψ/Ψ_w) is equal to the algebraic sum of the potentials – Solute potential (Ψ_s), pressure potential (Ψ_p), matric potential (Ψ_m) and gravitational component (Ψ_g – when present).

$$\Psi_w = \Psi_s + \Psi_p + \Psi_m + \Psi_g$$

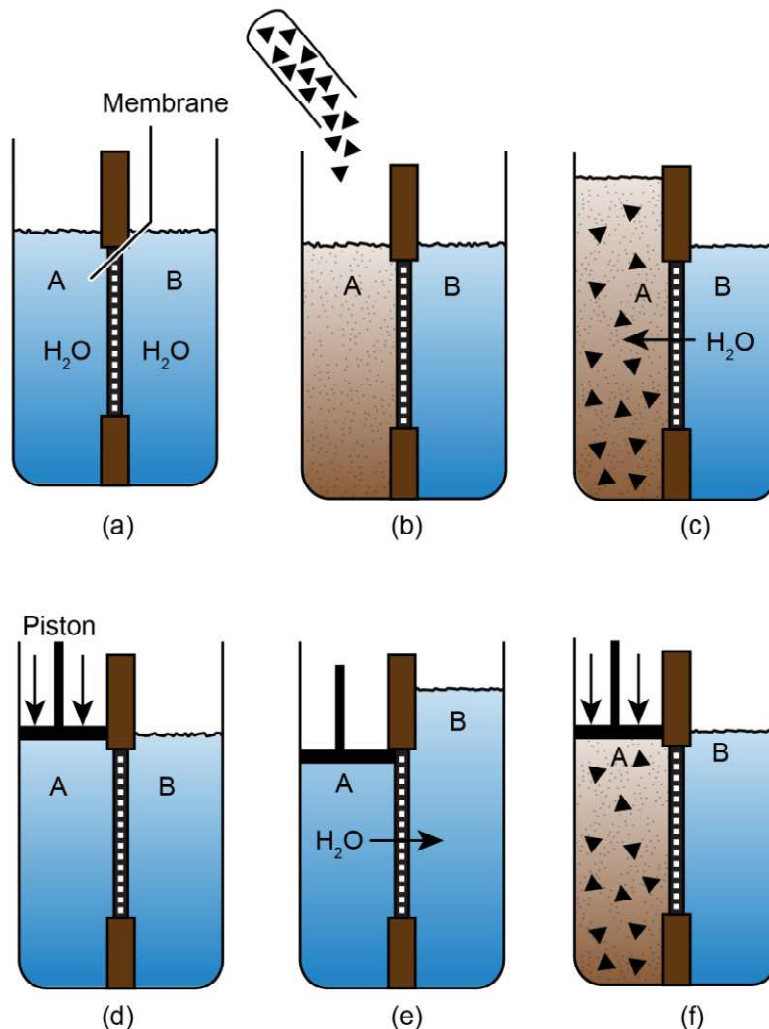
Let us consider each of these components in details:

Effect of Solute

Let us take pure water in two chambers A and B separated by a semi-permeable membrane (Fig. 1.3a). The water potential of both the chamber is zero. Addition of solute in chamber A (Fig. 1.3b) would reduce the free energy of water and the water potential will fall below zero. Consequently, water will move from B to A (Fig. 1.3c). The effect of dissolved solutes on water potential (Ψ_w) is called **osmotic potential** (Ψ_π). It can be estimated numerically if we know the osmotic pressure of the solution. The two are related as

$$\pi = -\Psi_\pi$$

For example, if π of solution is 5 bars then Ψ_π would be –5 bars.



Osmolality = moles of total dissolved solutes per litre of water – (mol L^{-1}). 6.02×10^{23} particles of the non-ionic solute will be present in one litre of solution with -22.7 bars at 0°C = 1 mole of non-electrolyte and -48.3 bars for the electrolyte sodium chloride.

Fig.1.3: Experiment to show the effect of solute and pressure on water potential.

In other words, osmotic potential or solute potential (ψ_s) represents impact of dissolved solutes on its water potential i.e. it records the decrease in the water potential or free energy of water due to the presence of solute particles (ionic or non-ionic). Under ideal conditions, the ψ_s is also equivalent to the maximum osmotic pressure which can be developed under ideal conditions but written with a negative sign as the dissolved solutes reduce the ψ_w of a solution with reference. Dilute non-ionic solutions, e.g., sucrose and glucose will have an osmotic potential (solute potential).

$$\psi_s = -RTC_s \text{ (see osmotic pressure Section 1.3.5 for values of R and T)}$$

Osmotic potential of 1.0 molal solution of sorbitol at 20°C will be [$\psi_s = -ci RT$]
 $: -(1.0 \text{ mol kg}^{-1}) (1.0) (0.00831 \text{ kg MPa mol}^{-1} \text{ K}^{-1} / 0.08205 \text{ kg atm mol}^{-1} \text{ K}^{-1}) (20 + 273 = 293 \text{ K}) = 2.43 \text{ MPa}$

The solute potential of the electrolyte will decrease by the degree of its dissociation over that of a non-electrolyte. To be expressed as ψ_s (electrolyte) = iRT . By this analogy, 0.33 M calcium chloride solution will have the same solute potential as 1.0 M sucrose.

Effect of Pressure

Let us now see the effect of pressure on water potential. As Fig. 1.3d illustrates, when pressure is applied the flow of water begins from chamber A

to chamber B through a semi-permeable membrane. This means that pressure increases the free energy of water and thus raises the potential of pure water above zero. The effect of pressure on water potential is called **pressure potential** and is designated as Ψ_p . The level of water in B rises due to increase in water potential of A (Fig. 1.3 e). Pressure potential is usually positive (is equal to turgor pressure). The values of turgor pressure are particularly high in leaf cells of crop plants during warm afternoons and in guard cells during stomatal opening.

In sharp contrast, xylem and in the walls between cells, a negative hydrostatic pressure or **tension** develops, leading to the value of Ψ_p becoming negative.

Now see the Fig. 1.3 where two chambers are present. What would happen if pressure were applied on the chamber containing solutes (Fig.1.3 d)? Now the Ψ_w will be affected by both solute and pressure. The solute would lower the water potential and pressure will raise the water potential. So, the flow of water from B to A would start decreasing (Fig.1.3 e). An equilibrium condition will reach when pressure potential Ψ_p will be equal but opposite in magnitude to osmotic potential Ψ_π and there will no net flow of water in the two chambers (Fig.1.3 f). This can be represented by the following equation:

$$\Psi_{w(B)} = (\Psi_\pi + \Psi_p)_B \quad \dots(1.1)$$

Let us suppose if Ψ_p is equal and opposite to Ψ_π . Then

$$\begin{aligned} \Psi_{wA} &= (\Psi_{\pi A} + \Psi_{pB}) \\ &= 0 \end{aligned}$$

We are going to discuss another important factor, i.e. the “Matric pressure”.

Effect of Matric Pressure

Water can get absorbed to the wettable surface of solids such as soil, wood, seeds, and cellular constituents. A force operates between solid-liquid interface and is called **matric suction** or **matric pressure**. The absorption process is accompanied by heat loss and results in decrease in free energy of water. In other words, the effect of matric forces on water potential is called **matric potential** (Ψ_m). Its value is always negative.

Matric potential can be viewed as a decrease in water potential on account of immobilization of water molecules due to their adsorption over the colloidal particles in the cytoplasm and cell wall. The value of Ψ_m may be appreciable in young cells, dry seeds, fruits, and some extreme xerophytes. The mature cells of mesophytes, however, show an extremely low Ψ_m value of -0.1 bars, and thus, can be ignored.

Likewise, in a well-watered soil Ψ_m is not very significant, however, when the soil is nearly drying Ψ_m determines water potential of the soil.

It is very natural that the water column will be forced to move downward due to the gravitational force. Thus height, density of water and the acceleration due to gravity will play a role to decide the equal and opposite force to counter this **gravitational potential**– Ψ_g . It has been calculated that the value of Ψ_g is quite insignificant (accounting for nearly 0.1 MPa in water potential) and is, therefore, generally not considered for water transport at the cell level.

So, we find that the Ψ_m is composed of the following main component forces:

$$\Psi_w = \Psi_\pi + \Psi_p + \Psi_m + \Psi \quad \dots (1.2)$$

Ψ = any other forces that may influence Ψ_w . Thus, water potential is equal to

$$\Psi_w = \Psi_s = \Psi_p$$

Resistances to Water Movement and Water Flux

We have explained earlier that if water potential drops from source to sink ($\Psi_{source} > \Psi_{sink}$) then there will be spontaneous flow of water. But we do not know the rate of this transfer i.e., flux. Let us learn how to calculate flux.

Flux describes any effect that appears to pass or travel (whether it moves or not) through a surface or substance. With reference to the transport phenomena, flux is considered as a vector quantity (having both magnitude and direction) which describes the magnitude and direction of the flow of a substance. The diffusive flux can be related to the concentration gradient using **Fick's first law**, which postulates that the flux moves from regions of high concentration to regions of low concentration. The magnitude of flux is proportional to the concentration gradient. In other words, the solute moves from a region of high concentration to a region of low concentration across a concentration gradient.

The flux for water flow is denoted by J_w which is volume of water flow through unit surface area per unit time. Water in plants flows from cell to cell and, also through cell walls.

When water moves from cell to cell the flow is the function of water permeability of the membrane. The flux of water is given by

$$J_w = L_p \Delta \Psi_w \quad \dots (1.3)$$

$$L_p = \text{permeability coefficient of limiting membranes}$$

$$\Delta \Psi_w = \text{difference in water potential at two points}$$

From equation (1.1) we know that

$$\Psi_w = (\Psi_\pi + \Psi_p)$$

$$\Delta \Psi_w = (\Delta \Psi_\pi + \Delta \Psi_p)$$

substituting the value of $\Delta \Psi_w$ in above equation (1.3)

$$J_w = L_p (\Delta \Psi_\pi + \Delta \Psi_p) \quad \dots (1.4)$$

Thus, the inward or outward rate of flow or water from cell to cell and tissues can be calculated from the above expression.

The water flux is directly proportional to $\Delta \Psi$ and inversely proportional to hydraulic resistance (R). In other words, higher the $\Delta \Psi_w$, more will be flux, but high R will decrease the flux.

In plants water will move through the pathway which offers least resistance. Between the two routes – cell walls and cell to cell, the membranes of the cells exert more resistance (because of low permeability) than the cell walls.

Fick's 1st Law :
Diffusive flux =
diffusion coefficient;
concentration
gradient

$$J = D_s \Delta / \Delta x$$

$$J = \text{Flux rate} \\ (\text{moles}/\text{m}^2)$$

$$\Delta C_s / \Delta x \text{ is} \\ \text{concentration} \\ \text{gradient} \\ (\text{moles } \text{m}^{-3}/\text{m})$$

$$D_s = \text{diffusion} \\ \text{coefficient } (\text{m}^2 \text{s}^{-1})$$

Hydrostatic pressure is the pressure exerted by or on a liquid above or below atmospheric pressure.

Therefore, water can flow relatively easily through cell walls. Water will not experience the resistance of plasma membrane when it moves from cell to cell via plasmodesmata. The xylem conduits which are not obstructed by cell membranes have least resistance and the rate of flow of water is very high. The ratio of resistance in xylem, cell walls and cell membranes is in the order of 0.3:1: 50. This explains why xylem is the pathway for long distance transport as has been observed experimentally. The resistance in xylem varies inversely with the diameter of xylem elements. The smaller the diameter of xylem greater will be the resistance.

In soil, pressure potential is insignificant and osmotic potential is zero because there are no membranes (solute and water move together). Hence, the driving force $\Delta\psi_w$ in soil is determined by the matric pressure.

$$\Delta\psi_{w(\text{soil})} = -\Delta\psi_{m(\text{soil})}$$

SAQ 3

- a) List the three factors that determine the value of ψ_w in plant.
- b) The water potential in a cup (ψ_c) containing salt solution will be

$$\psi_c > \psi_w$$

$$\psi_c < \psi_w$$

$$\psi_c = \psi_w$$
- c) What will be the water potential of a plasmolyzed cell if its osmotic pressure is 7.9 MPa?
- d) Fill in the blank spaces in the following statements with appropriate words:
 - i) At full turgor ψ_w of a cell will be.....
 - ii) The net flow of water movement in system will stop when ψ_p will be equal and to ψ_π .
 - iii) Greater matric suction will the water potential in a system.

1.3.9 Gradients of Water Potential

The absorption of water by a plant involves the water relations of an individual cell, a group of cells and finally of the whole plant. Therefore, we will consider water relations at different levels of organization. We have already discussed the long-distance transport of water.

Movement of Water in a Single Cell

Isolated cells, single celled organisms and root hairs absorb water directly from their surrounding media. Let us consider an ideal parenchymatous cell. The vacuole occupies up to 90% of the cell and contains cell sap which is a dilute solution of salt and other small molecules. Due to osmotic potential the cell sap has a lower water potential than pure water. When such a cell is

placed in pure water a gradient develops due to the difference in water potential. This results in the movement of water inside the cell (Fig. 1.4). However, in no time the concentration of sap also decreases which lowers the osmotic potential of the sap. Thus, the difference between the potential of pure water and cell sap gets reduced. This lowers the force by which water enters the cell. We can represent the relationship with the following equation:

$$\Psi_w(\text{outside the cell}) = (\Psi_\pi + \Psi_p) \text{ inside the cell}$$

where, Ψ_w is total water potential of the system, Ψ_π is the osmotic or solute potential and Ψ_p is pressure potential due to cell wall pressure or turgor pressure. At full turgor pressure the sum of Ψ_p and Ψ_π is zero. Hence Ψ_w is zero.

The driving force F that causes water to move can be represented by the following equation:

$$F = \text{gradient } (\Psi_c - \Psi_e)$$

Where Ψ_c is the total water potential of the cell including that of the cell sap and Ψ_e is the total water potential of the external medium. If the latter is pure water, then its value will be zero. In that case the driving force will be equal to Ψ_c . However, as the water will move into the cell Ψ_c will be regulated by Ψ_π and Ψ_p . In this relationship the elasticity of the cell wall would also play an important role. The volume of the cell would increase upto a certain limit with the dilution of the cell sap and this will increase the total osmotic potential. This in turn would influence the force developed due to the gradient between the cell and the medium.

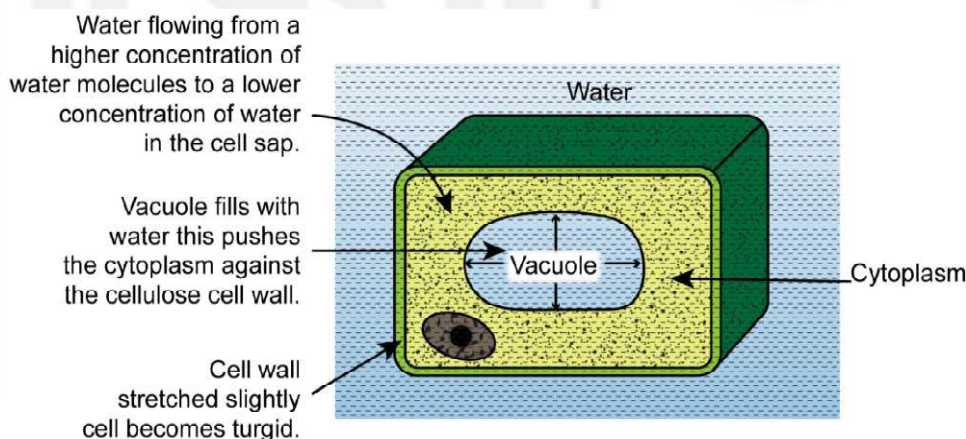


Fig. 1.4: A single cell surrounded by water (diagrammatic).

In the cells of root, leaf and other parts of plant, the external medium is the water in the cell walls and intercellular spaces (apoplast) which is under atmospheric pressure and has very low osmotic potential. But the matric forces exerted by the cell walls are higher, therefore, the water potential in apoplast is determined by matric forces exerted in cell wall.

It must be now clear to you that the osmotic status of the cell changes in response to gain or loss of water. The Fig.1.5 below (called Hofler diagram) illustrates the relationship between Ψ_w , Ψ_s , and Ψ_p (along with DPD, OP and WP) in response to gain or loss of water.

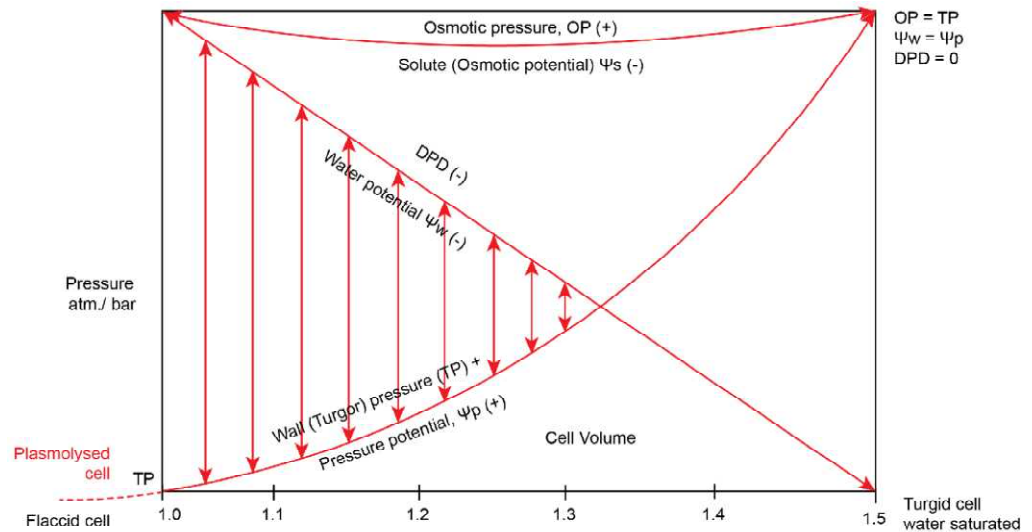


Fig.1.5: Hofler diagram depicting the relationship between Ψ_w , Ψ_s , and Ψ_p (along with DPD, OP and WP)

Placed in a hypotonic solution, endosmosis causes the expansion of the central vacuole, thereby swelling the protoplast and a rise in turgor pressure or pressure potential. At full turgidity turgor pressure (TP) rises to a level which is equal to osmotic pressure(OP). Currently Ψ_w or diffusion pressure deficit (DPD) becomes zero, and there is no further entry of water into the cell

	$DPD = OP - TP$	$\Psi_w = (-)\Psi_s - (+)\Psi_p$
	$\Psi_w = \Psi_s + \Psi_p$	
At full turgidity	$TP = OP$	$[\Psi_s = \Psi_p]$
	$DPD = 0$	$\Psi_w = 0$ (A)

Alternatively, when a turgid cell is immersed in a hypertonic solution, loss of water results. Due to water loss the solute potential of the cell sap becomes slightly more negative (slight increase in OP). The pressure potential or turgor pressure decreases and at full flaccidity, becomes zero. At this point the water potential shows maximal decline to equal to osmotic potential. (B)

In a flaccid cell

	$DPD = OP - TP$	$\Psi_w = (-)\Psi_s - (+)\Psi_p$
	$\Psi_w = \Psi_s + \Psi_p$	
Since	$TP = 0$	Since $\Psi_p = 0$
	$DPD = OP$	$\Psi_w = \Psi_s$

Water Relations of a Tissue

In higher plants no cell exists in isolation separate from others. Even a root hair which is projected outside into the surrounding medium is attached with other cells on all there maining sides. In a transverse section, it would appear to be surrounded on the three sides by other cells. Thus, the water relations of a root hair are governed on one side by the surrounding medium and on the other by other cells. Let us just consider two cells A and B joined with each other through a common cell wall. If these two cells individually have the same total water potential, then there will not be any net exchange of water between them. This can be shown as follows:

The total water potential of cell A (Ψ_{wA}) will be equal to the sum of its osmotic potential and pressure potential ($\Psi_{\pi A} + \Psi_{pA}$). Let us say that for the cells A and B, these values are

$$\Psi_{wA} = (\Psi_{\pi A} + \Psi_{pA})$$

$$\Psi_{wB} = (\Psi_{\pi B} + \Psi_{pB})$$

In a cell the matric forces are much less. Here the Ψ_w is lowered because of Ψ_{π} . If the Ψ_w in the vacuole is lower than Ψ_w in apoplast, then water flows inwards.

Now, the driving force (F) will be the difference in the water potential of two cells.

$$F = (\Psi_{wA} - \Psi_{wB})$$

If Ψ_{wA} is equal to Ψ_{wB} the gradient will be zero and so will be the driving force (F). Therefore, no net exchange of water will take place between the cells A and B. However, you must realize that the same value of Ψ_{wA} and Ψ_{wB} does not necessarily mean that the two cells should have the same osmotic potential and the same turgor pressure or wall pressure.

On the other hand, if the total water potential of cell B is lower than that of cell A, a driving force will develop which would cause influx of water into cell B till the two cells attain the same water potential. Now, this example can be extended to a larger number of cells which are connected with each other in tissue. If there are 20 cells beginning from 1 to 20, they will attain an equilibrium amongst themselves, depending on their total water potential in the same way as discussed for the two cells attached to each other. Under such conditions a situation may come when water absorption and movement will come to a standstill.

To illustrate this point by an example, if two adjacent cells A and B have the following values. Cell A has an osmotic potential (Ψ_s) of -9 bars and its pressure potential (Ψ_p) is equivalent to 6 bars, while the cell B has its corresponding values of -12 bars and 4 bars respectively, the flow of water will be :

$\Psi_w = \Psi_s + \Psi_p$		
A	$\longrightarrow$	B
$\Psi_w = -9 + 6$		$\Psi_w = -12 + 4$
$= -3 \text{ bars}$		$= -8 \text{ bars}$

Since water moves from less negative (higher) Ψ_w to more negative (lower) Ψ_w , movement of water will be from cell A to cell B.

Water Relations of a Whole Plant

Let us now consider the water relations of a plant considering leaves, stem, and roots, that provide a continuum for water in soil-plant-atmosphere system. The total water potential in the atmosphere could be very low, depending upon the temperature and humidity. Table 1.1 shows an approximate magnitude of water potential in the soil-plant-atmosphere-system.

Table.1.1: Water Potential.

Component	Water Potential (Bars)
Soil	-0.1 to -20.0
Leaf	-5.0 to -50.0
Atmosphere	-100 to -2000

It is clear from the data in Table 1.1 that the difference in total water potential in the soil-plant-atmosphere system would generate a driving force for water movement from the soil through the plant to atmosphere. If this continuum is broken, the driving force would automatically disappear.

1.4 SUMMARY

- Water is the key molecule for the maintenance of life on the earth. Plant has about 85 to 90% made up of water. The quantities of water used by plants are enormous.
- Water is a good solvent and reactant in the cell. For plants water is also crucial because the hydrostatic pressure of water provides turgidity to the cells, so that the soft stem parts can stand erect.
- Water moves up into the trees due to negative hydrostatic pressure created in xylem by the transpiring leaves. The continuity of water column in the xylem is maintained because of cohesive property of water.
- In plant tissue water molecules move through two routes – apoplast and symplast. Apoplast transport is through non-living portion of the plant and symplast is through cell to cell via plasmodesmata.
- Water status in a plant is expressed by water potential (Ψ_w). The rate of flow and the direction of movement depend upon the gradient of water potential between the two points. Water tends to move from a region of high-water potential to a region of low water potential.
- Water potential is affected by solute concentration, pressure and absorptive or matric forces. Addition of solute lowers the water potential while increased pressure on water causes a rise in water potential. Absorptive forces between solid-liquid interfaces lower the water potential. Water potential is computed as the algebraic sum of component forces: osmotic potential (due to solute), pressure potential (due to hydrostatic pressure) matric or suction potential (due to adhesive forces).
- In a cell both solute concentration and turgor pressure can have offsetting effect on water potential. The combined effect of the two in cells can build up the gradient and determine the rate of flow and direction of water movement.
- The gradient of water potential – the driving force for water movement in soil is due to differences in matric potential. This gradient also occurs along the cell walls.

1.5 TERMINAL QUESTIONS

1. Differentiate between:
 - a) Diffusion and Osmosis
 - b) Osmotic Potential and matric potential
 - c) Apoplastic and symplastic pathway
 - d) Imbibition and plasmolysis
2. Write brief notes on:
 - a) Aquaporins
 - b) Units of water potential
 - c) The concept of water potential and its components
 - d) Unique properties of water
3. What is water potential? Discuss its components and derive a relationship between them with the help of Hofler diagram.
4. Explain how the osmotic pressure vary in electrolytes and non-electrolytes.

1.6 ANSWERS

Self-Assessment Questions

1.
 - a) Heat of vaporization
 - b) Cohesion
 - c) 10 picoseconds
 - d) Dipoles
 - e) Germination
 - f) 0.239 calorie
2.
 - a) hypertonic
 - b) semi-permeable membrane;
 - c) maximum, Osmotic potential.
3.
 - a) Osmotic potential (Ψ_{π}), pressure potential (Ψ_p) and matric potential (Ψ_m).
 - b) $\Psi_p = \Psi_w$ the Ψ_c of salt solution in a cup will be zero because there are not membranes present. Therefore, its water potential is equal to pure water.
 - c) -7.9 MPa.

We know that $\pi = \Psi_{\pi}$. Therefore, the osmotic potential will be -7.9 MPa. Since $\Psi_p = 0$, therefore, $\Psi_w = \Psi_{\pi}$.

- d) i) zero
- ii) opposite
- iii) lower

Terminal Questions

1.
 - a) Refer to Section 1.3.
 - b) Refer to Subsection 1.3.2.
 - c) Refer to Section 1.4.
 - d) Refer to Subsection 1.3.2 and 1.3.8.
2.
 - a) Refer to Subsection 1.3.4.
 - b) Refer to Subsection 1.3.8.
 - c) Refer to Subsection 1.3.8.
 - d) Refer to Section 1.2.
3. Refer to Subsection 1.3.8 and 1.3.9.
4. Refer to Subsection 1.3.5.



UNIT 2

WATER TRANSPORT |

Structure

2.1	Introduction Objectives	2.7	Root Pressure and Guttation
2.2	The Pathway of Transport of Water	2.8	Physiological Processes Affected by Plant Water Status
2.3	Ascent of Sap		Soil Characteristics
2.4	Transpiration and its Significance Stomatal Number and Distribution		Water Absorption and Transpiration
2.5	Mechanism of Stomatal Opening	2.9	Summary
2.6	Factors Affecting Transpiration	2.10	Terminal Questions
		2.11	Answers

2.1 INTRODUCTION

At one time it was thought that man would be able to colonize the moon and other planets. However, even before man landed on moon his hope was shattered. The reason, there was not the atmosphere containing gases which sustain us on the earth nor was there any water, which is essential for life. Life originated in water of the oceans and remains a key molecule in maintaining life on earth.

The intimate relationship between water and plant is apparent from the greenery in areas where water is abundant and barren deserts in areas where it is in extreme deficit. Among the environmental factors – light, temperature, water, and soil, it is the water that significantly limits plant growth in virtually all environments.

You have studied the unique properties of water and its movement across the cells by diffusion and osmosis in Unit 1. You were also made familiar with the components of water potential that govern the movement of water into the cells. These concepts will help you to understand better the pathways of transport of water in plants described in the present unit.

The quantity of water absorbed by plant is enormous and is far greater than taken by animals of the same weight. This is because the loss of water by plants through transpiration is also colossal (approximately 97 percent). A major challenge before plant physiologists is to find ways to decrease water losses and increase the water use efficiency by the plants.

Plants can move water to great heights in trees. Even the tallest tree *Sequoia sempervirens* (113.1m, and still growing) found in California faces no problem in moving water to its top leaves. This was puzzling for the scientists. They tried to find out the forces that move water to such great heights.

We have the following questions before us regarding water relations of plants:

- i) What is the force that drives the flow and direction of water in plants?
- ii) What are the forces that drive water to great heights in trees?
- iii) What are the factors that control the opening and closure of stomatal aperture? and
- iv) Why do plants transpire so much water in an apparently wasteful way?

In this unit we will try to find answer to these questions.

Objectives

After studying this unit, you would be able to:

- ❖ draw and explain radial movement of water from soil to roots and long-distance transport from xylem to leaves;
- ❖ list the factors that affect water potential and explain their significance in the transport of water in soil-plant-atmosphere system, and explain how differences in water potential, affect the direction of water movement;
- ❖ calculate water flux and resistance using mathematical expression;
- ❖ discuss various resistances that impede water flow in plant and explain their significance to the plant, describe the factors that affect water absorption and water loss; and
- ❖ relate the structure and properties of stomata to their function, explain how changes in turgor bring about opening and closure of stomata, and list factors that control movement of stomatal aperture.

2.2 THE PATHWAY OF TRANSPORT OF WATER

Plants absorb water from soil by roots, which are the specialized organs for this purpose. Roots possess numerous unicellular root hairs present at the zone of maturation, where maximum absorption of water takes place Fig.2.1a, b). The radial movement of water is shown in Fig.2.2.

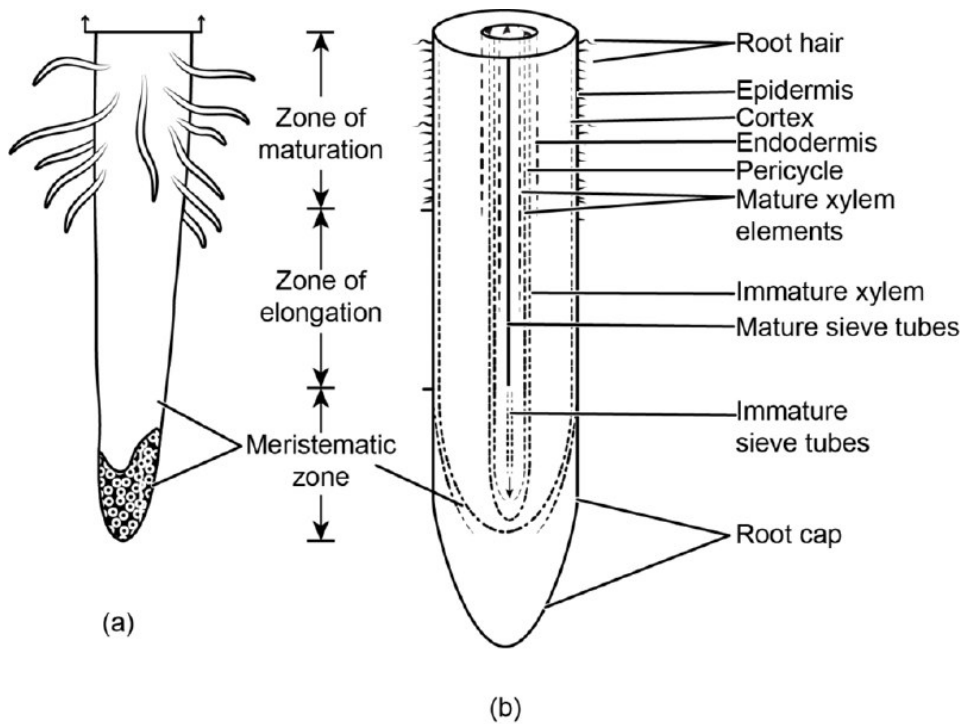


Fig. 2.1: a) General morphology of root tip showing root cap, meristematic zone, zone of elongation and zone of maturation with root hairs; b) location of various tissues including xylem and phloem.

Entry of water into the root hairs follow a particular sequence: adsorption of capillary water on the outer surface of the thin cellulosic cell wall of root hair; imbibition through the cell wall; endosmosis into the root hair protoplast via **aquaporins** located on the plasma membrane. Entry of water into the root hairs is on account of the existence of a water potential gradient. Root cells, with dissolved inorganic and organic substances have a much lower water potential than water outside. This facilitates the entry of water into the root hairs along the water potential gradient, i.e., from a higher to a lower water potential.

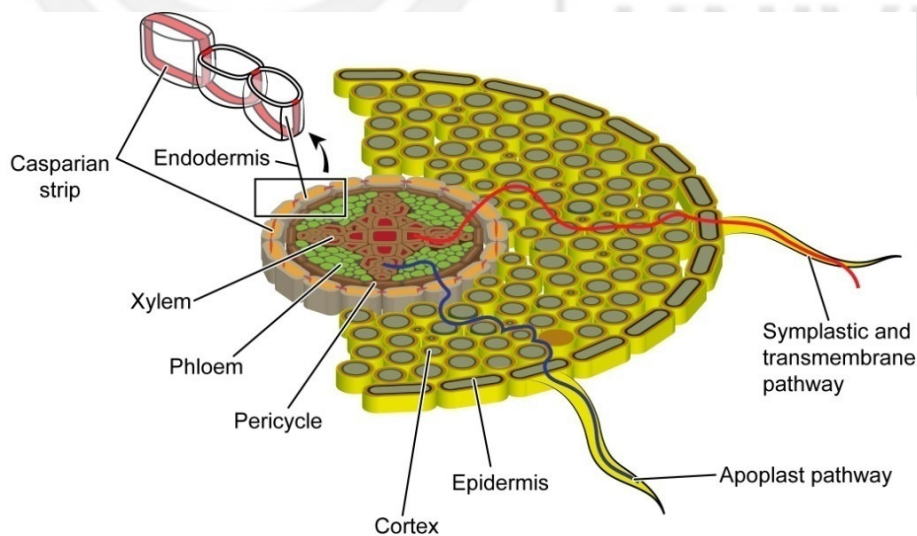


Fig. 2.2: The radial path of water (After Taiz *et al*).

At most stages of their journey water molecules have the option of moving either through the protoplast or through cell walls by following any of the three pathways at any time starting from the epidermis to the endodermis, i.e., the apoplast, symplast, and transmembrane pathway (Fig. 2.3).

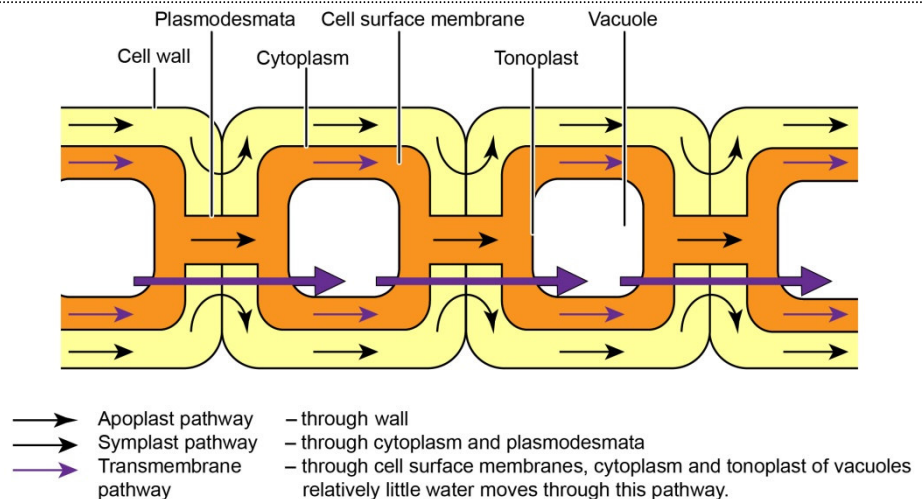


Fig. 2.3: Different pathways for diffusion of water.

1. Water may move through the cell walls, extracellular spaces, without crossing any membranes on its way across the root cortex. Later water passes through the intercellular spaces and non-living cells of the xylem. This is called the **apoplastic pathway**. Since the non-living portion of a cell is called the apoplast, it mostly involves the cell wall. The cell wall is composed of hydrophilic substances like cellulose, hemicellulose, pectin, lignin and hydrophilic proteins and carbohydrates, polymers of gum and mucilage.
2. Water may first enter the epidermal cells of the root by crossing the plasma membrane and then move along through the cytoplasm of the cortical cells, the endodermic, pericycle and finally into the xylem vessels and/or tracheids. This entire living portion of the plant interconnected by plasmodesmata – the protoplasmic bridges between the cells in called **symplasm** and the route is designated as **symplastic pathway**.
3. The transmembrane pathway is the third route by which water enters the cells, in series, i.e., first entering the cell on one side, going out from the other side: entering the next cell and so on. So, the plasmalemma of each cell must be crossed twice. This type of route may also involve the tonoplast (membrane covering the vacuole).

As mentioned above, water may not move along a set chosen path. Instead, its movement is directed by the gradients and the resistance. Water could switch on from apoplastic to symplastic and back in different regions till it reaches the endodermis. At this layer of cells, all further routes are obstructed except the symplastic one. In other words, the apoplastic movement of water is stopped because of the presence of a band of **Casparian strips** on the radial and transverse walls (not on the tangential walls) of the endodermis. The Casparian strips are impregnated by suberin, which is a hydrophobic, wax-like substance that is impermeable to water. Since the continuity of the apoplast pathway breaks here, water and solutes are forced to pass through the plasma membrane to cross the endodermis (by the symplastic route). At this point movement of water takes place mostly by water channels Aquaporins (Fig. 2.4).

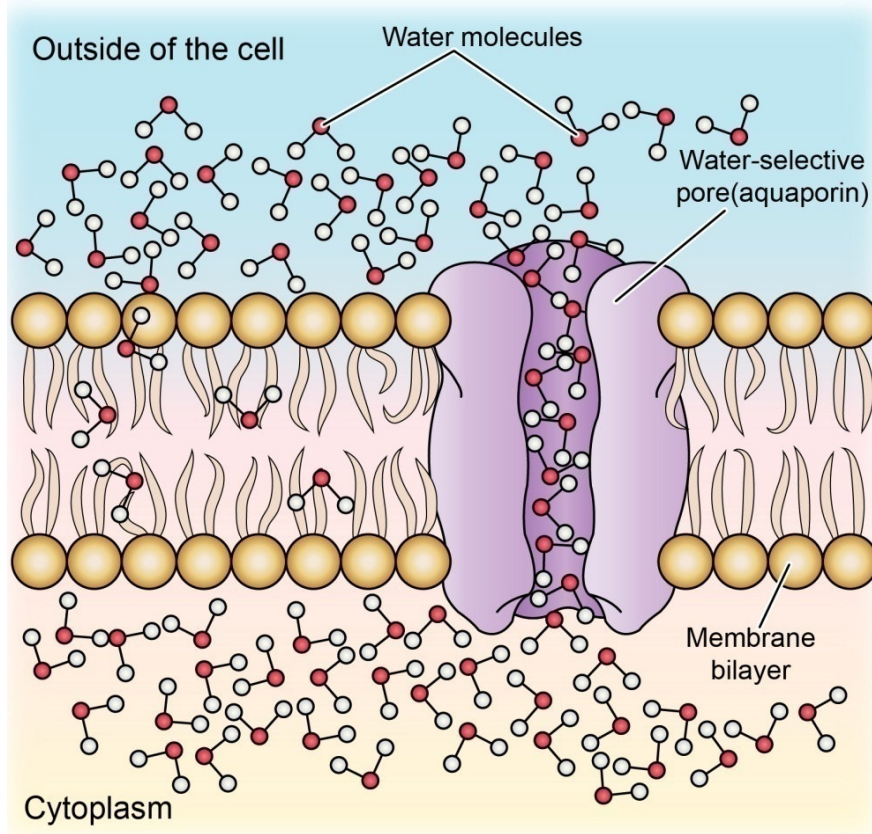


Fig. 2.4: Aquaporins for bulk flow of water (from Taiz *et al*).

Aquaporins are specialized water-selective channels present in the membranes and are composed of integral membrane proteins called as the family of major intrinsic proteins (MIPs). Aquaporins were discovered in 1991 by Agre (Nobel Prize in 2003). These channels facilitate a much faster diffusion movement of water into the plant cells (Fig. 2.5a). Aquaporins possess properties like specificity, hydrophobic interactions and size exclusion which ensure that only the water molecules, and not even protons, OH or other ions can enter these channels. A very high rate of water movement (approx. 109 molecules of H_2O per second/channel) has been recorded. However, conditions like intracellular pH and Ca^{2+} levels and state of phosphorylation regulate the permeability of such membranes by reversible “gating” (i.e., transition between open and closed state)(Fig. 2.5c). Aquaporins in plant membranes show highly conserved proteins and the water transport through these conduits can be regulated through gene expression via hormones. Such a regulation helps plants cells to modulate their permeability in response to altered environmental conditions like flooding or drought, soil salinity, nutrient deprivation, and chilling along with diurnal cycles. In addition, aquaporins have a distinct role to play in cell enlargement, opening and closing of stomates, epinastic and seismonastic movements, seed germination and anther dehiscence.

Interestingly, the aquaporins of plants, microbes and animals exhibit a highly conserved structure. A single aquaporin is a tetramer comprising four subunits, each with a molecular mass of 23 to 31 kDa. Each subunit, in turn, has six α -helices joined by 5 intervening loops spanning the membrane (Fig 2.5 b).

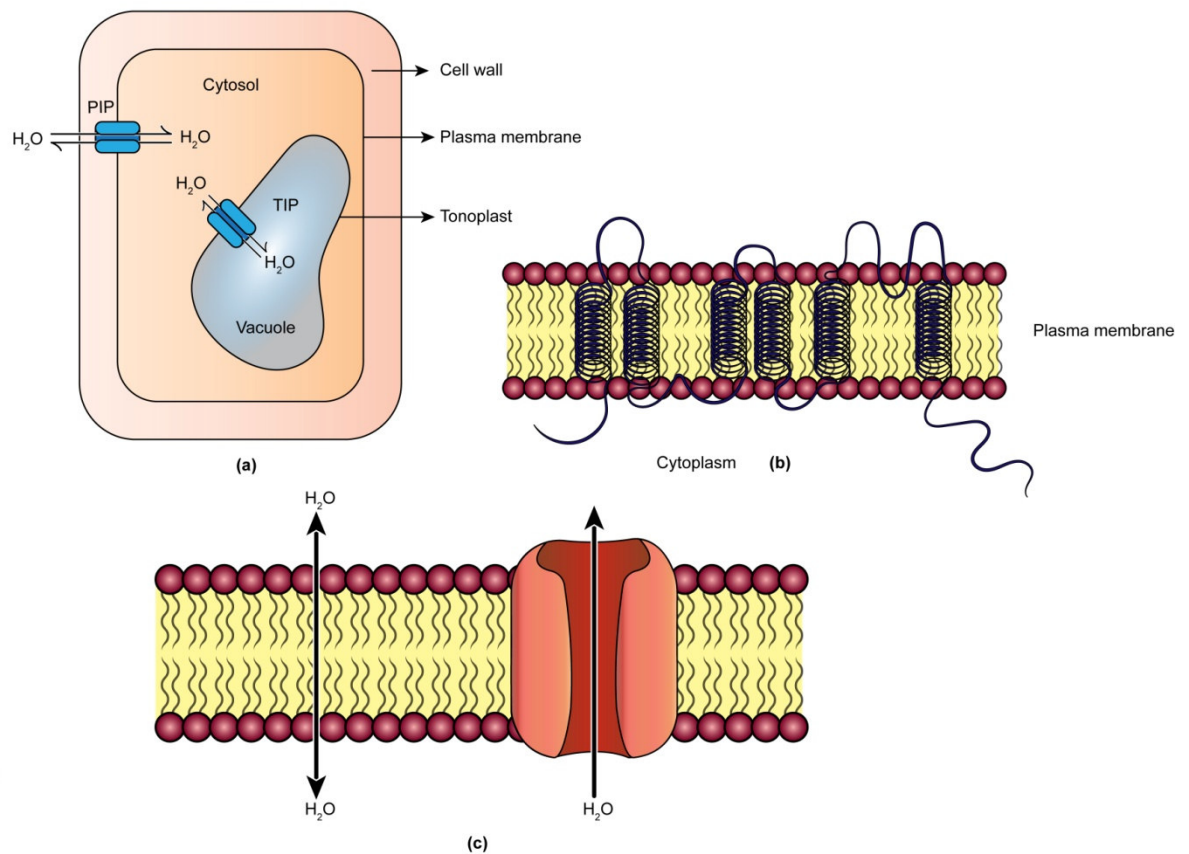


Fig. 2.5: a) Plant aquaporins present in the plasma membranes (PIPs) as well as in the tonoplast membrane (TIPs); b) Each of the four subunits of aquaporins span the membrane six times; c) Enhancement of water flow by gated aquaporins (After Hopkins and Hüner).

SAQ 1

Fill in the blanks.

- pathway traverses through intercellular spaces, cell walls and even lumens of non-living cells.
- Water is forced to adopt a symplastic or a transmembrane route at the endodermis by
- Movement across cell membranes are facilitated by

2.3 ASCENT OF SAP

It is a familiar fact that water runs downhill. But water absorbed by plant, along with dissolved minerals, which is called **sap**, is raised upwards from the soil by roots to great heights to top of tall trees (upto 110-120 meters) as in *Sequoia*, *Eucalyptus* and many conifers. Raising of sap up the plant is called **Ascent of Sap**. It is obvious that large quantities of water must be reaching the top against the force of gravity. Since substantial force is required to move water to such great heights, initial work focused on the participation of atmospheric pressure, capillary action, and root pressure as the driving forces for ascent of sap.

Let us now study this aspect with the help of an experiment. If a long tube closed at one end was to be filled with water and inverted in a beaker, the water column up to approx. 10.3 m could be supported at atmospheric pressure (Fig. 2.6) (nearly 101 kPa at sea level). Elevating water to the top of tall trees would require a force which is at least 10-15 times in magnitude i.e., 1.0 to 1.5 MPa. Moreover, forces of gravity, resistance offered by irregular wall surfaces, perforation plates of the column will add further to the resistance to finally require a force 2-3 MPa to make water reach to the top of the tallest trees.

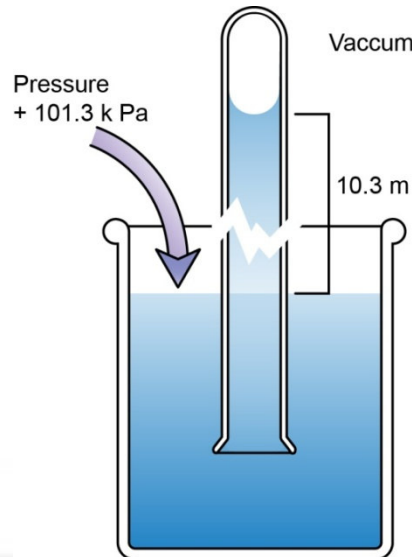


Fig. 2.6: Water column could rise to a maximum of 10.3 m on account of atmospheric pressure (After Hopkins and Hünér).

This was one of the puzzles of early plant physiologists. They tried to know what forces are involved that drive water upwards and how could they be generated.

They considered two possibilities: i) Water is either pushed up by the driving forces that might develop at the bottom (roots), or ii) it is pulled up by the force created at the top (leaf) of the plant. Moreover, it was to be established whether the forces of ascent of sap were being generated by living cells or that it was due to the physical forces themselves. Based on these premises, three types of theories were mooted a) **Vital theories**, b) **Root pressure theories**, and c) **Physical theories**.

The vital theories (given by Westemeier, Godlewski, Janse, Bose, Molisch and others) believed that ascent of sap was primarily on account of the activities of living cells lining the xylem.

Subsequent experiments by plant physiologists disproved the vital force as the basis of ascent of sap. Water moves through the dead xylem cells was first shown by Edward Strasburger in 1891, who sawed an oak tree at the base close to the ground. The cut end of the tree was placed in water containing picric acid or CuSO_4 (poisonous to living cells).

Though the chemicals killed the bark and other living cells, the movement of water was uninterrupted. The water is observed to move into the root system and upward through xylem. Now, we know that xylem vessels form an intricate plumbing network whose supply lines extend to all parts of the plant.

Root pressure (term given by Stephan Hales) was also considered by some earlier workers to be the main driving force behind ascent of sap. The hydrostatic pressure or force developed due to accumulation of water absorbed by roots which can push the sap upwards. It can be measured by a simple manometer. The average magnitude of root pressure is about 2 atm which is just not sufficient to account for ascent of sap in tall trees (which would require at least 20 atm for this purpose). Moreover, root pressure is rare in gymnosperms and is even negative in actively transpiring plants in summers.

Of course, the capillary rise is due to adhesion between water molecules and the polar groups along the glass capillary wall.

It was an Irish Botanist Josef Boehm (1809) who demonstrated by a simple experiment that if water were to be evaporated from a closed system like a porous pot connected to a set up as shown in Fig. 2.7, mercury could be lifted to a height of only 100 cm. The theory was discredited because water could reach only till a certain height. Moreover, narrower the bore, greater will be the rise of water column. If this be true, then all tall trees must have vessels with narrowest bore, (say $0.15\ \mu\text{m}$ dia) which is not true. Also, the gymnosperms do not have vessels. Instead tracheids with several porous septa are present. Another prerequisite for capillarity is the presence of a free surface. But in nature, vessels are not in direct contact with soil.

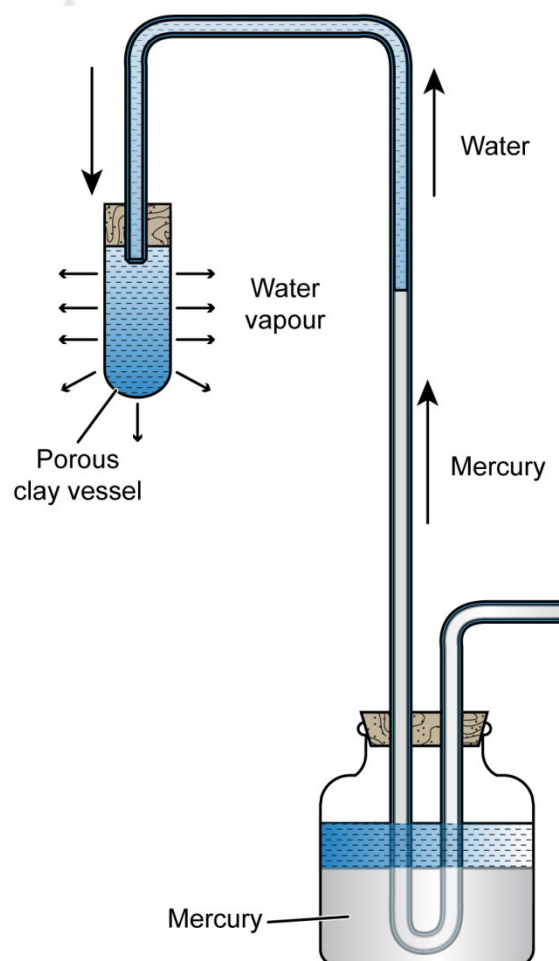


Fig. 2.7: Demonstration of the principle of water movement in the plant stem by a physical model. Evaporation of water from the porous clay pot exerts more pulling force than simple suction. It can pull mercury behind it to a height of more than 100cm.

As mentioned in the earlier discussion above, the water column supported by atmospheric pressure (1.0 atm) can only go upto its barometric height, i.e., 10.3 m/34 ft. This theory also failed to explain ascent of sap in tall trees.

The earliest of the physical theories was propounded by Sachs in 1878, who believed that water is imbibed through the cell wall materials and then translocated upwards. So, the wall was the important route. However, we now know for sure that the translocation of sap is through the lumen of vessels.

An Irish Botanist, Henry H. Dixon and his collaborator, a physicist, J. Jolly repeated the experiments of Boehm using a transpiring leaf instead of porous pot and got the same results. A similar experiment shown in Fig. 2.8 depicts the rise of water in plants. Dixon and Jolly proposed a theory in 1894 which Dixon published in a book form with many experimental details in 1914. Their work is now known as the famous **Cohesion Theory/ Transpiration Pull and Cohesion Tension Theory of Ascent of Sap**.

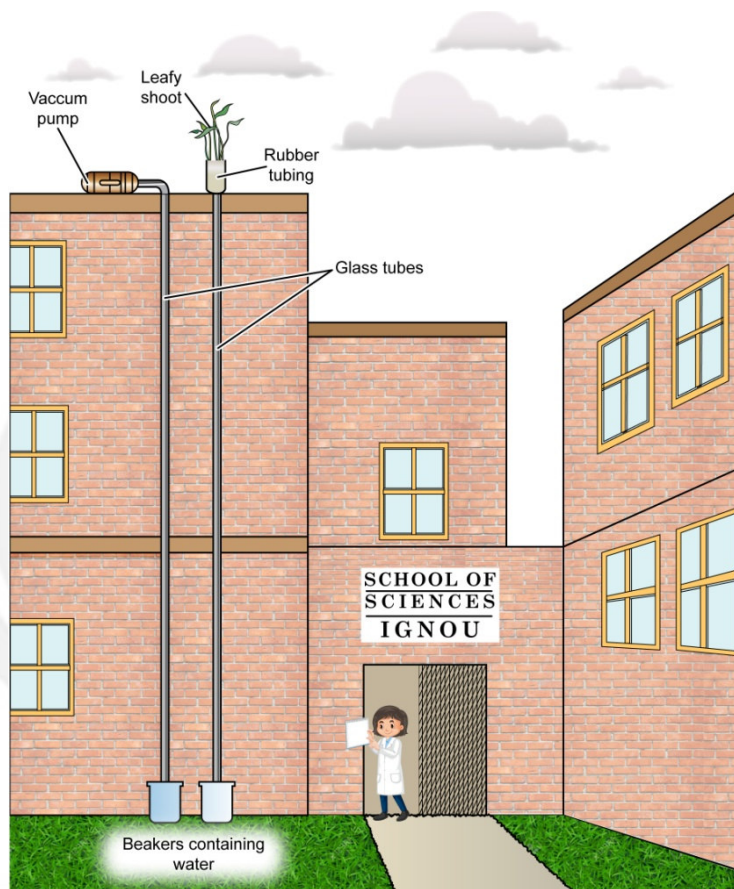


Fig. 2.8: Experiment to show the cohesion-tension theory of water movement in the plant. Two long slender tubes dipped in water from below are extended up to the height of the building. A vacuum pump fails to suction the water to this height while a tiny leafy shoot succeeds. A short piece of rubber tubing is used to connect the cut tip of a leafy shoot to a slender glass tubing (14m length and 0.5 mm in diameter) which is dipped in a beaker containing water and dye. The entire system is made continuous water filled air proof system.

Transpiration Pull and Cohesion Tension Theory

The theory is a combination/ collective result of three important parameters/processes viz., i) Continuous water column in the xylem channels, beginning from the water absorbing zones of roots till portions of leaves from where water is transpired, ii) Unique cohesive force between adjacent molecules of water and adhesion between water molecules and the cells walls

An actively transpiring tree creates a pressure gradient ranging from 0.01 to 0.05 MPa m⁻¹ (0.0987-0.493 atm m⁻¹). In case of transpiring trees well supplied with water the driving force is 0.015 MPa m⁻¹ (0.148 atm m⁻¹).

which gives a very high tensile strength to water (45-207 atm), and iii) Generation of transpiration pull or tension due to transpiration which creates negative pressure between one end of the system and the other resulting in the bulk flow of water through the xylem.

It is the evaporation of water from the leaf which creates a negative pressure or tension that constitutes the driving force for water movement. As water evaporates from the leaf mesophyll cells, it results in a decreased (more negative) water potential (Ψ_w) in these cells which are directly exposed to the air spaces of the leaf.

As a result, water from adjacent cells enters the mesophyll cells. This process continues in the mesophyll where alternately the cells must draw water from the leaf veins. As a result, xylem comes in a state of negative pressure or tension and this is alternately transmitted to the root system through the **unbroken column** (continuous) of water. Thus, the driving force is the gradient in decreasing water potentials (differences in $\Delta\Psi_w$) which develop from the soil through the plant to the gas liquid interface of stomates.

Water possesses exceptionally high tensile strength, which is a measure of the maximum tension a material can withstand before it breaks. This is largely due to the strong intermolecular cohesive force, on hydrogen bonding. You have read about this property in Unit 1.

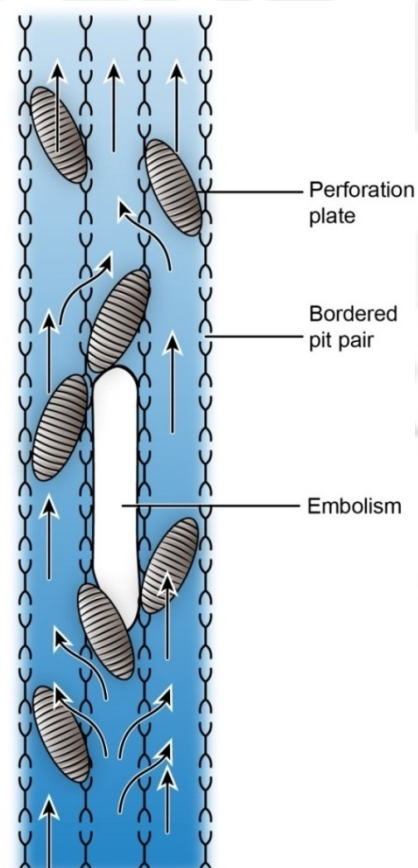


Fig. 2.9: Diagrammatic representation of the bypassing of embolized xylem element.

Pure water is known to withstand tension of -25 to -30 MPa at 20°C. This figure is nearly ten times more than the tension required to pull sap in an uninterrupted continuous column to the top of the tallest trees (-2.5 to 3.0 MPa). Air also tends to be pulled through microscopic pores in the xylem vessel

walls due to increase in tension. This phenomenon is called “**air seeding**”. Many a times the dissolved gases in the water column come out of the solution, coalesce, and form bubbles in the xylem. This process is called **cavitation**. If the bubble is large, it may even cause obstruction to the column by breaking the continuity. This is called **embolism** (Fig.2.9). Moreover, there are thousands of parallel, interconnected capillaries of xylem to circumvent this bubble and to maintain continuity.

High cohesive force of water helps to resist being pulled apart. If water is indeed moving through an upward pull, it would be expected to recede in the xylem, if its continuum is broken by cutting a branch above the ground level. The state of water in xylem is analogous to a stretched rubber band. Suppose if we cut a stretched rubber band at some point, it would immediately recede in both directions from the point where it is cut. A similar receding occurs in xylem vessels and water pulls away from the cut point. The pulling force of water column or tension in the xylem can be measured by a **Scholander pressure bomb** (Fig. 2.10) by forcing the xylem sap back to the original point of applying pressure.



Fig. 2.10: Demonstration of tension in xylem sap. When a twig is cut the tension in xylem pulls the sap away from the cut point. The pressure applied in Scholander Pressure Bomb force xylem sap back to the cut point.

Highly hydrophilic cell wall polysaccharides make the xylem wall surface to bind with water. This matrix is formed due to attraction between the slightly positive side of the polar water molecules and the net negative charge of the wall surface. Water is held by hydrophilic wall surface with tensions to the order of -100 MPa to -300 MPa. Thus, even the gravity exerted due to the sheer weight of the water column cannot tear away the water molecules from the cell wall.

It is important to point out here that the dead cells of the xylem are greatly strengthened with cellulose fibrils encrusted with lignin. Such strengthening of walls can be compared with reinforced concrete and thus the xylem cells are strong enough not to collapse when water is pulled through them.

Studies on the movement of water at various times of the day also suggest that the “motor” for ascent of sap lies in the leaves of the tree. The velocity of sap flow in the wood was also measured by inserting a heating element into the xylem. After a time interval, the distance covered by the heated xylem sap was measured by a thermocouple. The velocity as high as 75-100 cm/h was recorded (Fig. 2.11).

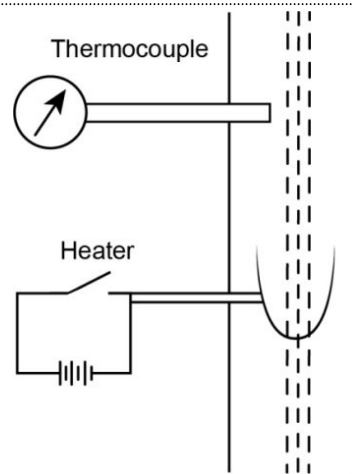


Fig. 2.11: Measurement of velocity of xylem sap. A small heating element can be inserted at a point in the trunk up to the xylem. After a time interval the upward movement of the sap can be detected by a thermocouple.

Although, the cohesion theory of water was proposed about a century ago, yet it holds good even today. Fig. 2.12 represents the Cohesion Transpiration pull Theory.

However, it explains only the long-distance transport of water through dead xylem tissue.

The movement of water from the soil through the plant into the atmosphere is in the form of a continuum involving different mechanisms of water transport. The pressure gradient ($\Delta\psi$) is responsible for the movement of liquid water in the soil and xylem.

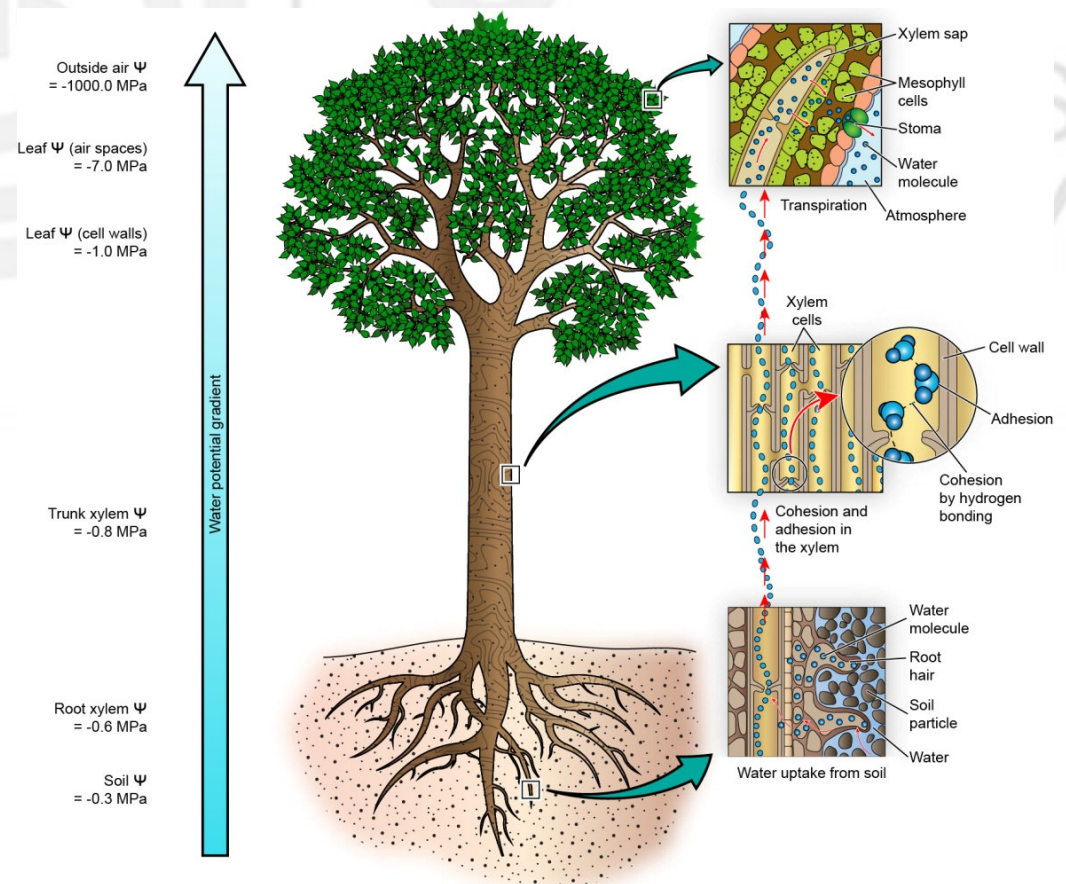


Fig. 2.12: Diagrammatic representation of cohesion-tension theory.

Water potential is the driving force for absorption and transportation of water by cells from soil to the xylem. Water is finally lost to the outside in a vapour form by diffusion. There is always a competition between the water held by capillary force in soil on one hand and negative pressures within the xylem due to transpiration on the other. Both these forces are directed in opposite directions. However, it is, the physical forces generated by sun-energized transpiration which ultimately wins this 'tug-of war' to transport water through this **Soil Plant Atmosphere-Continuum** (see Fig. 2.13).

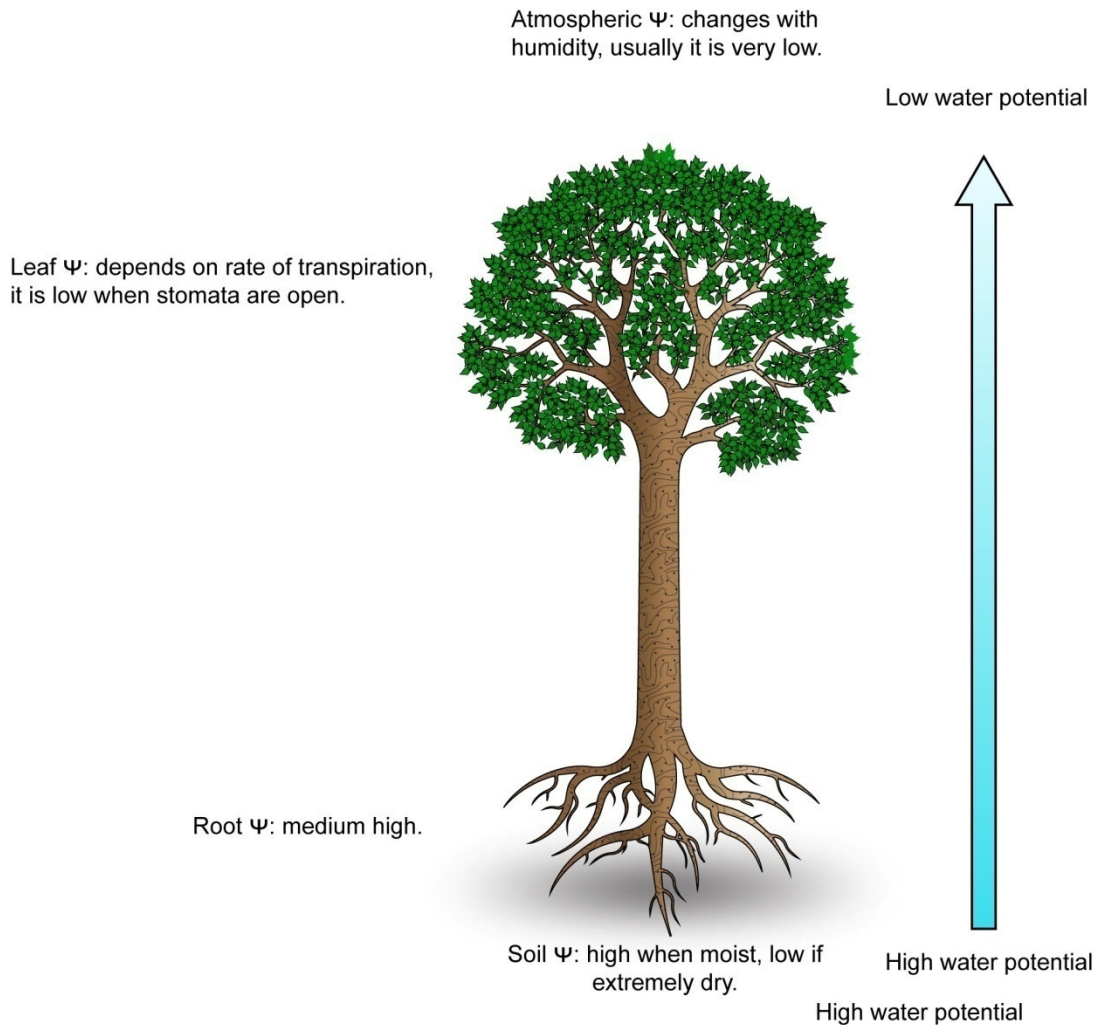


Fig. 2.13: Water transport in Soil-Plant-Atmosphere Continuum (SPAC).

SAQ 2

- a) Which of the following statements are true or false?
- The amount of unavailable water retained by a unit weight of soil under controlled conditions is called Field capacity.
 - The water uptake by the roots decreases due to low temperature and low O_2 tension.
 - As the root hair absorbs water from the soil, its DPD decreases.
 - The water potential is less negative in the xylem than in the soil water.

- b) Fill in the blanks with appropriate words/terms:
- i) are filamentous outgrowths which greatly increase the surface area of roots.
 - ii) The three pathways, viz., ... and are possible through which water can flow from the root epidermis to the root endodermis.
 - iii) The most acceptable theory for the ascent of sap among other features considers the and properties of water.
 - iv) The vital theories of ascent of sap were disproved by who used picric acid to kill the living cells.
 - v) Formation of bubbles in the xylem column or may sometimes break continuity of the water column, a phenomenon called
 - vi) present in endodermis force water to take a symplast or transmembrane route.

2.4 TRANSPIRATION AND ITS SIGNIFICANCE

Water may usually become a limiting factor for plants as they use this resource in large amounts, much more than animals do. It has been estimated that more than 97% of the water absorbed by roots of a plant gets transported through the plant and finally is lost as evaporate through the leaves. Only a small fraction, say about 2% is consumed in photosynthesis, and the remaining 1% in rest of the metabolic processes vital for life. This loss or evaporation of water in a vapour form, from the exposed aerial parts of a plant is called **Transpiration** (Box 2.1), which literally means Latin : *trans*– across, *spirare* – to breathe).

Box 2.1: Water lost through transpiration

If we consider the amount of water lost by transpiration, many plants lose huge quantities of water. For example, an Egyptian cotton plant loses nearly 1 litre of water every day through transpiration which would mean that an acre of such plants would transpire equivalent to 1cm of rain per acre per day. A sunflower plant would lose 2 Kg of water per day and this figure would be nearly 75 Kg per day in Beech tree. A maize plant (which is a C₄ plant and grows under relatively dry conditions) loses 200 liters of water during its lifetime. This is roughly 100 times more than its body weight. Also, trees of Birch, Elm and Silver Maple and Neem may lose very large amounts of water in transpiration.

Magnitude of Transpiration

Since most of the water absorbed by the roots is lost to the atmosphere, and only a small fraction is utilized for growth and accumulation of dry matter, a quantitative expression, **Transpiration Ratio (TR)** is used to explain the efficiency of transpiration. It is also called as *transpiration coefficient*.

Transpiration ratio is the ratio of the amount of water lost by the plant and the amount of dry matter accumulated (i.e., amount of CO₂ gained through stomata/amount of CO₂ assimilated by photosynthesis).

$$\text{Transpiration ratio} = \frac{\text{Moles of H}_2\text{O lost}}{\text{Moles of CO}_2 \text{ gained}}$$

C₃ plants lose much larger amount of water (over 400 molecules for every CO₂ molecule) to accumulate a single unit of dry matter, as compared to the C₄ plants which are more water economical or efficient. For example, Alfalfa (C₃) may have a high TR of 900 whereas a millet (C₄) will have a value around 250, lower TR values. In CAM plants the value of transpiration ratio is the lowest, i.e., nearly 50.

Even lower TR values are seen in CAM plants as their stomata open during night and close during day. And transpiration is much less in night, so the values of TR are around 50.

Although transpiration can take place from any exposed part of a plant, it is essentially more efficient from the open surfaces of the aerial parts of a plant. This is because evaporation of water from open surface meets less resistance while evaporation of water from leaves faces considerable resistance. Most of the transpiration (50-97%) occurs through leaves (**foliar transpiration**). Leaves have many microscopic pores called stomata which function as pathways of diffusion of water vapours from the moist interiors to the comparatively dry outside atmosphere. So, the intercellular spaces in the mesophyll cells come in direct contact with the atmosphere through these specialized pores. About 5% of the water is lost from the leaf through the cuticle. This is called **cuticular transpiration**. The amount of cuticular transpiration varies in plants depending on the thickness of the cuticle. In woody plants and some fruits there are lenticels opening within the bark that function in gas exchange. You have studied about lenticels in BBYCT-133, Unit 6. The water loss through these cells is called **lenticular transpiration**. The total water loss through lenticels is only a small fraction (0.1%) of the total transpiration. Sometimes, the suberized cork present in bark can also lose water by a slow process of imbibition. The water loss in some cases may be as much as the lenticular transpiration.

You must be thinking as to whether transpiration is really significant? Transpiration has many distinct advantages and importance in several vital functions and processes in a plant. Let us summarize the positive and the negative aspects of transpiration.

Main advantages of transpiration are:

- It exerts transpiration pull on water channels thus bringing about the ascent of sap.
- Removal of excess water absorbed by the plant and maintenance of optimum turgor.
- Passive water absorption.
- Cooling effect on the plant.
- Translocation of minerals.
- Extensive root development.
- Formation of assimilation products like latex, alkaloids, resins, and pigments.

Some of the disadvantages of excessive transpiration are:

- Reduction in growth due to less availability of water to the young and meristematic cells.
- Wilting of leaves and reduction in photosynthesis.
- Water stress may lead to breakdown of organic compounds.
- Desiccation, shedding of leaves and synthesis of ABA.
- Fall in metabolic rate and reduced yield.

Curtis, (1926) rightly called transpiration as a “necessary evil” or “an unavoidable evil” (Steward 1959).

2.4.1 Stomatal Number and Distribution

The cross-section of a leaf shown in Fig. 2.14 a shows the position of a typical stoma (plural stomata) which however, differs from species to species, with respect to the size of the pore, structure and size of the guard cells and depth and size of the stomatal cavity. You have studied about the stomatal structure in detail in Unit 5, BBYCT-133. Recall the structures by going through these units once again. As indicated in the diagram 2.14 b, water evaporates from wet mesophyll cell walls that border intercellular spaces, the vapours then diffuse out through sub-stomatal cavity and stomatal pores to the air outside the leaf.

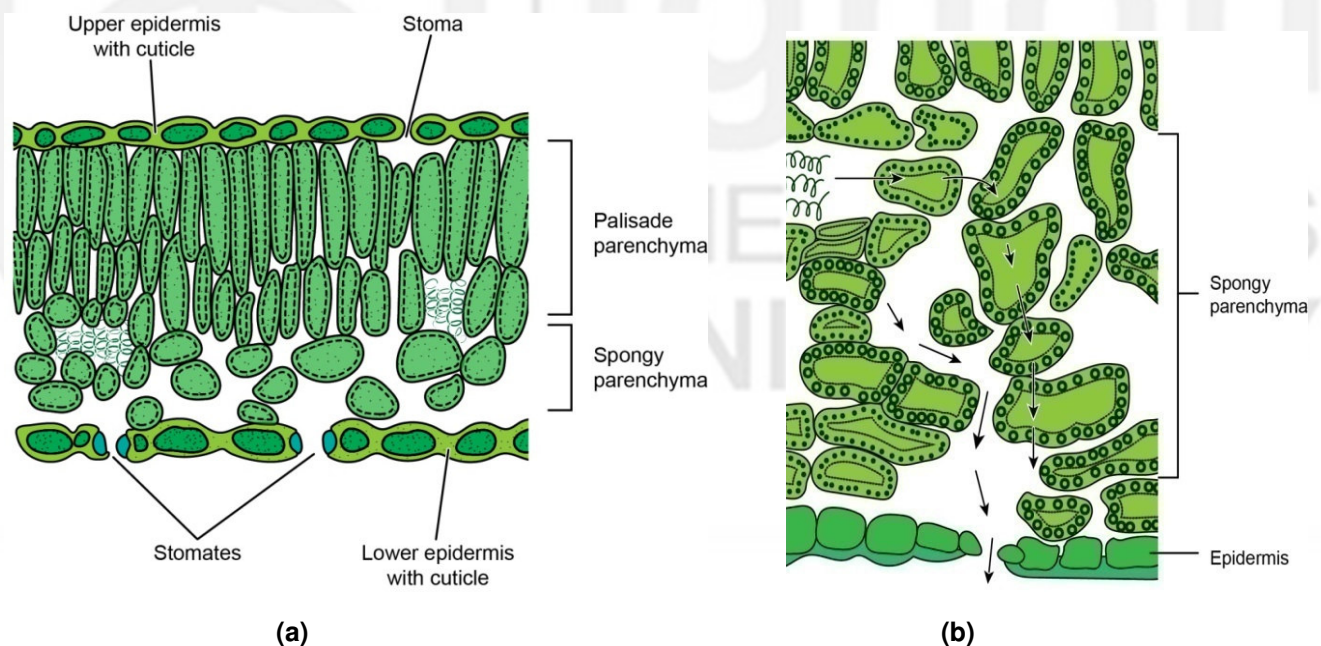


Fig. 2.14: a) Cross-section of a leaf showing the relationship of stoma with other leaf tissues; b) A stoma enlarged to show the path of water vapours from leaf to atmosphere.

The water potential gradient develops in the sub-stomatal cavity, stomatal pore, boundary layer and the atmosphere. During transpiration, the sub-stomatal cavity has relatively much higher water potential as compared to the atmosphere; therefore, the water vapours move out. This in turn lowers the water potential of the sub-stomatal cavity. Consequently, the cells surrounding the sub-stomatal cavity evaporate water through their cell walls. Depending upon the water potential of the environment, the water potential of the sub-

stomatal cavity and the surrounding cells is lowered. This gradient eventually acts as a 'pull' on the water column which maintains continuity through the vascular bundles of the leaf. The intercellular spaces also play an important role in this respect because they are in continuity with the sub-stomatal cavity and cause a gradient quickly.

When water evaporates through leaves it experiences considerable amount of resistance (Fig. 2.15) which can be grouped into the following two categories: a) leaf resistance (internal resistance), and b) air boundary resistance (external resistance).

The components of leaf resistance are cuticle, mesophyll cells, intercellular spaces of the leaf and stomata. The cuticular resistance is maximum followed by stomatal resistance and air boundary layer resistance. The pathway of movement of water vapours is analogous to the electric current moving in circuit, the greater the resistance, the smaller the flow of water and vice versa.

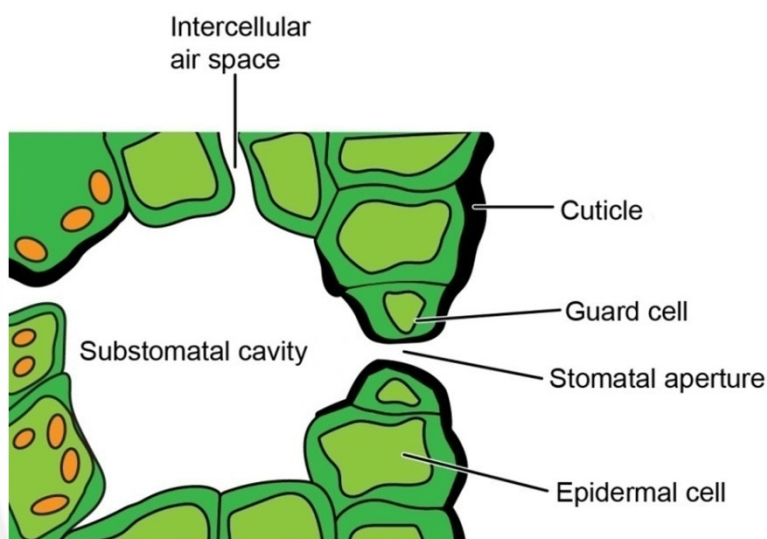


Fig. 2.15: Schematic cross-section of a leaf showing different resistance.

The cuticle forms outermost surface of the leaf and resists the evaporation of water vapour and entry of carbon dioxide necessary for photosynthesis.

Stomata perform the following main functions.

- i) They allow entry of CO_2 necessary for photosynthesis.
- ii) They control water loss through transpiration and thus protect the plant from desiccation.
- iii) At higher temperature (above 35°C) they allow higher transpiration which cools the plant.

Stomatal resistance is most important because gas exchange between leaves and external atmosphere takes place entirely through stomatal pores.

Stomatal resistance depends mainly on the size and shape of the stomatal cavity and size of stomatal aperture. Can you tell which pore size, small or large would exert greater resistance? Of course, smaller the pore greater would be the resistance for the outward movement of water vapour. On an average a standard pore measures about $20\text{ }\mu\text{m}$ in length and about $11\text{ }\mu\text{m}$ wide at its widest point when fully open.

Water loss from the leaf also depends on the number of stomata per leaf. One of the aims of agricultural scientists is to find ways of minimizing the water loss from the plants without significantly reducing carbon intake, to increase the efficiency of water use. One way is through a study of the number of stomata per leaf. Some investigations have been carried out keeping the following questions in mind:

- i) How the stomata are distributed and how is it related to water loss and carbon dioxide intake?
- ii) How can water loss be reduced without seriously impairing carbon dioxide intake?
- iii) Is it possible to evolve varieties which possess characteristics associated with low evaporation of water?

Stomata are classified based on:

- a) Distribution
- b) Periodicity of opening and closing (time of opening and closing)

Distribution: The distribution of stomata varies on both the surfaces of a leaf. You have already studied in the Unit 5 of BBYCT-133 on that leaves can have stomata either on both surfaces (**amphistomatic**), only on the upper surface (**epistomatic**), only on the lower surface (**hypostomatic**), or may have no/or few stomates (**astomatic**).

Five types of plants have been classified based on the distribution of stomates on the two surfaces of leaves:

- i) *Apple and Mulberry type* : Leaves **hypostomatic** e.g., apple, *Morus* (mulberry), *Ficus benghalensis* (Pipal), *Oxalis*, peach, *Nasturtium*, walnut, oak.
- ii) *Potato type* : Leaves **amphistomatic** but more stomata on the lower surface. e.g., Potato, tomato, sunflower, brinjal, cabbage, pea.
- iii) *Oat type* : Leaves amphistomatic with nearly equally distributed stomata on both the surfaces of the leaf. E.g., Oat and other monocots, *Verbena* and *Kochia*.
- iv) *Nymphaea or Water Lily type* : Leaves are **epistomatic**; occur mostly in aquatic plants with floating leaves, e.g., *Nelumbo* (Lotus), and *Nymphaea* (water lily), *Victoria regia* (giant water lily).
- v) *Potamogeton type* : Leaves are either **astomatic** or with rudimentary/functionless stomata. Occur in submerged aquatic plants, e.g., *Hydrilla*, *Potamogeton*, *Ceratophyllum*.

There are two parameters normally used for expressing the distribution of stomata. Stomatal frequency and stomatal index.

Stomatal frequency: The number of stomata per unit area. Plants differ in their stomatal frequency (1000-60,000/ cm²). In monocots the lower surfaces usually have the same frequency but in dicots the lower surface usually has

higher frequency than the upper surface. However, the frequency of stomata can change with the position of leaves on the plant. Even within the same leaf, stomatal frequency may increase from the midrib to the margins and from leaf base to the apex.

Stomatal Index: There exists a correlation between the number of stomata and the total number of cells (epidermal cells including stomata). This is referred to as stomatal index (SI). It is defined as the percentage number of stomata as compared to all the cells (epidermal + stomata) in a unit area of leaf.

$$SI = \frac{S}{E + S} \times 100$$

SI = Stomatal Index, S = Number of stomates per unit area, E = Number of epidermal cells per unit area.

The stomatal index is independent of the environmental conditions, age of the plant, or the position of the leaf. Since stomatal index is a constant parameter for a species, it is of great taxonomic importance.

Periodicity of Opening and Closing: Depending upon the stomatal behaviour (periodicity of opening and closure), plants here been divided into three broad categories by Lottfield (1921).

- i) **Barley or Cereal Type:** These types of stomata are generally presents in monocots. Their stomata remain closed throughout the night and open during the day only for a few hours.
- ii) **Alfalfa or Lucerne Type:** Plants of this group function as neophytes under drier conditions e.g., *Brassica*, *Alfalfa*, pea, beet, radish
- iii) **Potato Type:** When water is available in sufficient quantities, the stomata of the plants of this group remain open throughout the day and night. However, the stomata may close for a few hours during daytime when transpiration increases e.g., *Potato*, *Cucurbita*, *Allium*, *Portulaca*.

SAQ 3

- a) Fill in the blank spaces in the following statements with appropriate words/terms:
 - i) is the ratio between the amount of water lost by the plant and the amount of dry matter accumulated/ CO₂ gained.
 - ii) Most of the transpiration occurs through in leaves.
 - iii) Among leaves, the offers resistance to flow of water.
 - iv) leaves show stomata on both their surfaces.
 - v) The stomatal is constant for a species and is good taxonomic character.

b) Match the contents of column A with those of Column B.

Column A	Column B
i) Water lily type stomata	1. Pore size
ii) Periodicity of stomatal opening	2. Bark
iii) Leaf resistance	3. Epistomatic
iv) Low transpiration ratio	4. <i>Potamogeton</i>
v) Lenticels	5. CAM plants
vi) Astomatic	6. Loftfield

2.5 MECHANISM OF STOMATAL OPENING

It is important to know that stomata open because of reversible turgor changes in the guard cells. Stomata open when turgor in the guard cells is high and close when it is low. The turgor would increase only if the solute content of guard cells would be higher than the neighboring epidermal cells. Falling of solute content would decrease turgor. Sucrose is the osmotically active solute in guard cells. How do the two guard cells on swelling form an aperture? You have already studied in Unit 5 of BBYCT-133 that each stomatal opening is surrounded by two kidney-shaped or reniform guard cells in dicots. In monocots, the two guard cells are dumb bell shaped. The cell wall of guard cells is peculiar because the cellulose microfibrils in the guard cells of dicots are radially arranged, i.e., from the center towards the periphery. In monocots, the cellulose microfibrils are arranged in the bulbous portions/parts of the guard cells (Fig. 2.16 a, b).

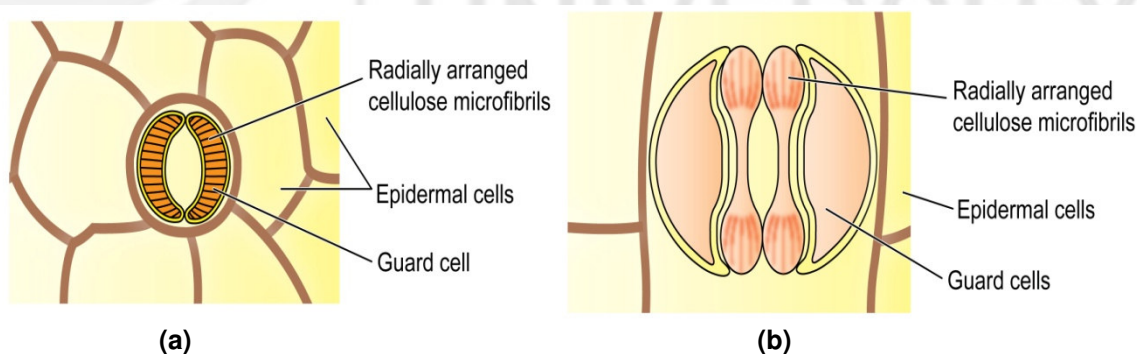


Fig. 2.16: Alignment of cellulosic microfibrils in the guard cells in a) dicot; and b) monocot stomata.

The microfibrillar arrangement in the kidney shaped guard cells of the dicots restricts the expansion of cell wall in the transverse direction. Since the inner wall (towards the pore) is thickened and less elastic than the outer wall, absorption of water from the surrounding cells makes the guard cells turgid and their thinner and weaker sides bulge outwards. As a result of this uneven longitudinal expansion cells are arched away from each other. The inner walls are pulled away from each other forming a pore in the center (Fig.2.17a).

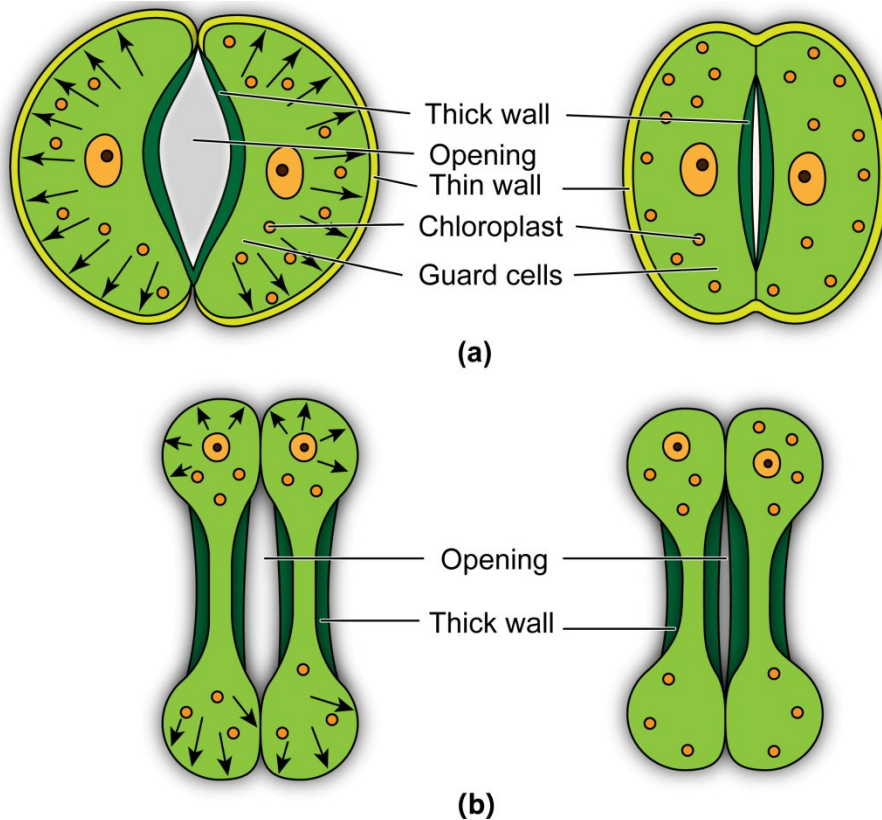


Fig. 2.17: a) Open and closed stomata in dicots; b) Open and closed stomata in monocots.

In monocots, high turgor pressure causes swelling of bulbous inflatable ends of guard cells, the two ends are pulled in opposite directions leading to the widening of the slit between them (Fig. 2.17 b).

The changes in the turgor pressure and size of the guard cells are facilitated by two-way solute transfer activity of subsidiary cells.

Before we discuss the control mechanism of stomatal movements, it is important to know that:

- i) Normally, stomata are open in the day and are closed at night (**photoactive**). However, a drop in supply of water leads to day time closure of stomata. Many succulents and even some non-succulents show **scotoactive** stomatal movements (opening in dark and closure during day) and involve organic acids.
- ii) Stomata open when the internal concentration of CO_2 drops and close when the internal concentration of CO_2 is maximum.
- iii) Dark CO_2 fixation (CAM plants, refer to Unit 7) occurs in the guard cells.
- iv) In guard cells there is a change of pH in light (day) and dark (night) which is associated with the interconversion of starch and sugar.
- v) Opening and closing of stomata are related to the osmotic potential of the guard cells and the permeability of membranes.
- vi) At the time of opening of stomata, there is an inflow of K^+ and Cl^- into the guard cells but when the stomata close, the guard cells lose these acquired ions to the surrounding cells.

- vii) Blue light brings about changes in stomatal movement by activating the H^+ -ATPase proton pumping, resulting in opening of stomata. The blue-light photoreception in the guard cells is brought about by the pigment Zeaxanthin.
- viii) The phytohormone Absciscic acid (ABA) closes the stomata in response to water stress. ABA signaling in the guard cells involves regulation of ion channels and plasma membrane ATPase.

There are two ways in which the relative turgor of guard cells may be altered. i) a decrease in osmotic potential or ii) a decrease in pressure potential. In both instances, the water potential of guard cells will fall and hence water from the neighbouring cells would move in. Alternatively, guard cells may face mechanical pressure from the subsidiary cells if they undergo a sudden change in their turgor.

Earlier theories regarding the stomatal movements have highlighted the role of plastids, CO_2 , starch- sugar interconversion, *phosphorylase* enzyme, pH, role of K^+ , Cl^- , H^+ and malate ions, and ATP-driven proton pumps.

Modern theories explaining the mechanism of stomatal movements which have found a lot of acceptability include the K^+ -pump (proton transport) concept by Levitt (1974), ABA signaling model by Blatt & Thiel (1993).

There is overwhelming evidence that osmotic potential of guard cells decreases on account of the following set of events, leading to light induced opening of stomata in non-succulents.

Light induced H^+ transport takes place from the cytoplasm into chloroplasts of guard cells. This results in generation of a negative potential. Due to this there is an influx of K^+ ions from the surrounding cells. Due to continued proton influx, pH of the guard cells chloroplast is lowered to 5 while pH of the cytoplasm is raised to between 8 and 9. At a higher pH CO_2 gets converted to HCO_3^- in the cytoplasm of mesophyll and guard cells.

Simultaneously, in photosynthesis CO_2 is utilized through *RuBPC* and results in decrease in CO_2 concentration. Within the guard cells chloroplasts, starch along with other carbohydrates gets converted into phosphoenol pyruvate (PEP) by the process of glycolysis. PEP now diffuses out into the cytoplasm where it reacts with HCO_3^- to form organic acid (OAA and then malic acid) in the presence of the enzyme *PEP carboxylase*. Malic acid produced by the reduction of OAA dissociates (ionizes) into malate anion $R(COO^-)_2$ and H^+ cations in the guard cells. The $K^+ - H^+ -ATPase$ pump comes into operation. ATP is utilized at the guard cell plasma membrane where H^+ and K^+ ions are exchanged. For every molecule of ATP hydrolyzed, a H^+ effluxes in exchange for one K^+ ion via the membrane bounded proton pump which enters the guard cell. Increased concentration of K^+ and malate ions in the vacuoles of guard cells results in a substantial decrease in osmotic and water potentials of the guard cells. Due to decrease in osmotic potential (i.e., increase in osmotic pressure) in guard cells, a gradient is created so that there is an influx of water from the adjacent subsidiary cells and endosmosis results in guard cells turgidity which eventually forces the stomata to open.

In addition, chloride ions (Cl^-) are the anions which alloy with malate ions to balance the K^+ ions in majority of the plants. Malate helps in maintaining the cellular pH as it replenishes the supply of protons lost by extrusion by releasing one proton per accumulation of each carboxyl group ($-\text{COO}^-$) into the cytosol.

Recently the role of sucrose and blue light has also been recognized in stomatal opening. Blue light induces biosynthesis of malate ions and proton efflux by the guard cells while sucrose accumulation helps in increasing the concentration of active solute particles in the vacuolar sap leading to endosmosis (Fig. 2.18).

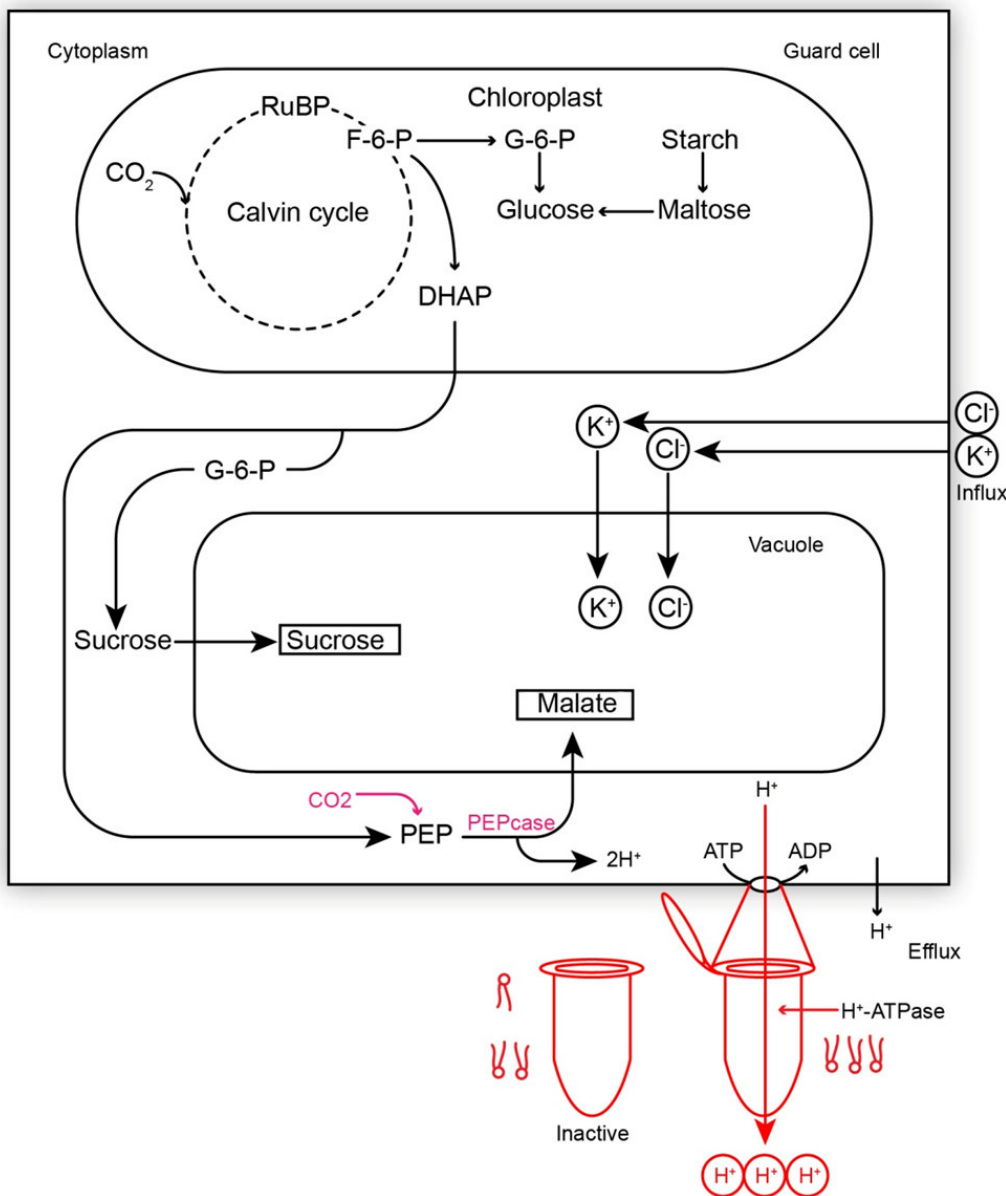


Fig. 2.18: A diagrammatic scheme for stomatal opening.

It is generally assumed that the events responsible for stomatal closure are reverse of the mechanism that you have studied above. However, it seems difficult to explain as to how do the stomata close rapidly in dark. Does only the passive leakage of ions from the guard cells sufficient to explain this phenomenon, or are there other specific metabolic pumps also operative?

There is accumulating evidence that ABA and Ca^{2+} play a vital role in the process which finally leads to the release of Cl^- and malate ions by inducing depolarization of the guard cell membrane. ABA increases the cytosolic Ca^{2+} concentration and alkalinization of the cytosol which results in enhanced activity of K^+ efflux. ABA also prevents blue-light induced stomatal opening by inhibiting the plasma-membrane H^+ - ATP ase, thereby contributing to membrane depolarization.

A simplified scheme for ABA signaling in stomatal guard cells is presented below (Fig. 2.19).

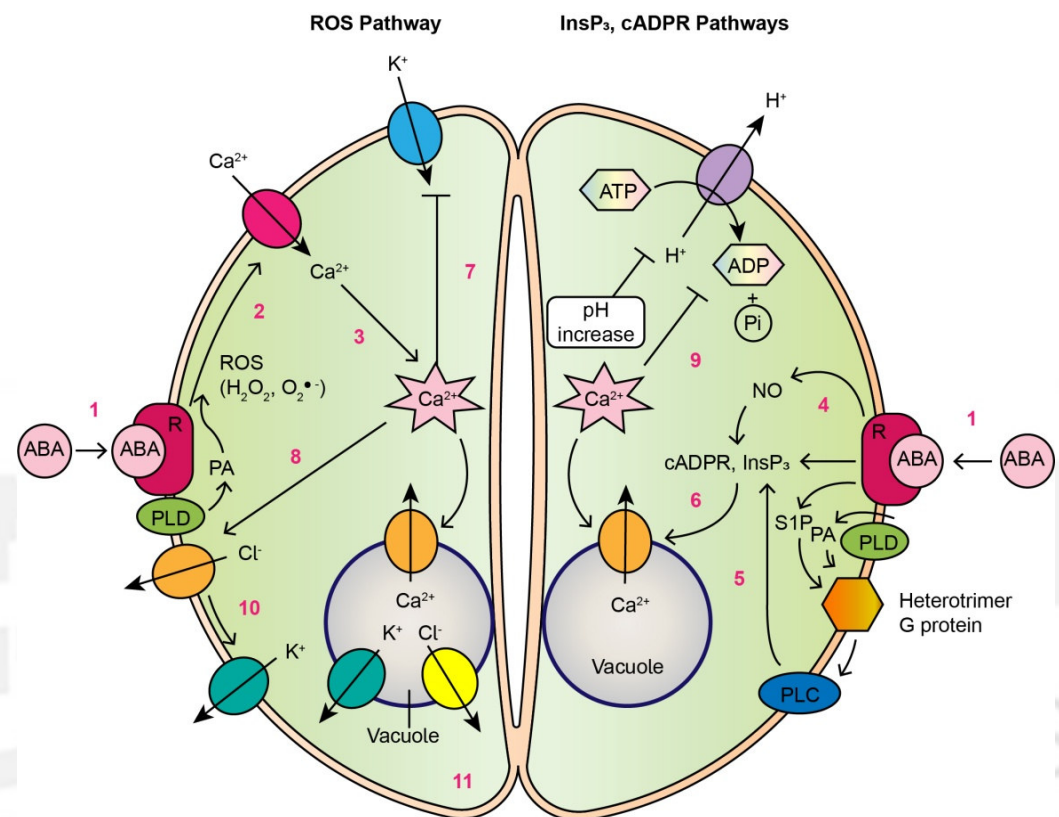


Fig. 2.19: ABA signaling model to explain stomatal movements (from Taiz *et al*).

In dark CO_2 gets accumulated in the guard cells or substomatal cavity as photosynthesis stops. A simplified scheme will make you understand the basic mechanism involved in ABA-signaled stomatal closure (after Taiz *et al*):

- ABA binds to its receptors on the guard cell membrane.
- ABA binding causes the activation of Ca^{2+} channel proteins on the plasma membrane through the formation of reactive oxygen species (ROS). ROS formation is also contributed by membrane-bound Phospholipase D(PLD)-mediated Phosphatidic acid (PA) production.
- As a result, Ca^{2+} influx takes place from the outside into the guard cell cytosol. This initiates the intra cellular calcium transients, which further promote the release of calcium from the vacuoles into the cytosol of guard cell.
- In addition, ABA binding also activates production of nitric oxide (NO), which in turn increases levels of cyclic-ADP-ribose (cADPR).

- v) ABA also increases the levels of inositol triphosphate (InsP_3) via a signaling pathway which includes sphingosine-1-phosphate (S1P), heteromeric G proteins, and phospholipases C (PLC) and D (PLD).
- vi) A rise in levels of cADPR and InsP_3 results in activating additional calcium channels on the tonoplast. Thus, more Ca^{2+} gets effluxed from the vacuoles. As a result, Ca^{2+} concentration in the cytosol increases further.
- vii) Rise in intracellular calcium concentration blocks the K^+ influx channels (K^+_{in}) on the plasma membrane.
- viii) Enhanced Ca^{2+} levels promote the opening of the Cl^- out (Cl^-_{out}) anion channels on the plasma membrane which result in **depolarization** of the membrane.
- ix) Proton-pump on the plasma membrane is also inhibited by this ABA-induced increased Ca^{2+} concentration of cytosol. The intracellular pH increases, which further depolarizes the membrane.
- x) Membrane depolarization further activates the K^+_{out} channels on the plasma membrane.
- xi) There is a mass transfer of K^+ and Cl^- or malate $^{2-}$ (anions) from the vacuoles into the cytosol which finally leave the guard cells through the plasma membrane.

There is a loss in turgidity of guard cells due to decrease in solute concentration and osmotic pressure. Water potential increases which causes exosmosis resulting in flaccidity of guard cells and ultimately the stomatal closure.

Scotoactive Stomatal Movement in Succulents (CAM Plants)

In CAM plants, the stomata open at night. This feature is of high adaptive value as such plants grow under water stress conditions and cannot afford to transpire. During night time, starch gets enzymatically converted to phosphoenolpyruvate (PEP) which traps both, the CO_2 evolved during respiration, as well as atmospheric CO_2 . You will study in Unit 7 of this course regarding the high CO_2 binding affinity of PEP in C_4 and CAM plants. In this reaction, malic acid is formed which gets accumulated in the vacuole. This organic acid provides a source of carbon for photosynthesis during daytime when the stomata are closed. As a result of malate accumulation water potential drops which initiates endosmosis, turgidity and finally opening of the stoma.

During daytime malic acid breaks down into CO_2 and pyruvate by *PEP case* activity. CO_2 enters the Calvin cycle for synthesis of sugars. Water potential increases resulting in exosmosis, which causes flaccidity of the guard cells leading to stomatal closure.

The scheme for opening and closing of stomata in a CAM plant is depicted in the Fig.2.20.

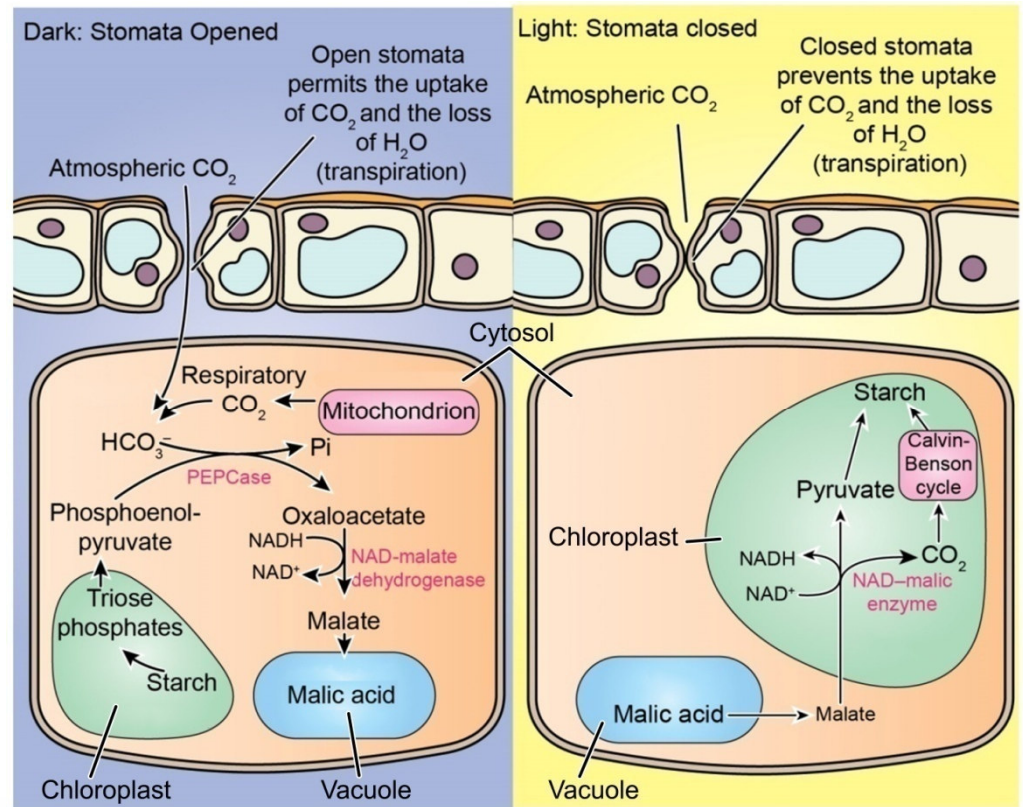


Fig. 2.20: Scotoactive movements of stomata in CAM plant (after Verma).

Factors Controlling Stomatal Aperture

You have studied that more than one factor can trigger the change in the turgor of the guard cells and bring about stomatal movements and control the size of stomatal aperture. The main controlling factors are:

- light,
- the level of CO_2 , and
- high temperature.

Now imagine the factors as hands that can open and close stomata by moving the turgor operated valve – the guard cells (see Fig. 2.21).

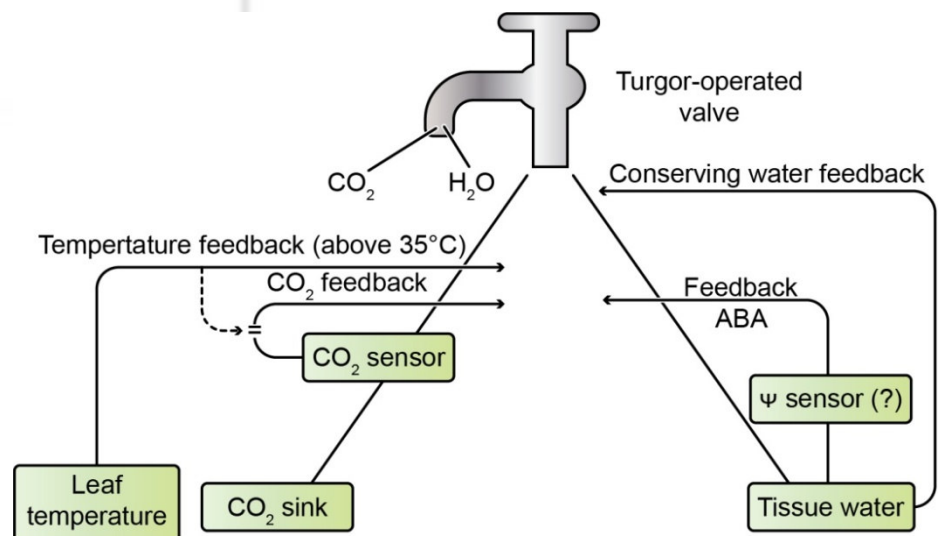


Fig. 2.21: A simplified model showing the effect of various factors on stomatal aperture. Ψ sensor is still unknown.

The stomata open when the level of CO_2 falls due to its rapid utilization in photosynthesis during the day. However, it is not known how guard cells sense the low level of CO_2 . The highest rates of photosynthesis are obtained when the conditions for light and temperature are optimum and plants are well watered. But if the water potential in the leaf falls under low level of CO_2 , then conserving water becomes more urgent than food production for the plant and the stomata close. In other words, the water status of plant overrides the control by CO_2 .

The rate of photosynthesis increases with increase of temperature but above 35°C cooling of the plant becomes necessary. Therefore, irrespective of CO_2 concentration stomata open widely above 35°C if there is no shortage of water. Here again the temperature overrides the control by CO_2 concentration. This makes sense because high temperatures are deleterious to the health of the leaves, and evaporation of water through transpiration can lower the temperature of leaves and plant.

It is important to point out that the water loss by a plant due to transpiration is very high usually in the range of $0.1\text{--}2.5\text{ g dm}^{-2}\text{h}^{-1}$ during daytime. However, it plays no major role in growth and development of plant. We have mentioned above that it cools the plant at high temperatures. Perhaps, the transpiration stream facilitates the availability of mineral ions to all parts of the plant. Soil contains minerals in very dilute concentration but in cells they get accumulated in much higher concentration due to active transport aided by their rapid transpiration stream.

Box 2.2: Antitranspirants

You have learnt that the external application of plant hormone abscisic acid (ABA) to leaves causes stomata to close. During water stress, increase in the level of ABA of the leaves has also been observed. Therefore, it is quite likely that ABA is an internal control for the regulation of water content. Phenyl mercuric acetate – a fungicide has been used as foliar spray in low concentrations (10^{-4}M) to bring about partial closure of stomata for about two weeks without visible damaging effect to the plant. Other chemicals such as colorless plastics, silicon oils and low-viscosity waxes are also used as foliar spray. These chemicals form on leaves a film permeable to CO_2 and O_2 but not to H_2O . Water-saving strategy by reducing transpiration though seems quite promising yet is a big challenge for plant physiologists.

Other substances which can reduce the stomatal opening are CO_2 and aspirin.

2.6 FACTORS AFFECTING TRANSPIRATION

Transpiration is influenced by i) Environmental or External Factors and ii) Morphological or Internal Factors.

The external factors include atmospheric humidity, light, temperature, air currents, atmospheric pressure along with the water availability in soil. The internal factors comprise the transpiring leaf area, stomatal structure, distribution and periodicity of opening, size shape, and orientation of leaves, along with their water content. In addition, the root shoot ratio and health of a plant are also important parameters.

One of the most important factors affecting transpiration is the water vapour pressure deficit of the air. It represents the difference in water vapour pressure in the inside and outside of leaf. When relative humidity is low, the atmosphere is dry and relatively less saturated. Under this greater vapour pressure deficit, the atmosphere assumes a greater capacity to absorb water from the plant. Under moist atmospheric conditions, rate of transpiration slows down as the water saturated atmosphere prompts less deficit in vapour pressure. Thus, there is a linear relationship between the vapour pressure deficit and rate of transpiration.

Most of the stomata (photoactive) open during light conditions and close during night. High light intensity also raises the temperature and permeability of the protoplasmic membranes facilitating transpiration. Stimulation of stomatal opening by blue light via cryptochromes, phototropins and zeaxanthin have been discussed in section 2.5.

An increase in atmospheric temperature results in enhancing the vapour pressure deficit of air by decreasing the relative humidity. This increases the saturated air inside the leaf, and results in an increase in the rate of transpiration. Water vapours will escape the leaf even when the atmosphere is relatively saturated on account of temperature induced higher vapour pressure in the leaves. More so, higher temperature induces a wider opening of stomata.

Soil temperature tends to affect the warm weather crops more than the cool weather ones. At low soil temperature of 5-8 °C, transpiration in the former drops to a minimum whereas not much effect is seen in the latter.

Air or wind currents tend to remove the partly saturated air from around the leaf resulting in enhanced transpiration. High speed air currents reduce transpiration by causing the closure of stomata.

Low atmospheric pressure induces increased rate of transpiration, as seen in plants growing at higher altitudes. However, the low temperatures prevailing in these areas offset this increase.

Availability of water will also be instrumental in influencing the rate of transpiration. High salt content in water also reduces water loss by a plant.

Transpiration is directly proportional to area of the transpiring surface, i.e., the leaf area, number, and distribution of stomata. In addition, the leaf texture, orientation for more exposure to light, are parameters that affect the transpiration process. Transpiration is low in plants with a low root/shoot ratio.

SAQ 4

- a) Fill in the blanks with appropriate words/terms.
- Scotoactive movement of stomata is on account of increase in during daytime due to breakdown of malic acid into CO₂ and
 - Stomatal opening is on account of decrease in osmotic potential and decrease in pressure potential, which causes a decrease in resulting in in guard cells.

- iii) Excessive loss of water from plants under dry conditions will lead to
 - iv) There is an influx of and ions during opening of stomata.
 - v) light is more effective than light in stimulating stomatal opening.
- b) List the features of guard cells essential for opening of stomata.

2.7 ROOT PRESSURE AND GUTTATION

You have studied in the above sections that plants may exert root pressure which can be as high as 0.05 to 0.2 MPa. This positive root pressure gets built up in the roots as solutes are being continuously absorbed and transported to the xylem. As a result, the xylem osmotic potential (ψ_s) as well as water potential decreases. Consequently, a positive hydrostatic pressure is developed in the xylem. This would never develop if the transpiration rates were high and water was being continuously lost to the atmosphere. So, when the absorption of water is in far excess than transpiration, root pressure manifests itself in the form of droplets of xylem sap exudates from edges of leaves during the morning hours. These so called “dew drops” escape the leaf margins through specialized pores called “hydathodes” which are associated with the vein endings. The loss or exudation of water as liquid droplets from the leaves or other intact/uninjured parts of a plant is called **guttation**.

Guttation is frequently seen in grasses, *Colocasia*, Strawberry, Balsam, *Nasturtium*, and many members of family Cucurbitaceae (Fig 2.22). Guttation is best seen at night under condition of high humidity. Guttation drops must not be mistaken for dew drops. Guttation drops are restricted to certain areas in leaves, occur on cloudy humid nights. The guttation drops normally contain water and dissolved inorganic and organic substances. Upon evaporation of the liquid, these substances get deposited in the form of salts and may even be injurious to the plant. On the other hand, dew drops can occur anywhere on a plant, and even on soil as they are just condensed water droplets.



Fig. 2.22: Guttation drops exuded out of hydathodes.

Some major differences between transpiration and guttation have been summarized in the table 2.1.

Table 2.1 : A comparison between transpiration and guttation.

Transpiration		Guttation	
1.	Loss of water occurs in the form of water vapour.	1.	Loss of water occurs in the form of liquid droplets.
2.	Water vapour is pure water.	2.	Liquid water is having organic and inorganic solutes.
3.	It occurs through stomata, lenticles or cuticles of the epidermis.	3.	It occurs through uncontrolled pores called hydathodes.
4.	Usually occurs by day.	4.	Usually occurs by night.
5.	It is beneficial to plants as it maintains body temperature by cooling effect.	5.	It is less significant to plants and sometimes causes injury to plants by deposition of salts on the leaf tips after evaporation. Later on these sites are attacked by bacteria/fungi/other pathogens.
6.	It is caused by transpiration stream and water potential gradient.	6.	It is caused by root pressure of plants.
7.	It is a controlled process.	7.	It is an uncontrolled process.
8.	It causes development of a negative pressure in the xylem of the plant.	8.	It is produced only when the xylem shows positive pressure.
9.	Transpiration continues even when the plants are facing water stress condition.	9.	It does not occur when the plants are facing water stress condition.
10.	Excess transpiration produces wilting.	10.	Excess guttation does not cause loss of turgidity.
11.	Transpiration occurs during dry period.	11.	Guttation occurs during humid period.

2.8 PHYSIOLOGICAL PROCESSES AFFECTED BY PLANT WATER STATUS

Water status of a plant is determined by two major factors: i) water absorption and ii) water loss. We deal with water absorption in this section and water loss in the following section.

Water absorption is regulated by soil factors, rate of transpiration and size and distribution of roots. The soil factors that regulate water absorption are: i) soil water content, ii) difference in water potential between soil and root. iii) concentration of soil solution, iv) soil temperature and v) aeration of the soil.

2.8.1 Soil Characteristics

The physical properties of soil govern water-holding capacity and water availability to the plant. First, we will study about various characteristics of soil.

The fine soil particles of clay have much more water-holding capacity than silt and sand. Addition of humus increases the water holding capacity of soil. Water moves through pores present in soil. The pores form because individual soil particles aggregate to form large particles of varying sizes called micelles. The pores are spaces left between micelles. The size of pores can be small (micropores) or large (macropores) depending upon the soil type. The pores get filled with water.

A soil, freshly wet with irrigation or rainwater cannot retain all the water and much of the water percolates through macropores due to gravity. This water has been termed as **gravitational water**. The remaining water that is held tightly by hydrogen bonds to the soil particles against gravitational forces is called **capillary water**. This water is available to the roots. Part of the capillary water that is held very tightly and is not available to the root is termed **hygroscopic water**.

The water content of soil is expressed as

$$\% \text{ of soil water} = \frac{FW - DW}{DW} \times 100$$

FW = Field weight of wet soil

DW = Dry weight (obtained by heating soil to 60 °C)

Field Capacity (FC)

Field capacity is the amount of soil moisture or water content held in soil after excess water has drained away and the rate of downward movement has decreased. In other words, it is the capacity of a field to hold the amount of water against gravitational forces. It is expressed as the percentage of the dry weight of the soil. Field capacity represents the upper limit of water availability and it differs from soil to soil. The capacity of clay soil on an average is about 40-50%, silt 20% and sand 5-10%.

Permanent Wilting point Percentage (PWP)

It is the percentage of moisture in the soil at which a plant wilts and does not recover unless water is added to the soil. PWP for clay is about 26%, for silt 10% and for sand 3-5%. It must be noted that PWP is used to express property of a soil not any feature of the plant.

However, the moisture content at which any plant shows permanent wilting need not be the same for all plants even in the same soil. This happens because some plants are prone to wilting at reasonable water content of soil while others wilt only when the moisture content is very much reduced.

The water status of a plant in terms of FC and PWP is significant only if the soil properties are known. This is because the soil determines the availability of water. However, it is describable as well as easier to express soil water status in terms of water potential so that soils become uniform with respect to water.

Soil Temperature

Soil temperature is known to influence water absorption and ultimately transpiration to a considerable extent. In many plants water absorption is

reduced sharply below 10°C. Water absorption also slows down above 25°C. In most instances, temperatures above 40°C in the rhizosphere does not support water absorption and plant may show signs of wilting. The following are the reasons suggested for the reduced absorption of water at low temperature: i) decreased root growth, ii) increased viscosity of water, iii) increased resistance of water into roots due to decreased permeability (increased hydraulic resistance) of cell membranes and iv) decreased metabolic activity of root cells.

Soil Aeration and Flooding

It is not unusual to observe some plants wilting while they stand in water. The following are the possible reasons of flood injury.

- i) Poor availability of O₂ and accumulation of high concentration of CO₂ around roots.
- ii) Changes in the pattern of ion uptake, resulting in the accumulation of some ions to toxic levels.
- iii) Accumulation of toxic substances in the root and/or around them.

Poor availability of oxygen affects respiratory activity of roots, thus lowering the ATP supply. In the following units you will study that ATP is required for the active uptake of ions. Low uptake lowers the osmotic potential of roots cells and hence water cannot enter because water potential ($\Delta\Psi_w$) between root cells and soil is not sustained. Increased concentration of CO₂ affects the permeability of membranes and thus affects water uptake adversely.

Among toxic substances are the metabolites produced because of anaerobic respiration. These could be alcohol or aldehyde and even ethylene. Plants usually become chlorotic under waterlogged conditions. This is apparently due to reduction in translocation of iron from roots to the top of the plant.

Root

Root system is directly related to the absorption of water and its growth under field conditions is very much influenced by soil. In dryland agriculture, particularly root structure has apparently greater significance. The rates of water absorption into roots of different plants differ in their stage of growth. Highest rates of water entry are associated with root hair and unsuberized roots and lowest with suberized woody root.

2.8.2 Water Absorption and Transpiration

The rate of water absorption is controlled by the rate of transpiration. A high-water potential in the atmosphere would reduce water loss from the plant and consequently slow down water absorption. But it does not follow if the water potential of the atmosphere is very low and soil is near drying. When the equilibrium in the soil-plant-atmosphere system is disturbed either due to soil or atmosphere, the plant can respond appropriately and can control water absorption or water losses. We discuss below some of these aspects.

Let us first see the relationship of water absorption and transpiration in a well-watered plant (Fig. 2.23). By and large all plants show a diurnal behaviour in

their rate of transpiration. There is a rapid increase in transpiration during the morning hours and a peak is reached in the early afternoon. Then a decline begins, and a minimum is reached during the night.

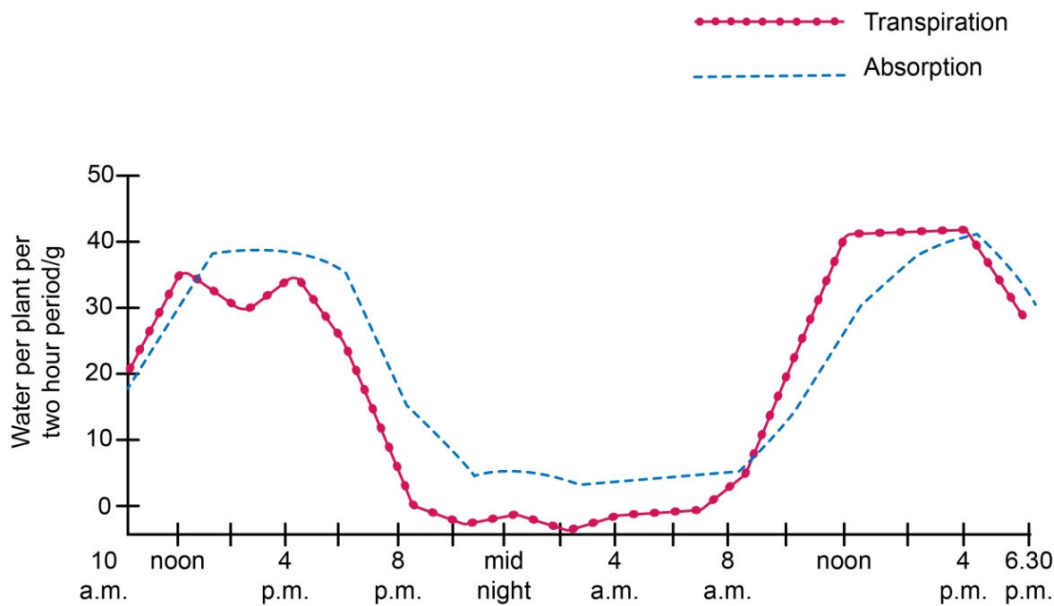


Fig. 2.23: Diurnal variations in rate of transpiration and absorption of water by a plant.

You may note in the graph that water absorption keeps pace with losses due to transpiration except around noon when it lags. Why is it so? You have studied that the resistance to the movement of water across the roots is generally higher than in the leaves because in roots, water moves through symplasm. In roots it faces high resistance of the membranes of cortical cell and that is why there is an absorption lag. This idea is supported by an experiment where water absorption was recorded after removing the roots of a plant. As shown in the graph (Fig. 2.24) the absorption lag is reduced greatly.

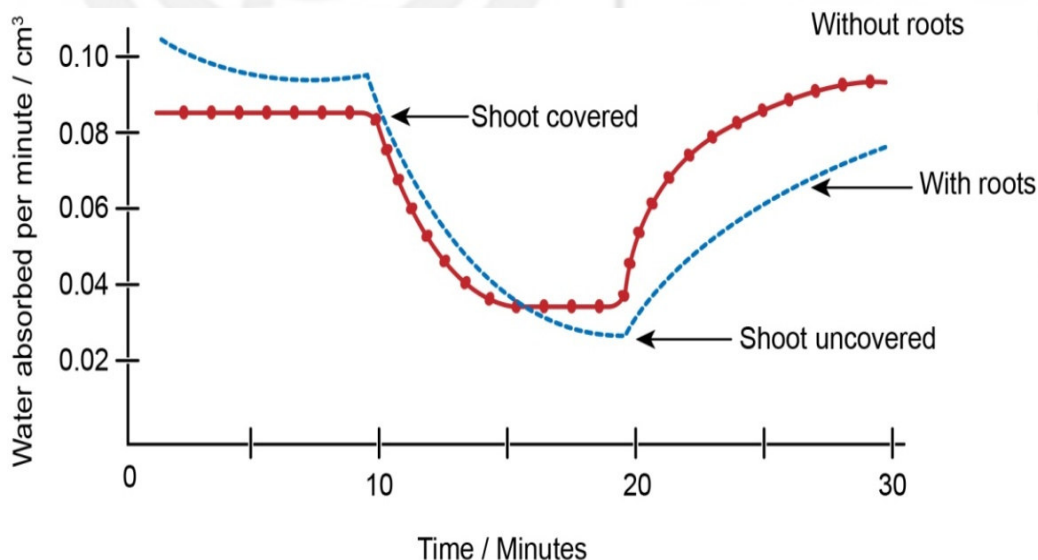


Fig. 2.24: Comparison of rate of water absorption in plants with and without roots. When transpiration is high (shoot uncovered), the rate of water absorption in a plant with roots is less than the plant without roots.

Let us now see what happens to water loss if soil is not moist. Ralph O. Slater (1967), a plant physiologist at Australian National University, Canberra,

studied the relationship between water potential of soil (ψ_w soil), root (ψ_w root) and leaf (ψ_w leaf) of a plant where water supply was withheld for five days. The graph (Fig. 2.25) shows probable changes in leaf potential, water stress around the noon time and recovery of lag in absorption at night. The water potential of the soil decreased almost linearly after second day. It must be pointed out that when plants are under water stress, water tends to move out of the cells that are not situated in the main pathway of transport of water. This causes reduction in turgor, i.e., cell volume. In fact, it was demonstrated a century ago by Josef Friedrich that there are daily fluctuations in the diameter of tree trunk, shrinkage in the morning and recovery in the diameter by night.

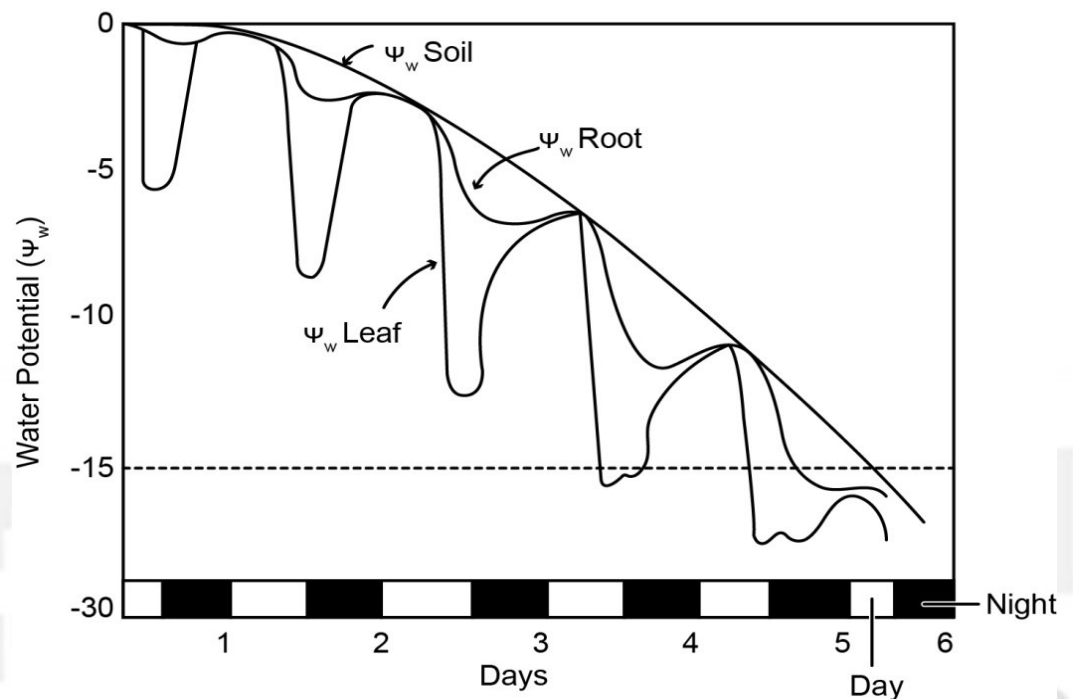


Fig.2.25: Diagram showing probable changes in leaf potential (ψ_w leaf) and root water potential (ψ_w root) of a transpiring plant rooted in soil allowed to dry from a water potential near zero to a water potential (ψ_w soil) at which wilting occurs.

SAQ 5

- a) Fill in the blanks with appropriate words/terms.
 - i) The guttation drops normally contain and
 - ii) Permanent wilting percentage is the percentage of at which any plant shows
 - iii) Specialized pores, the, which are associated with vein endings exude guttation droplets.
 - iv) Flood injury in plants can be due to high concentration of and low concentration around roots.
 - v) Water absorption sharply at temperatures below 10°C due to increased viscosity of water and hydraulic resistance of cell membranes.

- b) Which among the following suggestions are desirable for growing healthy plants? Put a tick mark for the correct ones.
- i) Excess use of fertilizers. []
 - ii) Saturation of plants, particularly growing in pot, with water. []
 - iii) High humidity area for water-sensitive plants. []
 - iv) Use of very cold water in warm season. []
 - v) Use of lukewarm water in cold season. []
-

2.9 SUMMARY

In this Unit you have studied that:

- Water movement within the dead xylem cells occurs along the gradient of hydrostatic pressure.
- There is resistance to water flow in the soil and plant. Water takes up the path of least resistance. Membranes of cells exert greatest resistance while xylem offers least resistance
- Water status of a plant depends upon water absorption and water loss due to transpiration. Soil characteristics, soil temperature, aeration, flooding, and structure of roots are the factors which affect water absorption.
- The rate of water movement in plant is determined by the rate of transpiration in a well-watered plant. Temperature, wind velocity, humidity of the air, light intensity and degree of stomatal opening affect the rate of transpiration. However, ultimate limit to transpiration is the supply of water from soil.
- Among stomatal, cuticular and lenticular transpiration, stomatal transpiration accounts for major water loss. Evaporation of water faces considerable resistance from the leaf – cuticle, mesophyll cell, intercellular spaces, stomata, and air boundary layer of the leaf.
- Stomata control water loss through transpiration, allow gas exchange and control temperature of the leaf. Some efforts have been made to reduce water loss through stomata without affecting CO₂ intake.
- Stomatal aperture is controlled by the changes in the turgor of guard cells. The turgor in turn is affected by the metabolism and the movement of K⁺ and other ions into and out of the guard cells. A proton pump powered by *ATPase* operates in the plasma membrane of the guard cell and the K⁺ uptake is a passive uptake.
- Stomatal aperture is controlled by the level of CO₂, light and high temperature. However, if conserving water or saving plants from deleterious effects of high temperature becomes more urgent than food production then these factors override the system.

2.10 TERMINAL QUESTIONS

1. Why do animals need less water than plants?
2. What would happen if a twig of a potted plant is cut into pieces and joined with Tygon tubing in such a way that it leaves an air gap between the cut ends? Will water continue to move?
3. A plant cell with an osmotic potential of -7.6 bars and pressure potential of 3 bars is placed on a slide with a drop of pure water, in which direction the water will flow? What is the ψ_{π} , ψ_p and ψ_w of the cell and water initially and after equilibrium?
4. Draw the structure of stomata and explain how stomatal aperture opens by the increase in the turgor of the guard cells.

2.11 ANSWERS

Self-Assessment Questions

1. a) apoplastic
b) casparian bands/strips
c) aquaporins
2. a) i) False, ii) True; iii) True; iv) False
b) i) root hairs
ii) apoplast, symplast, transmembrane
iii) adhesion, cohesion
iv) Strasburger
v) cavitation, embolism
vi) casparian bands
3. a) i) transpiration ratio
ii) stomata
iii) cuticle, maximum
iv) amphistomatic
v) index
b) i) epistomatic
ii) Lottfield
iii) pore size
iv) CAM plants
v) bark
vi) *Potamogeton*

4. a) i) water potential, CO_2 , and pyruvate
 ii) water potential, turgidity
 ii) wilting
 iv) K^+ , Cl^-
 v) blue, red
- b) i) a thickened cell wall, facing the pore; ii) presence of chloroplasts; iii) supply of ATP; iv) presence of a proton pump in the membrane; v) capacity to generate higher turgor pressure, lower water potential than adjacent cells.
5. a) i) water, dissolved substances
 ii) moisture, permanent wilting
 iii) hydathodes
 iv) CO_2 , O_2
 v) decreases, increased.
- b) iii) and v) are right suggestions.

Terminal Questions

- In animals a great deal of water is recycled through the body in the form of blood plasma and other fluids in vertebrates. In plants more than 90% of the water absorbed by leaves is lost into the air as water vapour through the process of transpiration.
- The plant will absorb water, but the upper part of the plant will wilt. This is because the air column between the two stem pieces does not provide a continuum. In plants the movement of water takes place only in liquid phase which can stand enormous pressure without breaking the liquid column.
- Water will flow into the cell until the water potential of cell and water drop is equal.

The following are initial and final (equilibrium potentials)

	Initial		Equilibrium	
	Water drop	Cell	Water drop	Cell
Ψ_π	0	-7.9	0	-7.9
Ψ_p	0	+3	0	+7.9
Ψ_w	0	-4.9	0	0

4. Refer to Section 2.5.

MINERAL NUTRITION |

Structure

3.1	Introduction	3.4	Role of Essential Elements
	Objectives		Macronutrients
3.2	Nutrient Elements of Plants		Micronutrients
	Plant Ash Analysis	3.5	Microbes, Mycorrhiza and Mineral Uptake
	Special Techniques to Study the Role of Mineral Nutrients	3.6	Summary
3.3	Classification of Elements	3.7	Terminal Questions
	Criteria of Essentiality of a Mineral Nutrient	3.8	Answers
	Biochemical Role and Physiological Function		

3.1 INTRODUCTION

We are dependent on plants for food, clothing and shelter, and most important of all oxygen, on which our life depends. Like man and animals, plants too need wholesome nutrition for healthy growth and development. While man and animals are mobile and can gather their food from wherever it is available, plants are stationary and manufacture their own food from simple inorganic nutrients, relying mostly on what they get from their immediate environment. For successful production of a healthy crop, an in-depth knowledge of their nutritional requirements is a must. In this unit we will find out what these requirements are, how plants obtain them from the surroundings and how they are transported to all parts of the plant body. By answering these questions, we can improve plant produce and have better understanding of plant growth.

Objectives

After studying this unit, you would be able to:

- ❖ list the essential macro and micronutrients that a plant must absorb to survive and grow;
- ❖ describe the role of essential elements in plants; and
- ❖ list deficiency symptoms of specific nutrients in a plant.

3.2 NUTRIENT ELEMENTS OF PLANTS

Let us first find out the chemical composition of plants and see which element, nature has selected to support their healthy growth. Only then we will be able to know the nutritional requirement of plants.

3.2.1 Plant Ash Analysis

As you know a major part of plant tissue is comprised of water. This we can demonstrate by taking a known amount of plant tissue and drying it for a few hours in an oven at a temperature of 65-80°C. If we condense and analyses the vapors coming out from the plant tissue, we will find that it is nothing but water. In fact, about 85-90 per cent of the tissue is composed of water. The part of the tissue which is left behind is called the **dry matter** and typically it is about 10-15 per cent of the original weight. The dry matter consists mainly of organic compounds. About 90% of the dry matter consists of plant cell walls, primarily cellulose and related carbohydrates. This can be eliminated in the form of gases on combustion at 600°C. The residue now left is the ash which varies in different plant tissue from about 1 per cent to 0.15 per cent of the dry weight. Detection of mineral elements can be made either by **wet digestion** or by **ash analysis** of the oven dried powdered plant material. Ash is dissolved in warm dilute HCl or HNO₃ and then subjected to various physical, chemical or physicochemical methods to detect and quantify the elements present, which include the modern techniques of atomic emission spectroscopy, X-ray emission spectroscopy, ion-chromatography and many more. Interestingly, a careful analysis of the ash shows that it contains almost all the chemical elements present in the soil surrounding the plant.

Now, the question is whether all the elements found in the ash are essential for the plant to lead a healthy life? How do we distinguish the essential elements from the non-essential ones?

3.2.2 Special Techniques to Study the Role of Mineral Nutrients

Studies on plant nutrition in the early times revolved around the elemental analysis of plant material and formulation of fertilizers for augmenting agricultural productivity.

Nicholas Théodore de Saussure was perhaps the first to demonstrate the dependence of plants on elements from soil. His experiments (mentioned in his book "*Recherches chimiques sur la végétation*" published in 1804) clearly indicated that plants obtained inorganic mineral nutrients using their root system. According to him, these minerals, along with nitrogen were vital for growth and development of plants. His ideas were later supported by the work of Sprengel and Boussingault, who also stated that soils deficient in one single element could be unproductive for plant growth. Liebig (1840) provided evidence for the essential role played by inorganic components of plant ash in plants. It was Sir John Lawes and John Gilbert (UK) who performed extensive field experiments on mineral nutrition and crop improvement in 1843. They

also successfully converted the insoluble rock phosphate to superphosphate (soluble phosphate). This led to the establishment of the use of nitrogen, phosphorus, and potassium (NPK) fertilizers by the end of the last century.

As far back as 1860, Sachs used an aqueous solution of mineral salts to study the minimal requirements of plants. He believed that soil was too complex a medium to study the requirements of each element to a plant. His initial work with a simple nutrient solution containing nine inorganic salts laid down the foundation of **solution** or **hydroponic culture**, where plants are grown in a defined nutrient solution.

It took almost another 50 years before the need for additional mineral nutrients was experimentally demonstrated. Gradually, use of more sophisticated analytical techniques detected many more elements to be added to this list.

Solution culture or Hydroponics (Gericke, 1937) is the technique of soilless culture where the plant roots are immersed in well-defined solution. In such cultures, the quantity and relative proportion of mineral salts can be precisely controlled and the contamination from other sources can be avoided (Fig. 3.1).

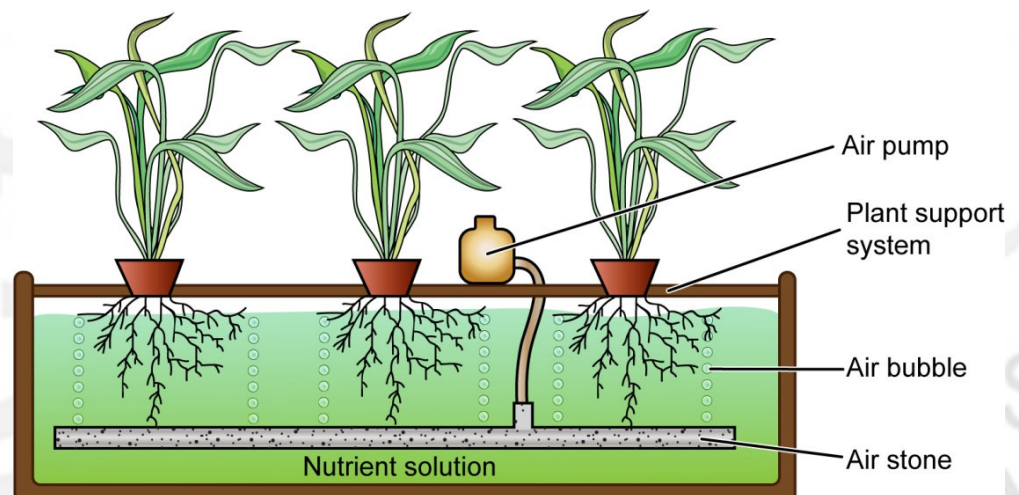


Fig. 3.1: Hydroponic growth system (Based on Taiz *et al*).

Plants are suspended by the base of the stem over a tank full of nutrient solution. Large quantities of the nutrient medium ensure minimum fluctuations in the concentration of elements supplied. A continuous aeration through a pump is needed into the medium to avoid any depletion of oxygen (anoxia) to the roots. The container is usually painted black to avoid light and minimize contamination due to growth of algae.

Hydroponics are being used extensively to grow vegetables in greenhouses on a commercial scale. Tomatoes, lettuce, sweet pepper, and seedless cucumbers grow vigorously in the presence of only fibrous roots (no resistance for them as in soil). Plants are grown with the supporting material of sand, gravel, vermiculite or expanded clay.

There are some disadvantages in using this technique. Under alkaline conditions the iron salts in the form of FeSO_4 or $\text{Fe}(\text{NO}_3)_2$ can precipitate out in the nutrient solutions as iron hydroxide. In the presence of phosphates, iron

phosphate can also be formed. Thus, iron would be unavailable in an ionic form. To avoid this problem, some **chelating agents** like citric acid or tartaric acid are added which can hold the iron and calcium cations by ionic forces to remain in a physically available form. Some compounds like **EDTA** (ethylene diamine tetraacetic acid or **DTPA** (diethylenetriaminepentaacetic acid /Pentetic acid) are popularly used as chelating agents in modern hydroponics and nutrient culture media.

The pH may fluctuate due to absorption of minerals by roots and selective depletion of nutrients. Various modifications have been made in the standard hydroponic system.

- Plants are grown with the non-nutritive supporting material of sand, gravel, vermiculite, acid- worked quartz, or perlite. Daily watering by fresh nutrient solution is done (**Slop culture**). Alternatively, the nutrient solution is made to drip slowly onto the culture from a reservoir (**Drip culture**).
- In other modifications, plants are grown in a trough or tube on a slight incline. An inlet pump supplies nutrient solution from a reservoir to the elevated end of this compartment. The solution flows down and returns to the nutrient recovery chamber from where it is recirculated. An air pump saturates the reservoir with oxygen (Fig. 3.2). This is called as the **Nutrient film growth system** as the nutrient medium flows in the form of a film and the roots are bathed in it.

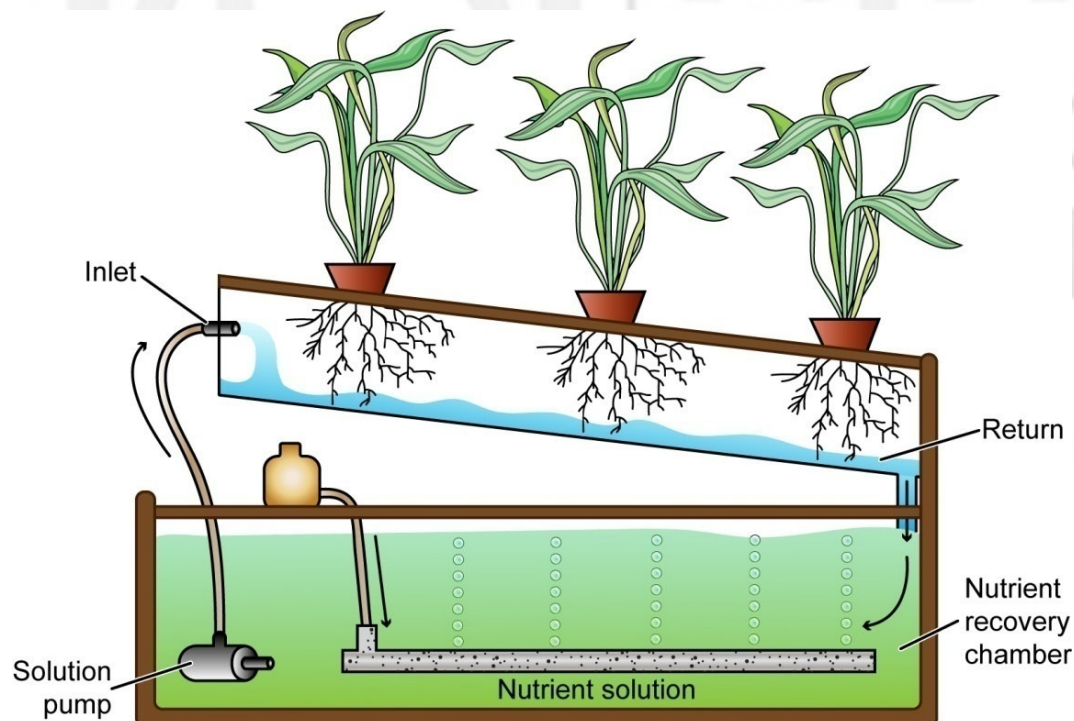


Fig. 3.2: A Nutrient film growth System (Based on Taiz et al).

- Another form of solution culture is the **Ebb and Flow System** where the nutrient solution periodically rises and recedes so that the roots also get exposed to moist atmosphere, in addition to nutrients (Fig.3.3). Both nutrient film and Ebbed flow systems provide good aeration to the roots.

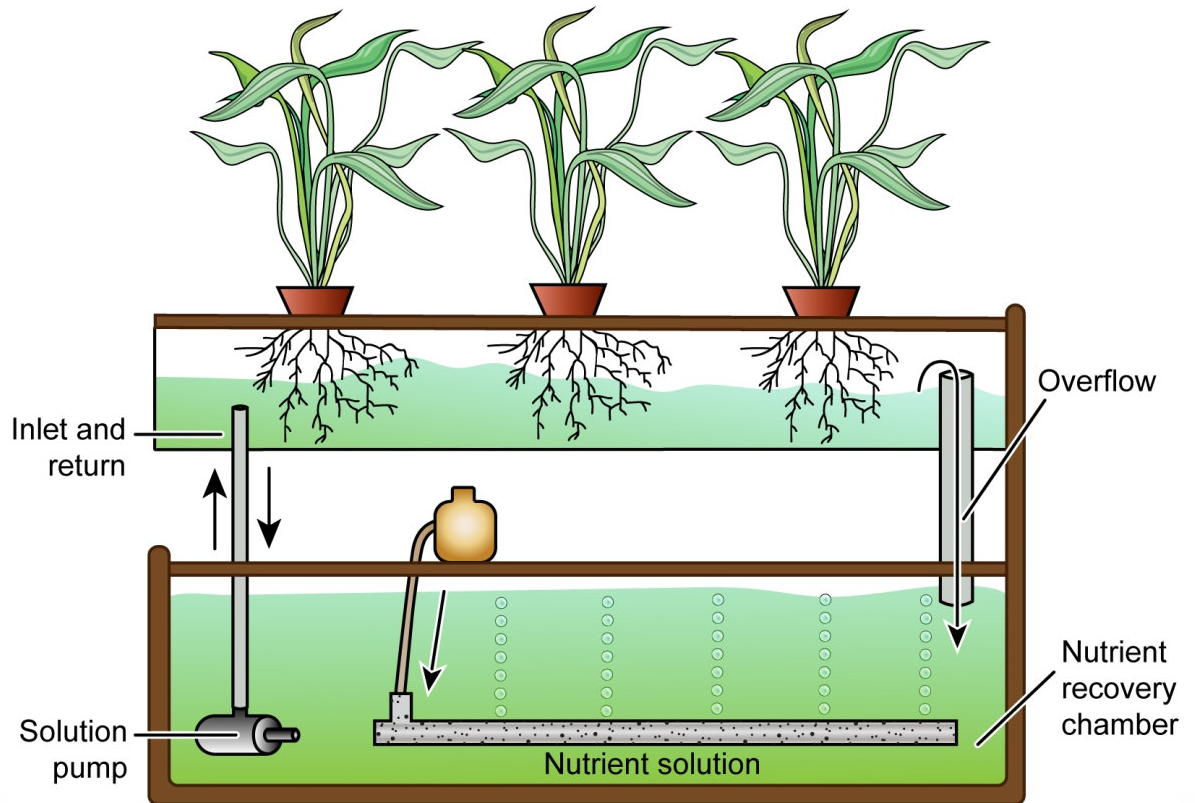


Fig. 3.3: An Ebb and Flow System (Based on Taiz *et al*).

- Aeroponics (Weathers and Zobel, 1992) is a further advancement where the roots of plants are suspended in air and are continuously sprayed with a nutrient solution. Although it is easier to manipulate the gaseous environment around the roots, much higher volumes of the nutrient solution are needed for this method (see Fig. 3.4).

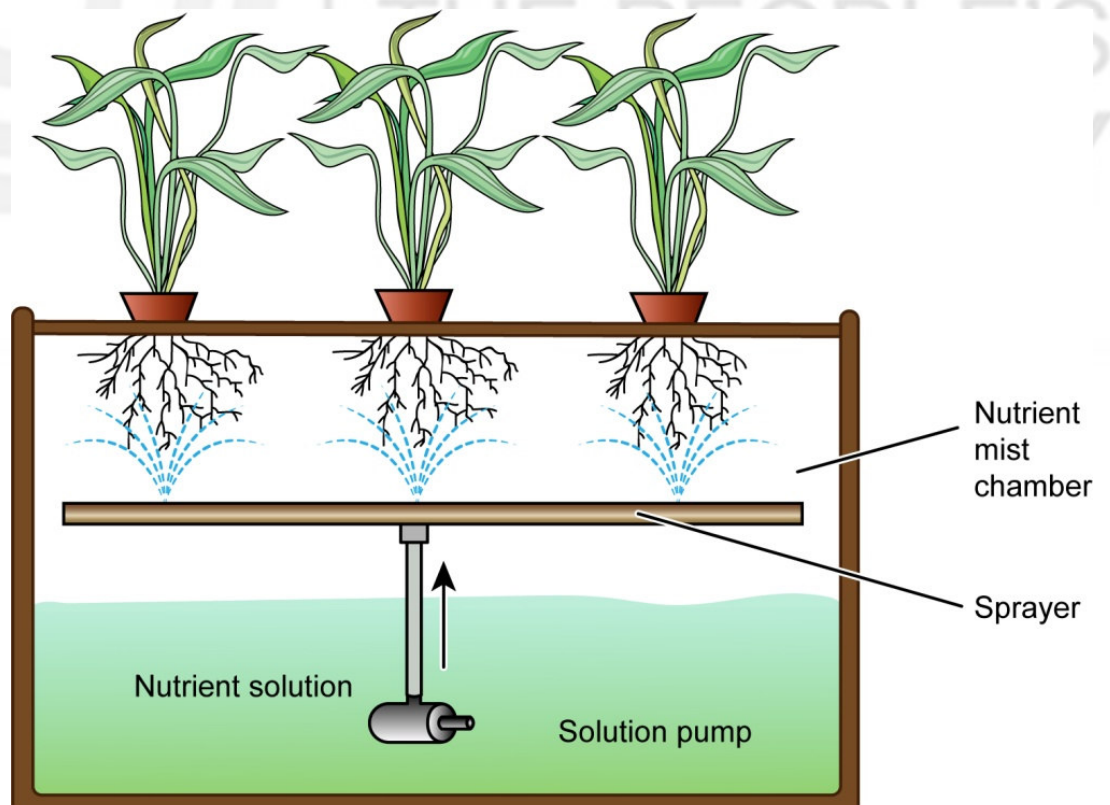


Fig. 3.4: An Aeroponic Growth System (Based on Taiz *et al*).

SAQ 1

Match the contents of Column I corresponding with those of Column II:

Column I	Column II
a) Gericke	1. Solution cultures
b) Lawes & Gilbert	2. Aeroponics
c) Sachs	3. Minerals and crop improvement
d) de Saussure	4. Hydroponics
e) Weathers & Zobel	5. Mineral nutrients from soil

3.3 CLASSIFICATION OF ELEMENTS

Out of the elements listed in the periodic table, only few have a role to play in the life of a plant. You will now study which of the elements are integral parts of plant body or metabolism.

3.3.1 Criteria of Essentiality of a Mineral Nutrient

Arnon and Stout (University of California, USA), as early as 1939, suggested certain criteria that an element must fulfill to be classified as **essential**. These criteria are listed below.

1. An element is essential if in its absence the plant cannot complete its life cycle and form viable seeds.
2. An element is essential if it forms a part of any molecule or a constituent of the plant that is essential for the plant. For example, nitrogen in protein, magnesium in chlorophyll and iron in cytochromes.
3. The element must act directly inside the plant, and not enhance or suppress the availability of some other element.

Epstein (1999) elaborated upon the 1st criterion that an essential element must have a clear physiological role.

Although the first two criteria mentioned above are sufficient to judge if an element is essential, the third criterion can eliminate doubtful candidates from the list. For example, the plant *Astragalus* is a selenium accumulator. When grown in seleniferous soils the element shows growth promoting effect. However, experiments have shown that this property is due to the ability of selenate ion to inhibit the absorption of phosphate, which otherwise is absorbed by the plant in toxic amounts. From a practical point of view, an element is considered essential if plants show deficiency symptoms when they are raised without that element in the medium even if the plants can form viable seeds. You may note that rigorous exclusion of elements, specially trace elements is very difficult as they can come from seeds themselves, from dust in air, or as contaminants of major salts.

Based on the above criteria, the following elements are essential or indispensable for the growth of higher plants (Table 3.1).

Table 3.1: Levels of essential elements required by most plants

Element	Chemical symbol	Concentration in dry matter (% or ppm)
Macronutrients obtained from water or carbon dioxide		
Hydrogen	H	6
Carbon	C	45
Oxygen	O	45
Obtained from the soil		
Macronutrients		
Nitrogen	N	1.5
Potassium	K	1.0
Calcium	Ca	0.5
Magnesium	Mg	0.2
Phosphorus	P	0.2
Sulfur	S	0.1
Silicon	Si	0.1
Micronutrients		
Chlorine	Cl	100
Iron	Fe	100
Boron	B	20
Manganese	Mn	50
Sodium	Na	10
Zinc	Zn	20
Copper	Cu	6
Nickel	Ni	0.1
Molybdenum	Mo	0.1

(**Source:** Epstein 1972, 1999).

The values for the non-mineral elements (H, C, O) and the macronutrients are in percentages. The values for micronutrients are expressed in parts per million).

The essential elements are classified based on their concentrations required by a plant.

- i) It also reflects the relative concentration required in nutrient solution. Two categories of essential elements have been traditionally recognized.
 - a) **Macronutrients** required in large amounts (more than 10m mole kg⁻¹ of plant dry weight). The first ten elements given in Table 3.1 are the

macronutrients. b) **Micronutrients/Trace elements** are required in relatively small quantities i.e., less than 10m mole kg^{-1} of plant dry weight.

- ii) Since most of the macronutrients constitute the structure of molecules, its requirement is high. On the other hand, the trace elements function mostly as regulators, and enzyme activators, which are required in small amounts.
- iii) This categorization is by no means suggestive of their relative importance to the nutritional needs of a plant. Trace elements are as important as the macronutrients, though the quantities needed are different.

Liebig (1940) demonstrated the essentiality of mineral nutrition in plant and proposed the “Law of minimum” which states that the yield of a crop plant is determined by the amount of the necessary element which is present in minimum quantity in proportion to the demand of the plant.

This classification though useful in some respects, is arbitrary and in many cases the difference between the contents of the two groups is not much. To give an example, the Fe and Mn contents of plant tissues very often are as high as the content of S or Mg. Hence, scientists are trying to evolve a more satisfactory classification based on physiological and biochemical parameters.

3.3.2 Biochemical Role and Physiological Function

Essential elements have also been classified based on their biochemical role and physiological function. According to Mengel & Kirkby (2001), the essential elements have been categorized into four basic groups (Table 3.2).

1. Nitrogen and sulfur constitute the first group of essential elements. Plants assimilate these nutrients via biochemical reactions involving oxidation and reduction to form covalent bonds with carbon and create organic compounds.
2. The second group is important in energy storage reactions or in maintaining structural integrity. Elements in this group are often present in plant tissues as phosphate, borate, and silicate esters in which the elemental group is covalently bound to an organic molecule (e.g., sugar phosphate).
3. The third group is present in plant tissue as either free ions dissolved in the plant water or ions electrostatically bound to substances such as the pectic acids present in the plant cell wall. Elements in this group have important roles as enzyme cofactors and in the regulation of osmotic potentials.
4. The fourth group, comprising metals such as iron, has important roles in reactions involving electron transfer.

This classification may also not be considered absolute as many elements possess several functional roles. On this account the elements placed under different groups would overlap.

Table 3.2 : Classification of plant mineral nutrients according to biochemical function

Mineral nutrient	Functions
Group 1	Nutrients that are part of carbon compounds
N	Constituent of amino acids, amides, proteins, nucleic acids, nucleotides, coenzymes, hexosamines, etc.
S	Component of cysteine, cystine, methionine. Constituent of lipoic acid, coenzyme A, thiamine pyrophosphate, glutathione, biotin, 5'-adenylsulfate, and 3'-phosphoadenosine.
Group 2	Nutrients that are important in energy storage or structural integrity
P	Component of sugar phosphates, nucleic acids, nucleotides, coenzymes, phospholipids, phytic acid, etc. Has a key role in reactions that involve ATP.
Si	Deposited as amorphous silica in cell walls. Contributes to cell wall mechanical properties, including rigidity and elasticity.
B	Complexes with mannitol, mannan, polymannuronic acids, and other constituents of cell walls. Involved in cell elongation and nucleic acid metabolism.
Group 3	Nutrients that remain in ionic form
K	Required as a cofactor for more than 40 enzymes. Principal cation in establishing cell turgor and maintaining cell electroneutrality.
Ca	Constituent of the middle lamella of cell walls. Required as a cofactor by some enzymes involved in the hydrolysis of ATP and phospholipids. Acts as a second messenger in metabolic regulation.
Mg	Required by many enzymes involved in phosphate transfer. Constituent of the chlorophyll molecule.
Cl	Required for the photosynthetic reactions involved in O ₂ evolution.
Mn	Required for activity of some <i>dehydrogenases</i> , <i>decarboxylases</i> , <i>kinases</i> , <i>oxidases</i> , and <i>peroxidases</i> . Involved with other cation-activated enzymes and photosynthetic O ₂ evolution.
Na	Involved with the regeneration of phospho enolpyruvate in C ₄ and CAM plants. Substitutes for potassium in some reactions.
Group 4	Nutrients that are involved in redox reactions
Fe	Constituent of cytochromes and nonheme iron proteins involved in photosynthesis, N ₂ fixation, and respiration.
Zn	Constituent of <i>alcohol dehydrogenase</i> , <i>glutamic dehydrogenase</i> , <i>carbonic anhydrase</i> , etc.
Cu	Component of <i>ascorbic acid oxidase</i> , <i>tyrosinase</i> , <i>monoamine oxidase</i> , <i>uricase</i> , <i>cytochrome oxidase</i> , <i>phenolase</i> , <i>laccase</i> , and plastocyanin.
Ni	Constituent of <i>urease</i> . In N ₂ -fixing bacteria, constituent of <i>hydrogenases</i> .
Mo	Constituent of <i>nitrogenase</i> , <i>nitrate reductase</i> , and <i>xanthine dehydrogenase</i> .

From the physiological point, essential elements in general serve the following three functions in plants.

Functions of Essential Elements

Essential elements in plants serve the following three functions:

1. **Osmotic Function:** Plant cells generally contain mineral ions 10 to 1000 times higher in concentration than the surrounding soil. That is why water enters the cells by osmosis and builds up turgor. You know that turgor maintains the shape and size of non-rigid plant part such as leaves. Potassium is a key ion in this respect. Changes in its concentration in guard cells affect turgor and thus result in opening and closing of stomata. Turgor is also essential for the growth of plant cells.
2. **Structural Function:** The elements nitrogen, phosphorus and sulphur absorbed from the soil are essential components of amino acids and nucleotides. The other two elements that are constituents of important compounds are Mg^{2+} and Ca^{2+} . While Mg^{2+} is part of chlorophyll molecule and Ca^{2+} is an integral part of the middle lamella and is thought to be essential for maintaining structural characteristics of cells walls. It also maintains the permeability of plasma membrane. In its absence cells begin to leak out. In recent years, a new regulatory role that Ca^{2+} plays in the cell in beginning to be appreciated. It plays the role of a second messenger.
3. **Biochemical Function:** Elements Mg, Mn, K, Ca, and Fe are cofactors for many enzymatic reactions. Fe is carrier of electrons in electron transfer chain. Phosphorus plays a great role in chemical reactions in a cell. Phosphorylated sugars are essential for photosynthesis and respiratory metabolism, and phosphorus in the form of phosphate is essential for the formation of the high-energy bonds of ATP.

Interestingly some naturally occurring elements other than the essential elements get accumulated in the plant tissue. This is because some plants may exhibit additional requirements and tend to require these elements in addition to the essential ones. Such elements are categorized as **Beneficial elements**. Incidentally, they cannot now be included into micronutrients/trace elements as the present analytical techniques do not give a very reliable concentration of these elements. The notable examples are sodium, selenium, and cobalt.

- Sodium has been shown to function like a micronutrient specifically the C_4 plants like *Atriplex vesicaria* where its deficiency leads to chlorosis and even necrosis of leaves.
- Silicon is beneficial to many species like grasses and *Equisetum*. Its presence helps to prevent lodging and resistance to fungal infections.
- Cobalt is a component of vitamin B_{12} (Cobalamin) which itself a part of many enzymes that participate in nitrogen fixation in microbes. Cobalt requirement has been demonstrated in both free-living as well as symbiotic nitrogen-fixing bacteria.

- Selenium is generally considered to be toxic to plants, yet many plants growing in poorly drained arid regions tend to accumulate the salts of this element. For example, a legume *Astragalus* can tolerate a very high concentration of selenium. High concentration of selenium is toxic to many plants.
- Aluminum has been reported to stimulate plant growth in isolated instances.
- Gallium has been found to have some role in the growth of the fungus *Aspergillus niger*.
- Vanadium is also needed in traces by the diatom *Scenedesmus*.

SAQ 2

- a) Which of the following statements are true? Write T for true and F for false in the given brackets.
- i) The matter which is left after drying the plant tissue is called ash. []
 - ii) Plants contain more calcium than phosphorus. []
 - iii) The elements that are found in concentration 9m mole kg^{-1} of dry weight are called micronutrients. []
 - iv) The element sodium plays a key role in maintaining the turgor of plant cells. []
- b) Fill in the blanks with appropriate words:
- i) The key element that performs osmotic function in plants is
 - ii) maintains the permeability of plasma membrane.
 - iii) Mg^{2+} is part of molecule.
 - iv) is carrier of electrons in electron transfer chain.

3.4 ROLE OF ESSENTIAL ELEMENTS

Macronutrients, as you can gather from the name that are the nutrients required by the plants in large amounts. These include nitrogen, phosphorus, potassium, sulfur, calcium and magnesium. These are obtained from the soil. Macronutrients play a very important role in plant growth and development. Generally, application of macronutrients increases yield, growth, and quality of crops because nutrients are involved in every step of plant life. Every macronutrient has its own character, and is therefore, involved in different metabolic processes of plant life. Their functions range from being structural units to redox-sensitive agents.

3.4.1 Macronutrients

Nitrogen (N)

In the atmosphere nitrogen (N) is present as gas (N_2) to the extent of 79%. However, plants with a few exceptions cannot use it. From the soil only a very small portion of nitrogen is available to plants. The available forms of nitrogen in soil are NO_3^- and NH_4^+ ions. Because of the numerous factors which affect nitrogen turnover in the soil, the concentration of N dissolved in the soil solution can change considerably. This is particularly true of NO_3^- . Usually the NO_3^- content in the soil solution is of major importance in plant nitrogen nutrition.

Nitrogen is the mineral that plants require in greatest amount. Nitrogen is an indispensable elementary constituent of important organic compounds like amino acids, proteins, and nucleic acids. Dry plant material contains about 2 to 4% nitrogen. In green plant parts, protein nitrogen is by far the largest N fraction and accounts for 80 to 85% of the total nitrogen. In vegetative parts, the proteins are mainly enzyme proteins, whereas in seeds and grains, special storage proteins make up the major protein fraction. Nitrogen is also an essential constituent of various coenzymes.

Although nitrate ion is preferred, plants can absorb NH_4^+ as well. Crops mainly take up NO_3^- even when NH_4^+ fertilizers are applied because of the rapid microbial oxidation of NH_4^+ to NO_3^- in the soil. The rate of uptake of NO_3^- is generally very high as plants require large amounts of nitrogen. An important difference between uptake of NO_3^- and NH_4^+ ion is in their sensitivity to pH. NH_4^+ uptake takes place best in a neutral medium and it is depressed as the pH falls. The converse is true for NO_3^- absorption; a more rapid uptake occurs at low pH. Gaseous ammonia may also be absorbed by the upper plant parts via the stomata, but the quantity is very small.

Within the plant, nitrate is reduced to ammonia before it is incorporated into amino acids and proteins. In Unit 12, you will learn about metabolism of nitrogen.

The form in which translocation occurs depends on the nitrogen source and metabolism in the root. Nearly all the NH_4^+ ions absorbed are assimilated in the root tissue and translocated as amino acids. Nitrate can be translocated as such to shoots and leaves but this depends on the nitrate reduction potential of the roots.

When the supply of nitrogen from the root is inadequate, nitrogen from older leaves is mobilized to feed the younger plant organs. For this reason, plants suffering from N-deficiency first show deficiency symptoms in older leaves.

Nitrogen deficiency is characterized by a poor growth rate. The leaves are small, and the older ones often fall off prematurely. Shoot growth is affected and branching is restricted. Leaves deficient in nitrogen show **chlorosis** (yellowing of leaves) which is evenly distributed over the whole leaf. **Necrosis** of leaves occurs when deficiency is severe. Deficiency symptoms of Fe, Ca, S

are also characterized by yellowish and pale leaves like nitrogen deficiency. In these deficiencies, however, the symptoms occur first in the younger leaves. Nitrogen deficiency in cereals leads to poor tillering, the reduction in the number of ears per unit area and the number of grains per ear.

Nitrogen fertilizers supply NO_3^- and NH_4^+ to the soil. Between the two, NH_4^+ is partially adsorbed on soil colloids and its uptake rate is usually lower than NO_3^- under field conditions. For this reason, most crops do not respond as quickly to NH_4^+ fertilizers as to NO_3^- application.

In paddy fields, nitrogen is lost because of denitrification. These soils should not be given NO_3^- containing fertilizers. Hence urea and NO_4^+ fertilizers are recommended. The main drawback of anhydrous ammonia is the special equipment required for its transport and application.

Phosphorus (P)

In soil, phosphorus occurs almost exclusively in the form of orthophosphate. Substantial amount of P is associated with the soil organic matter. The major P-containing ions in soil solutions are HPO_4^{2-} and H_2PO_4^- , when the soil pH is less than 7.0.

Roots are capable of absorbing phosphate from solution low in phosphate content. The phosphate content of roots and xylem sap is about 100-1000-fold higher than that of the soil solution. This shows that phosphate is absorbed by plants against a very steep concentration gradient. Phosphate is highly mobile in the plant and can be translocated in upward or downward direction.

Inositol is a sugar (hexose) alcohol.

The inorganic form of phosphate found in plants are orthophosphate and to a minor extent, pyrophosphate. The organic form of phosphates are compounds in which the orthophosphate is esterified with hydroxyl groups of sugars and alcohols or bound through a pyrophosphate bond to another phosphate group. You know that phosphorylated sugars and alcohols are the major intermediary compounds of metabolism. Phosphate is also present in phospholipids. The nucleotide phosphates - ATP, UTP, GTP, CTP supply energy to various endergonic processes including active ion uptake and the synthesis of various organic compounds.

Another important phosphorus containing compound is **phytin** which is mainly found in seeds. It is Ca or Mg salt of phytic acid and is formed during seed formation. Phytic acid is the hexaphosphoric ester of inositol. Immediately after pollination there is an increase in P transport towards the developing seeds. Phosphorus in the phytin of seeds is regarded as a P reserve. During seed germination phytin is mobilized and converted into other phosphate forms that are needed in the metabolism of young plants.

Plants suffering from P deficiency are retarded in growth. In cereals tillering is reduced. Generally, the symptoms of P deficiency appear in the older leaves which become darkish green in color. The stems of many annual plant species suffering from P deficiency develop reddish color due to enhanced formation of anthocyanin pigments.

Potassium (K)

The main source of K^+ for plants comes from withering of potassium containing minerals. Potassium released by withering dissolves in the soil solution. It can then be taken up by plants or adsorbed onto soil colloids.

In plants, K^+ is an important cation. It is taken up by the plant at high rates through K^+ channels present in the membranes.

The concentration of K^+ in the cytoplasm is about 100 mM which is 5-10 times higher than K^+ concentration in the vacuole. The phloem sap is rich in K^+ . It is the most abundant cation present in phloem. As the solutes of the phloem sap can be translocated both upwards and downwards in the plant, K^+ movement is bidirectional.

Potassium is necessary for meristematic growth. It is involved in controlling water status of plants and maintains cell turgor. There is less water loss from plants supplied with K^+ due to a reduction in transpiration rate. As you know K^+ plays an important role in opening and closing of stomata. Plants inadequately supplied with K^+ have impaired stomatal activity. It is also involved in the translocation of photosynthates. The main biochemical function of K^+ is the activation of various enzymes.

Potassium deficiency does not immediately result in visible symptoms. At first there is only a reduction in growth rate. Chlorosis and necrosis appear later. These symptoms generally appear on the margins and tips of older leaves. Plants suffering from K^+ deficiency show a decrease in turgor. They easily become flaccid under water stress. Resistance to drought is, therefore, poor. Inadequate soil K^+ levels can be corrected using K^+ fertilizers. The most widely used and cheapest potash fertilizers is potassium chloride (KCl) which is known commercially as muriate of potash.

Sulphur (S)

Sulphur is present in the soil in inorganic and organic forms. The inorganic forms of sulphur in soil consists mainly of SO_4^{2-} . In arid regions, soil may accumulate high amounts of sulphur salts such as $CaSO_4$, $MgSO_4$, and Na_2SO_4 . Under humid conditions however, SO_4^{2-} is present either in soil solution or is adsorbed on soil colloids.

The organic S of the soil is made available to plants by microbial activity. In this process of mineralization H_2S is formed which under aerobic conditions readily undergoes auto oxidation and forms SO_4^{2-} . In anaerobic media, however, H_2S is oxidized to elemental S by chemotrophic sulphur bacteria such as *Beggiatoa*, and *Thiothrix*. Further oxidation of sulphur results in the formation of H_2SO_4 . As a result, increase in soil acidity can occur.

Plants mainly absorb S in the form of SO_4^{2-} . It is mainly translocated in an upward (acropetal) direction. Downward (basipetal) movement of S is relatively poor. There is now a considerable evidence to show that plants can utilize sulphur dioxide also.

Many plant species contain a small amount of volatile sulphur compounds. These are mainly di- or polysulphides. In onions these compounds are responsible for the lachrymatory (tear producing) effect. The main component of garlic oil is diallyl-disulphide.

The most important sulphur containing compounds are cysteine, methionine, lipoic acid, coenzyme A, biotin, thiamin, and ferredoxin (an electron carrier, a type of non-heme iron-sulphur protein). Sulphur forms disulphide bridges in polypeptides.

In field crops sulphur deficiency and nitrogen deficiency are sometimes similar and becomes difficult to distinguish. In plants suffering from S deficiency the rate of plant growth is reduced. Generally, the growth of the shoots is more affected than root. In contrast to nitrogen deficiency, chlorotic symptoms occur first in the younger, the most recently formed leaves.

Although the content of S in crops is like phosphorus content, S application does not play an important role as P fertilization. This is because SO_4^{2-} is not strongly bound to soil particles as phosphate and is thus more available to plants. In addition, substantial amounts of S can come from the atmosphere or from fertilizers which contain S along with other major nutrients being applied e.g. ammonium sulphate or potassium sulphate. The most important sulphur containing fertilizers are gypsum, superphosphate, ammonium sulphate and potassium sulphate.

Calcium (Ca)

Soils differ very widely in their Ca content. Plant species may be classified into calcicoles and calcifuges. The calcicoles are those growing on calcareous soils whereas the calcifuge species grow on acidic soils poor in Ca.

Generally, Ca^{2+} concentration of the soil solution is about 10 times higher than that of K^+ but the uptake rate of Ca^{2+} is usually lower than that of K^+ . This low Ca^{2+} uptake is because Ca^{2+} can be absorbed only by young root tips in which the cell walls of the endodermis are still not suberized. The uptake of Ca^{2+} can also be competitively depressed by K^+ and NH_4^+ which are rapidly taken up by roots. Calcium is translocated in an upward direction in the xylem with the transpiration stream. It is translocated only in very small concentrations in the phloem. Once Ca is deposited in older leaves it cannot be mobilized to the growing tips.

In the absence of Ca^{2+} , growth rate is reduced and after a few days the root tips become brown and gradually die. Ca^{2+} is required for cell elongation and cell division. It is essential for the stabilization of newly synthesized membranes. In the absence of Ca^{2+} , membrane permeability increases to such an extent that inorganic and organic constituents can diffuse out of the cell causing considerable damage to the cells. In whole plants, this disorder occurs first in the meristematic tissue such as root tips, growing points of the upper plant parts and storage organs.

Most of the Ca^{2+} present in plant tissues is located in the apoplast and in the vacuoles. The Ca^{2+} concentration of the cytoplasm is low. The maintenance of low cytoplasmic Ca^{2+} is of vital importance for the plant cell because evidence shows that high Ca^{2+} may inhibit various cytoplasmic enzymes, and precipitate as Ca-phosphate. The maintenance of low Ca^{2+} is achieved by pumping Ca^{2+} out of the cytoplasm into the apoplast or into the vacuole.

In the cytoplasm, the function of Ca^{2+} is related to calmodulin, which is involved in the activation of many enzymes by allosteric induction. Calcium is present in plant tissues as free Ca^{2+} adsorbed to indiffusible ions such as carboxylic, phosphoric, and phenolic hydroxyl groups. It is also present as Ca oxalates, carbonates, and phosphates. These compounds often occur as deposits in cell vacuoles. Calcium in the cell wall is associated with the free carboxylic groups of pectins.

Calcium deficiency is characterized by a reduction in growth of meristematic tissues. The deficiency can be first observed in the growing tips and young leaves. They become deformed and chlorotic. At a more advanced stage necrosis occurs at the leaf margins. The affected tissue becomes soft due to dissolution of the cell walls. Brown colored substances accumulate in intracellular spaces and in the vascular tissue where they can affect the transport mechanism. In apple the disease is called 'bitter pit' because the surface of the apple is pitted with small brown necrotic spots. In tomato the disease is known as 'blossom end rot' and is characterized by a cellular breakdown at the distal end of fruit.

Magnesium (Mg)

Magnesium like Ca^{2+} is present in high concentrations in the soil solution. Generally the concentration in soil solution is higher than that of K^+ but the rate of uptake of Mg^{2+} is much lower than uptake rate of K^+ , Mg^{2+} is very mobile in the phloem and can be translocated from older to younger leaves or to the apex.

Mg is constituent of chlorophyll molecule. It is a cofactor in almost all phosphorylation reactions. Mg^{2+} forms a bridge between the pyrophosphate structure of ATP or ADP and the enzyme molecule. The activation of ATPase by Mg^{2+} is brought about by this bridge formation. Another key function of Mg^{2+} is in the activation of ribulose biphosphate carboxylase. Light triggers the import of Mg^{2+} into the stroma of the chloroplast in exchange of H^+ thus providing optimum conditions for the carboxylase reaction. Mg deficiency inhibits protein synthesis.

Mg is mobile in the plant and deficiency always begins in the older leaves and then moves to the younger leaves. Intervinal yellowing or chlorosis occurs and in extreme cases the areas become necrotic.

Mg application is recommended for all crops growing on soils with less than 25 ppm exchangeable Mg. The major Mg fertilizers are, magnesium limestone (MgCO_3 – 5 to 20% MgO), ground burnt magnesium lime (Mg oxide – 10 to 33% MgO), kieserite ($\text{MgSO}_4 \cdot \text{H}_2\text{O}$ – 27% MgO), Epsom salt ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 16% MgO) and Magnesite (MgCO_3 – 45% MgO).

Silicon (Si)

In view of lack of any direct experimental evidence, some scientists have grouped Silicon as **beneficial elements** in place of essential element.

Plant species may be divided into Si accumulators and non-accumulators. The accumulators include paddy rice (*Oryza sativa*), horse tails (*Equisetum arvense*) and members of the Pinaceae, all of which contain 10-15% SiO_2 in

the dry matter. Other cereals, sugarcane, and several dicots with 1 to 3% SiO_2 are also included in this category. The non-accumulators are most of the dicots including the legumes with less than 0.5% SiO_2 .

Necrosis of older leaves and wilting associated with higher rates of transpiration are the typical deficiency symptoms. There is little biochemical evidence to justify Si as an essential element for higher plants, however, it shows several well-established beneficial effects on plant growth. In plants well supplied with Si, cuticular water loss is lowered because of the epidermal accumulation of silica. In cereals, the presence of silicon is important for keeping the leaves erect and decreasing susceptibility to lodging. In rice, a significant relationship is observed between the Si content of the straw and the yield of rice. Silicon especially promotes the formation of reproductive organ in rice. The important silica fertilizers are soluble silicates, sinter phosphates and Ca silicate slags.

SAQ 3

- a) Fill in the blanks in the following sentences:
- The form of nitrogen preferred by plant is.....
 - Largest fraction of N present in plant is in the form of
 - The symptoms of nitrogen deficiency first appear in..... leaves.
 - Calcium is mainly translocated through
 - The plants suffering from P deficiency develop colour in stems.
 - is a highly mobile element in plants.
 - controls the water status of plant and maintains cell
 - Basipetal movement of is relatively poor.
- b) List four magnesium fertilizers.
- c) Match the content of Column I corresponding with those of Column II.

Column I	Column II
i) Magnesium	a) deficiency affects cell division and cell elongation.
ii) Calcium	b) cofactor in all phosphorylation reactions.
iii) Sulphur	c) component of coenzyme A.

3.4.2 Micronutrients

Micronutrients are nutrients that are required in very small amount for normal plant growth. In comparison to macronutrients; micronutrients are required in

very small amounts. Here we are going to describe nine elements and their roles as micronutrients. These are Fe, Mn, Zn, Cu, Mo, B, Cl, Co and Ni. Although vital, some micronutrients can be highly reactive and toxic, so plants control their uptake, distribution at subcellular level.

Iron (Fe)

Iron is present in all soils. Soluble Fe in soil is extremely low in comparison with its total content. Soluble inorganic forms are Fe^{3+} , $\text{Fe}(\text{OH})^{2+}$ (hydroxoferric ion), $\text{Fe}(\text{OH})_2^+$ (dihydroxoferric ion) and Fe^{2+} . Fe must be reduced before it can be taken up by the roots. Iron in the free spaces of roots may be present in the ionic form or as chelate. The reduction of Fe^{3+} chelate destabilizes the complex and the resulting Fe^{2+} can be absorbed. The rate of Fe reduction is pH dependent and is higher at low pH.

The uptake of iron is competitively inhibited by other cations, like Mn^{2+} , Cu^{2+} , Ca^{2+} , Mg^{2+} , K^+ and Zn^{2+} . Iron is not readily mobile between the various plant parts. Green plants lacking Fe become chlorotic in the younger plant parts, whilst older parts remain green. Younger parts are, therefore, dependent on a continuous supply through the xylem. The major form in which Fe is translocated in the xylem is considered to be as ferric citrate.

The well-known function of Fe is in enzyme systems in which heme forms the prosthetic group. Here, Fe plays a somewhat similar role to Mg in the chlorophyll molecule. The heme enzyme system includes catalase, peroxidase, cytochrome oxidase and the various cytochromes. Another important compound containing Fe is the non-heme iron protein 'ferredoxin' which is an iron sulfur protein. It is an electron carrier in photosynthetic light reactions.

The deficiency of Fe and Mg are similar as both are characterized by a failure of chlorophyll production. Iron deficiency, however, unlike Mg deficiency, always begins in the younger leaves, the darker green veins contrasting markedly against a lighter green or yellow background. The youngest leaves may often be completely white and totally devoid of chlorophyll. In the leaves of cereals, the deficiency is shown by alternate yellow and green stripes along the length of the leaf.

Iron toxicity is particularly a problem in flooded rice soils, since within a few weeks of flooding it may increase 500-1000 folds. The iron toxicity in rice is known as '**Bronzing**'. In this disorder the leaves are first covered by tiny brown spots which develop into a uniform brown color. This frequently occurs in rice leaves containing excessively high Fe concentrations.

In the treatment of Fe chlorosis, the addition of inorganic Fe salts to the soil is mostly without effect for the Fe is rapidly made insoluble as oxides. Foliar treatment with ferrous salts is also not satisfactory. Iron chelates are more effective and can be used as fertilizers applied to the soil or as a foliar spray.

Manganese (Mn)

Mn^{2+} and Mn oxides in which Manganese is present in trivalent or tetravalent forms are the important soil fractions. In soil solutions, divalent Mn is the most important form.

When soils are waterlogged a reduction of Fe^{3+} to Fe^{2+} occurs which is accompanied by an increase in solubility. Anaerobic bacteria which use Fe oxides as electron acceptors in respiration, bring about this reduction. This process is of particular interest in paddy soils where it can lead to high Fe^{2+} concentrations. Such high concentrations often produce toxic effects in rice plants.

Mn^{2+} resembles Mg^{2+} in its biochemical functions. Both ions bridge ATP with the enzyme complex. Decarboxylases and dehydrogenases of the TCA cycle are also activated by Mn^{2+} . Nevertheless, Mn^{2+} is not specific for these enzymes and can be substituted by Mg^{2+} . The most well documented role of Mn in green plants is in the Hill reaction of photosynthesis where a manganese protein catalyses water splitting and O_2 evolution.

Mn deficiency causes disorganization of the lamellar system of chloroplasts. Mn deficiency symptoms resemble Mg deficiency, as in both cases interveinal chlorosis occurs in the leaves. In contrast to Mg deficiency, however, Mn deficiency symptoms are first visible in the younger leaves whereas in Mg deficiency older leaves are first affected. Organic soils high in pH are particularly low in available Mn and it is in crops growing on these soils that Mn deficiency often occurs. Application of Mn salts to the soil, e.g. MnSO_4 , usually does not alleviate the deficiency because the applied Mn^{2+} is rapidly oxidized. Spraying 1 to 5 kg MnSO_4/ha is the best way of removing the deficiency in most crops. Of the organic Mn carriers, Mn-EDTA gives the best response.

Zinc (Zn)

Recently a very special role that Zinc plays in gene activation has been discovered. There are proteins which act as transcription factors. These are folded in the form of fingers with zinc liganded at the bottom of each finger. Interestingly, as soon as the fingers touch the DNA the gene is turned on.

In its function, Zn^{2+} resembles Mn^{2+} in that it brings about substrate binding and conformational changes in enzymes. Several enzymes are thus activated in the same way by Mn^{2+} , Mg^{2+} , or Zn^{2+} .

Zn is actively involved in the nitrogen metabolism of the plant. In Zn deficient plants, protein synthesis and protein levels are markedly lowered, and amino acids and amides accumulate. Zn deficiency affects protein metabolism through the inactivation of RNA polymerase. It affects structural integrity of ribosomes and enhances RNA degradation by increasing RNAase activity. Zn is also required for the synthesis of indole acetic acid from tryptophan.

Plants suffering from Zn-deficiency often show chlorosis in the interveinal areas of the leaves. They become pale green, yellow or even white. In fruit trees leaf development is adversely affected. Unevenly distributed clusters or rosettes of small stiff leaves are formed at the ends of the young shoots. Frequently, the shoots die off and the leaves fall prematurely. In apple trees the disease is known as '**rosette**' or '**little leaf**' and in rice as **Khaira disease**.

Zn toxicity may occur in areas of Zn ore deposits. Some plant species are, however, Zn tolerant and can withstand high soil Zn levels.

Zinc deficiency is one of the most common micronutrient deficiencies and it is becoming increasingly important in areas of high yield agriculture. Plant species and even cultivars vary considerably in the susceptibility to Zn deficiency.

Zn deficiency can be alleviated either by spraying or by soil application of Zn fertilizers. ZnSO_4 is the most used fertilizer because of its high solubility. On acid sandy soils it is preferable to spray the crop because ZnSO_4 is very easily leached from soil.

Copper (Cu)

Copper occurs in the soil almost exclusively in its divalent form. Copper is absorbed by the plant only in very small quantities. Zn inhibits the uptake of Copper and vice versa. It is not definitely known whether Cu is taken up as Cu^{2+} or as copper chelate. Copper is not readily mobile in the plant although it can be translocated from older to younger leaves.

Copper has several attributes which control its biochemical behaviour. Cu bound to protein participate in redox reactions which are mostly dependent on the valency change ($\text{Cu}^{2+} + e^- \longrightarrow \text{Cu}^+$).

The most important copper containing proteins are plastocyanin, superoxide dismutase, amine oxidases, ascorbic acid oxidase and lactase. Cytochrome oxidase, the terminal oxidase in the mitochondrial transport chain is one of the most well studied Cu containing enzymes. As in case of Fe, high concentration of Cu is found in chloroplasts. There is also a specific requirement of Cu in symbiotic N_2 -fixation. In the absence of Cu, nodule development and N_2 -fixation are depressed.

Cu deficiency symptoms appear first in the younger leaves. In cereals, the deficiency appears first on the leaf tips. The plants develop a bushy habit with white twisted tips accompanied by a reduction in panicle formation. Copper chelates can be used either as foliar spray or as soil dressing to overcome the deficiency.

Molybdenum (Mo)

Molybdenum is absorbed as molybdate (MoO_4^{2-}) ion by plants. Its uptake can be competitively reduced by (SO_4^{2-}). The requirement of plants for Mo is very low. Mo is an essential component of two major enzymes, nitrogenase and nitrate reductase. The functional mechanism of both enzymes probably depends on valency changes of Mo.

The most important function of Mo in plant metabolism is in nitrogen assimilation. Mo deficiency resembles N-deficiency. Older leaves becoming chlorotic first, but in contrast to N-deficiency, necrotic symptoms rapidly appear at the leaf margins because of nitrate accumulation. In Brassicaceae, in extreme deficiency of Mo, leaf laminae are not formed and only the mid rib is formed. The leaf thus appears like a whip and for this reason the deficiency is called 'Whip tail' disease.

Boron (B)

Boron (B) is absorbed by the plants as undissociated boric acid (H_3BO_3) and as borate ions (H_2BO_3^-). It has convincingly been shown that boron is an essential element for higher plants. Unlike other plant nutrients boron is not known to be a component of any enzyme. Boron is necessary for sugar translocation. It combines with sugar to form sugar borate complex which can easily be transported.

Abnormal or retarded growth of the apical growing points is the first deficiency symptom. The youngest leaves are malformed, wrinkled or are often thicker and of darkish blue green in colour. The leaves and the stem become brittle indicating a disturbance in transpiration. As the deficiency progresses the

terminal growing point dies and flower and fruit formation is restricted or inhibited. Boron is known to have a role in germination of pollen and formation of pollen tubes. Thus, plants growing on boron deficient soils show a disturbance in pollen germination and impairment of fruit formation. In some plant species, the affected growth of pollen leads to parthenogenesis. The most well-known B deficiency symptoms are 'crown' and 'heart rot' in sugar beet.

Boron deficiency is more pronounced in a wide range of crops under wide climatic conditions than deficiencies of any other micronutrient. The most well-known boron fertilizer is borax ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$). Borated super phosphates are also available. Boric acid (H_3BO_3) is frequently applied as leaf spray particularly when the soil is potentially capable of fixing high amounts of boron.

Chlorine (Cl)

The role of Cl in plant is not much understood. Chlorine anion (Cl^-) is required in Hill reaction, the water splitting reaction of photosystem II about which you will study in Unit 5 and 6. In the presence of Cl^- , both the evolution of O_2 and photophosphorylation are enhanced. Cl^- may also influence photosynthesis indirectly via its effect on stomatal regulation of the guard cells.

Wilting of leaves at the margin is a deficiency symptom of chloride. Cl^- deficiency is noticed only rarely. The presence of Cl^- in the atmosphere and in the rainwater is more than enough to meet the demand of the crops. In facts, its presence in excess in plants is a more serious problem. Crops growing on salt affected soils often show symptoms of Cl^- toxicity. These are burning of leaf tips or margins, bronzing, premature yellowing, and abscission of leaves.

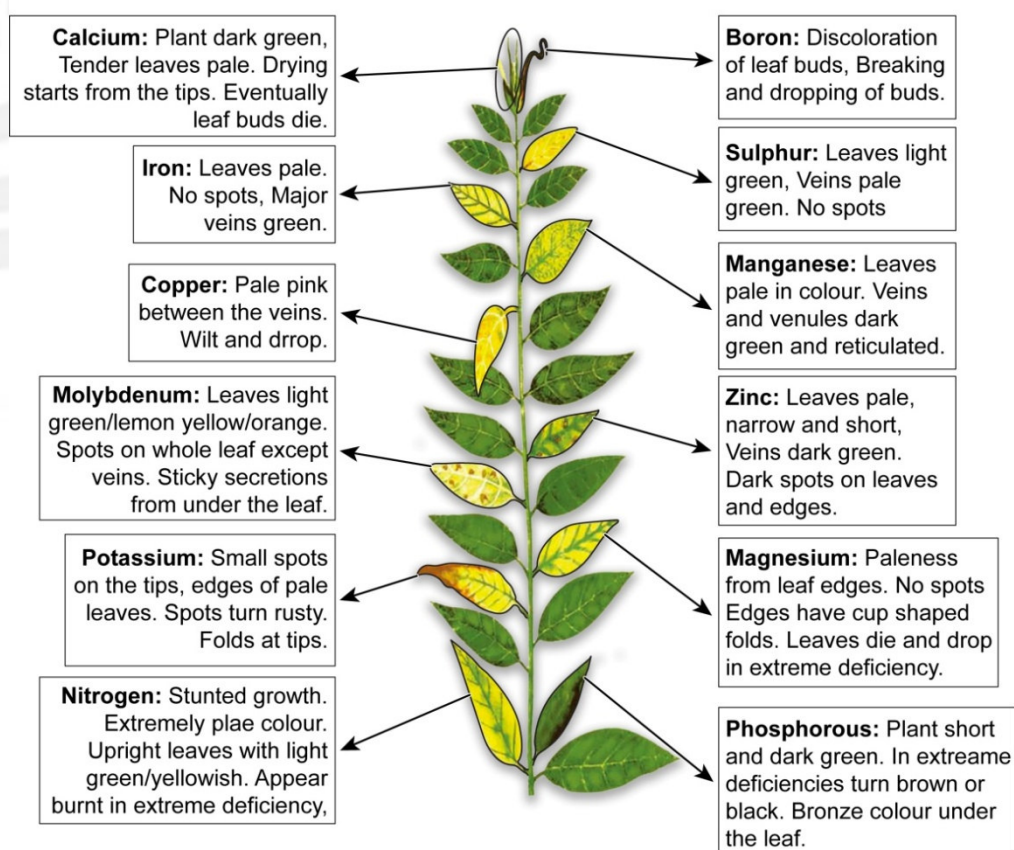


Fig.3.5: Deficiency chart of some nutrients. Colours are only indicative and may vary from plant to plant.

Cobalt (Co)

The Co concentration in the dry matter of plants grown in soil normally lies around 0.02 to 0.5 ppm. In soils the content varies from 1 to 40 ppm. Cobalt is not readily mobile in the plant.

Co is essential for symbiotic N_2 -fixation. Increasing the supply of Co increases rhizobial growth, N_2 -fixation, and the formation of leghemoglobin in nodules. Co is essential component of vitamin cyanocobalamin.

Nickel (Ni)

Nickel has been recently added to the list of essential trace elements. It constitutes the prosthetic group for the enzyme *urease*. It is believed to be important for mobilization of nitrogen during seed germination. In addition, this element is required for the microbes for its *hydrogenase* enzyme.

Table 3.2 : Deficiency symptoms of elements

Symptoms	Element Deficient
A. Older leaves affected (i) Effects mostly generalized over whole plant, lower leaves dry up and die. Plants light green, lower leaves yellow, drying to brown, stalks become short and slender. (ii) Effects mostly localized, mottling or chlorosis, lower leaves do not dry up but become mottled or chlorotic, leaf margins cupped or tucked. - Leaves mottled or chlorotic, sometimes reddened, necrotic spots, stalks slender. - Mottled or chlorotic leaves, necrotic spots small and between veins or near leaf tips and margins, stalks slender. - Necrotic spots large and general, eventually involving veins, leaves thick, stalks short.	Nitrogen Phosphorus Magnesium Potassium Zinc
B. Young leaves affected (i) Terminal buds die, distortion and necrosis of young leaves. - Young leaves die at tips and margins. - Young leaves light green at bases, die from base, leaves twisted.	Calcium Boron
(ii) Terminal buds remain alive, but chlorotic or wilted, without necrotic spots. (iii) Young leaves wilted, without chlorosis, stem tip weak. (iv) Young leaves not wilted, Chlorosis occurs. Small necrotic spots, veins remain green. - No necrotic spots. - Veins remain green. - Veins become chlorotic	Copper Manganese Iron Sulphur

Deficiency of Nickel results in accumulation of urea in leaves which leads to necrosis of leaf tips.

The levels of mobility of different elements determine to a larger extent whether the younger or older tissues will be affected first. K, N, Mg, P, Cl, Na, Zn and Mo are mobile elements. On the other hand, Ca, S, Fe, B and Cu are immobile. The following table illustrates the pattern of appearance of nutrient deficiency symptoms.

SAQ 4

- a) List three molecules that contain heme iron in their prosthetic group.
- b) State whether the statements are True or False:
 - i) The iron toxicity in rice is known as 'bronzing'.
 - ii) Mn^{2+} and Mg^{2+} resemble in their biochemical functions.
 - iii) Mn deficiency symptoms are first visible in older leaves.
 - iv) Zn is not actively involved in N metabolism.
 - v) Very low concentration of copper is found in chloroplast of the cell.
 - vi) The 'whip tail disease' in Brassicaceae is caused because of Mo deficiency.
 - vii) Boron is necessary for sugar translocation.
 - viii) Cl has no influence on photosynthesis.
 - ix) Silicon promotes the formation of reproductive organ in rice.
 - x) Cobalt is not readily mobile in the plant.
- c) List three important compounds containing copper.

3.5 MICROBES, MYCORRHIZA AND MINERAL UPTAKE

Roots are not the only living components residing in soil and utilizing its nutrients. The rhizosphere also contains a vast variety of microbes which also compete for these mineral nutrients. The rhizosphere is rich in bacteria as the zone is rich in nutrient content. In addition, mucilage secretion by the roots attracts more colloidal matter from soil. **Mucigel** is a general term given to a mixture of root secretions, bacteria, and colloidal soil particles. These microorganisms do also enter a mutually beneficial relationship (symbiotic) with roots in the form of mycorrhizal associations.

Roots absorb different mineral ions in different areas, through uptake of some ions may be greater in the apical region, or zone of elongation or at the root hairs. Absorption of minerals from soil creates a nutrient depletion zone

around the rhizosphere. This must be overcome both by the ability of root to grow into fresh soil and to increase its capacity of mineral ion uptake. Association of roots with mycorrhizal fungi may enhance the nutrient availability to the root system and help to overcome the problem of nutrient depletion.

Mycorrhizae are widespread in the natural vegetation. They are associated with root systems of over 83% of dicots, 79% monocots, and all gymnosperms. You have also read about mycorrhizae in Unit 3 of BBYCT-133 course.

There are two types of Mycorrhizae:

The **Ectotrophic** Mycorrhizal fungi form thick sheath the form of a mantle around the roots. Some of the hyphae penetrate the cortex of the root and surround them forming a zone called **Hartig net** (Fig. 3.6). On the other end the mycelium extends into the soil. This association improves the capacity of the root system to absorb nutrients as the fungal hyphae, being thinner than root hairs, can reach beyond the nutrient depleted zones in the soil. Most of the mycorrhizal fungi are higher Basidiomycetes and Ascomycetes.

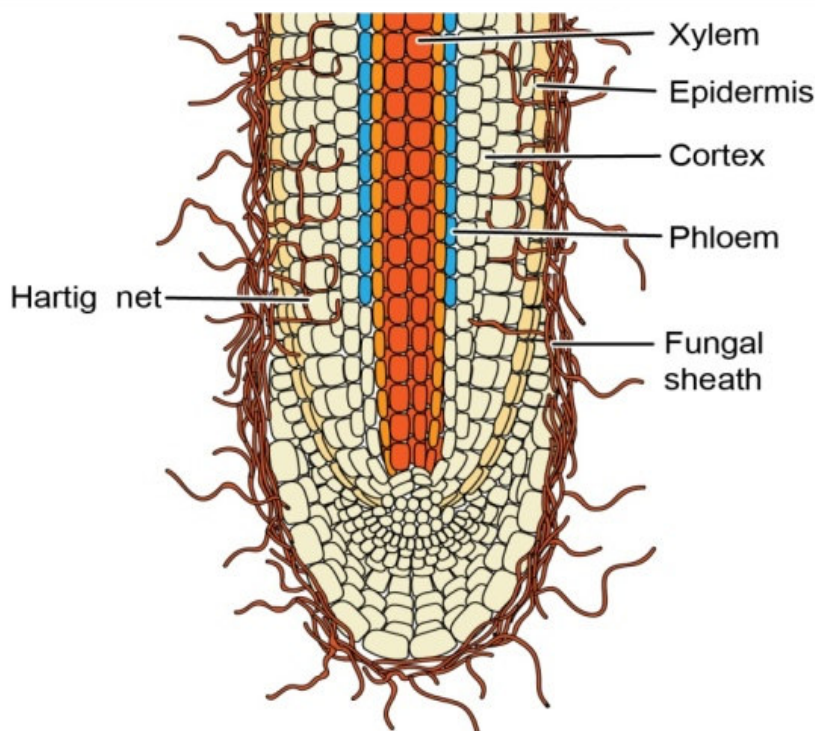


Fig. 3.6: Ectotrophic mycorrhizal fungi with mantle and Hartig net (From Taiz et al).

Another form of mycorrhizae viz., the **endotrophic** mycorrhizae or **arbuscular** mycorrhizal fungi grow in less dense arrangement and do not make a compact mantle fungal mycelium around the root. Instead, their hyphae grow and penetrate within the root by a manner like the one adopted by the symbiotic nitrogen fixing bacteria (discussed in Unit 13 on Biological Nitrogen Fixation). Once inside the root tissue, the fungal hyphae grow in the intercellular spaces and penetrate the individual cortical cells forming oval **vesicles** or branched **arbuscules**. Maximum nutrient transfer between the fungus and the host plant takes place at the arbuscules (Fig. 3.7).

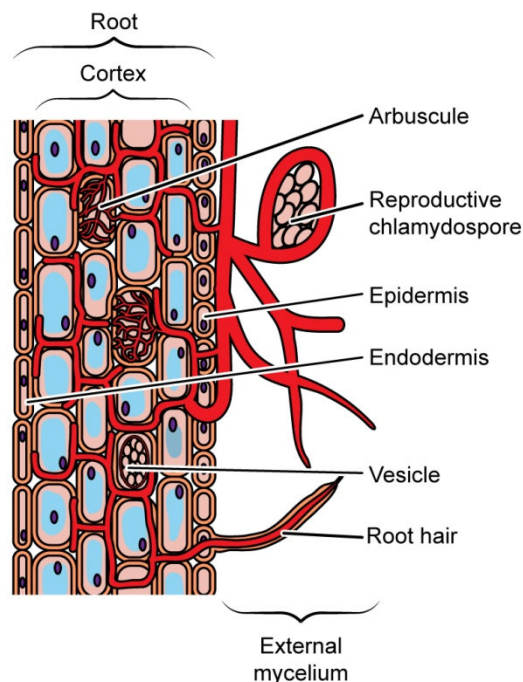


Fig. 3.7: Arbuscular mycorrhizal fungal – root association (From Taiz *et al*).

On the outside the mycelium extends much away from the nutrient depletion zone thereby benefitting the plants. Such arbuscular mycorrhizae are found in most of angiosperms and gymnosperms and major agricultural crops. This symbiotic association facilitates the uptake of water, phosphorus and microelements like zinc and copper.

3.6 SUMMARY

In this unit you have studied that:

- On an average mineral elements account for about 1.5 per cent of fresh weight of plant. Not all the elements detected may be essential for a plant. Essential elements are necessary for the completion of the plant's life cycle.
- Besides, C, H, and O that make the backbone of organic molecules, N, P, K, Ca, S, Mg and Si are required by plants in a relatively large amounts and are referred to as macronutrients, whereas Fe, Cu, Mn, Zn, Cl, B, Co, Ni and Mo are required in lesser amount and are referred to as micronutrients.
- Mineral elements perform structural osmotic and biochemical functions in plants.
- Plant obtains carbon, hydrogen and oxygen from CO₂ and water. Other elements are absorbed by roots from the soil in ionic form.
- Nitrate and ammonia are two nitrogen sources of plants. Between the two, NO₃⁻ is the preferred source and is absorbed at low pH. Nitrate may be translocated as such or may get reduced to nitrite in the roots before translocation. The deficiency symptom chlorosis appears first in older leaves and plants show poor growth rates.

- Phosphorus is absorbed by plants against steep concentration gradient. It is highly mobile. Its deficiency manifests by the formation of anthocyanin pigments and by the older leaves becoming darkish green.
- Potassium is required in large amount because it maintains turgidity of cells. In its deficiency plants resistance to drought is poor.
- Sulphur is an important constituent of several compounds in plants. Its deficiency results in poor growth rates.
- Calcium is required for cell elongation and cell division. In its deficiency the permeability of membranes increases and thus causes damage to cells.
- Mg and Mn are cofactors in phosphorylation reactions. Mg is part of chlorophyll. Mn and Cl are necessary for an important reaction in photosynthesis.
- Iron, copper, and Mo are constituents of proteins that take part in redox reactions. Iron and Mg both are necessary for chlorophyll synthesis, zinc for protein synthesis and Cobalt for N_2 -fixation.
- The root fungal symbiotic relationships in the form of mycorrhiza facilitate the procurement of minerals for the plant, especially phosphorus. In turn the plants provide the mycorrhizae with carbohydrates.

3.7 TERMINAL QUESTIONS

1. What is hydroponics? How it is set up and what are the disadvantages of this technique?
2. Describe the three criteria on which element can be classified as essential.
3. Describe the functions of essential elements.
4. Differentiate between macro and micronutrients and make a list of these nutrients.
5. Write a note on mycorrhizal fungi.

3.8 ANSWERS

Self-Assessment Questions

1.
 - a) mineral nutrients from soil
 - b) minerals and crop improvement
 - c) solution cultures
 - d) hydroponics
 - e) aeroponics

2. a) i) False; ii) True; iii) False; iv) False
- b) i) potassium
- ii) calcium
- iii) chlorophyll
- iv) iron
3. a) i) NO_3^-
- ii) proteins
- iii) older
- iv) xylem
- v) red
- vi) Magnesium
- vii) K^+ , turgor
- viii) sulphur
- b) The major Mg fertilizers are, magnesium limestone (MgCO_3 – 5 to 20% MgO) ground burnt magnesium lime (Mg oxide – 10 to 33% MgO), kieserite ($\text{MgSO}_4 \cdot \text{H}_2\text{O}$ – 27% MgO), Epsom salt ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 16% MgO) and Magnesite (MgCO_3 – 45% MgO)
- c) i) cofactor in all phosphorylation reactions.
- ii) deficiency affects cell division and cell elongation
- iii) Component of coenzyme A.
4. a) Any three of the following : cytochrome oxidase, catalase, peroxidase, cytochrome *f* and cytochrome *c*.
- b) i) True; ii) True; iii) False; iv) False; v) False;
- vi) True; vii) True; viii) False; ix) True; x) True.
- c) plastocyanin, cytochrome oxidase, lactase, ascorbic acid oxidase, superoxide dismutase.

Terminal Questions

1. Refer to Section 3.2.
2. Refer to Subsection 3.3.1.
3. Refer to Subsection 3.3.2.
4. Refer to Subsections 3.3.1, 3.4.1 and 3.4.2.
5. Refer to Section 3.5.

UNIT 4

NUTRIENT TRANSPORT |

Structure

4.1	Introduction Objectives	4.4	Radial Movement of Ions in Roots
4.2	Nutrient Absorption Nutrients and the Soil Uptake of Mineral Ions Movement of Nutrients in the Root Free Space	4.5	Long Distance Transport – Xylem Loading and Unloading
4.3	Transport of Ions across Plasma Membrane Simple Diffusion Membrane Transport Proteins Pumps and Active Transport	4.6	Summary
		4.7	Terminal Questions
		4.8	Answers

4.1 INTRODUCTION

In Unit 3 you have studied about the essentiality and role of macro and microelements in a plant. You were also made familiar with the deficiency symptoms arising in plants as well as the toxic effects of certain elements. The present unit aims to describe the physical and chemical principles governing mineral ion uptake across the cell wall and membranes of the roots. The role of aquaporins and membrane proteins has been discussed in detail along with the functioning of ion channels and pumps.

Objectives

After studying this unit, you would be able to :

- ❖ Describe the active and passive transport of mineral ions across the membranes;
- ❖ List the various routes like pumps, channels, and gates for the transport of mineral ions; and
- ❖ Describe the xylem loading and unloading.

4.2 NUTRIENT ABSORPTION

Except for carbon, hydrogen and oxygen which are provided by CO_2 and water, all the other elements essential for plants are provided by the soil. Hence, in this unit we will confine our discussion to those elements which are acquired from the soil by the plants.

4.2.1 Nutrients and the Soil

Early experiments on mineral uptake were performed by Hoagland, Stout and Arnon in 1923. They showed that minerals were taken up from the soil primarily in ionic form. The rate of uptake of different ions by roots varied and one ion influenced the uptake of other ions. As soil is the medium for the storage and exchange of mineral ions, its properties, ion exchange capacity, pH and the presence of different cations and anions affect the availability of ions to the plant. In other words, the presence of a certain mineral ions in abundance in the soil cannot ensure its availability to the plant because ions may adhere to clay or precipitate out of the solution as insoluble salts. The soil with high water holding capacity generally has high mineral holding capacity as well. The fine particles of clay and humus possess a relatively large surface to volume ratio and are negatively charged. In addition, to the high specific surface area these colloids also possess many charges (mostly negative) on the colloidal surfaces. Thus, the colloidal particles provide a very large surface area (upto 10000-fold) which allows the mineral elements in the soil solution to interact. Hence, they have higher ion-binding capacity than the soil composed of coarse particles. Figure 4.1 shows the colloidal clay crystals (micelles) with innumerable negative surface charges. The cations are loosely bound to negative charges by ionic bond and can exchange rapidly and reversibly with those in the soil solution. H^+ ions have greater affinity for charged soil particles than Ca^{2+} , Mg^{2+} or K^+ ions. Therefore, these cations are released in soil water by H^+ ions and made available for uptake by roots. The acidity of soil also increases due to respiration because CO_2 released reacts with soil water to form carbonic acid.

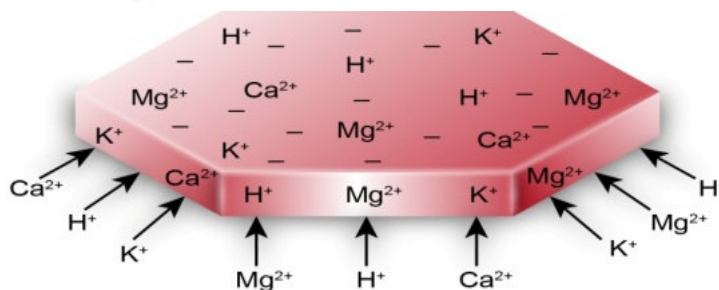


Fig. 4.1: Diagrammatic representation of the outside surfaces of a clay micelle. Note many negative surface charges and the various cations surrounding them.

Since acidification of soil increases the concentration of hydrogen ions in the soil which have a much stronger attraction for the colloidal surface charges on the clay micelle, they tend to displace other cations into the soil solution (Fig. 4.2).

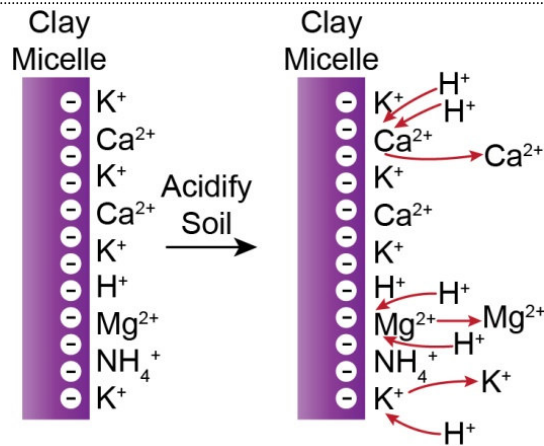


Fig. 4.2: Absorption of cations to the negatively charged soil particles. With an increase in soil acidity, the additional H^+ tend to displace other cations into the soil solution (From Hopkins & Hüner).

One of the simplest colloidal clays is **kaolinite** (alumino silicate) $Al_2Si_2O_5(OH)_4$. Ionization of alumina and silica at the edge of the clay particles gives rise to large number of negative charges. In addition, the colloidal carbon in soil also carries negative charges as it is derived mostly from the exposed carboxyl and hydroxyl groups of lignin and carbohydrates.

The ion exchange capacity of mineral ions is affected by the pH of soil which in turn affects the availability of different ions to the plant as shown in the graph below (Fig. 4.3).

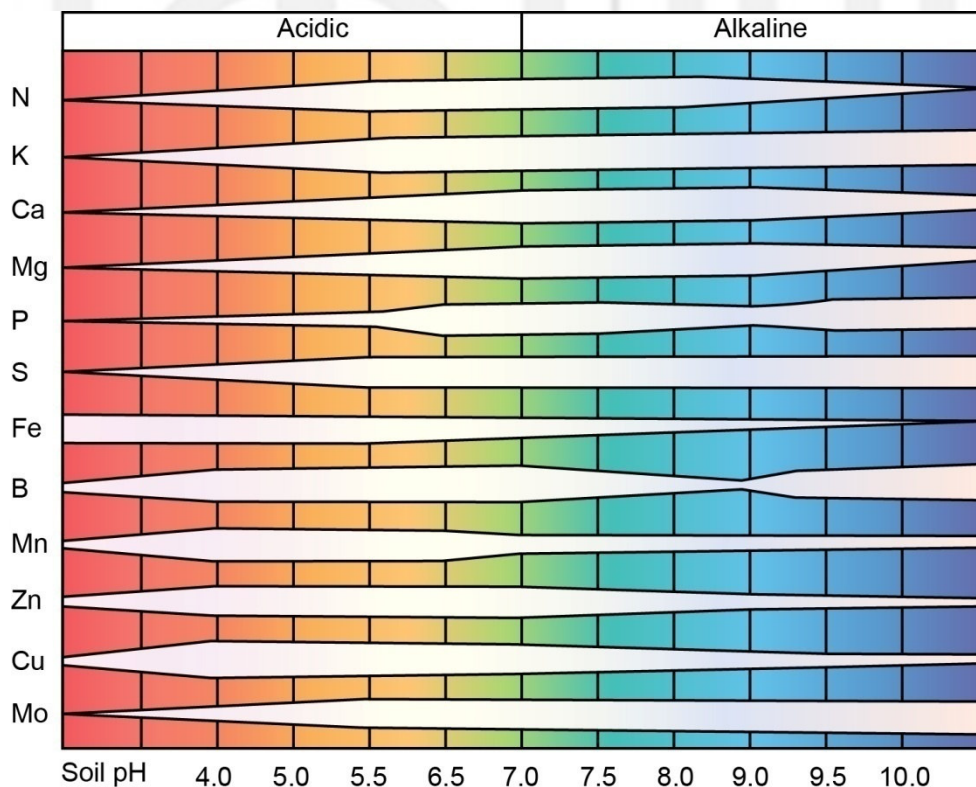


Fig. 4.3: The effect of soil pH on the availability of different ions to the plant. The wider the bar greater the availability.

4.2.2 Uptake of Mineral Ions

Let us now see, what happens to one solute concentration in the root cells when plants are raised for a few days in a nutrient solution of known

concentration. Data from a typical experiment are given in Table 4.1. Two plants, maize and beans are selected for comparison.

Table 4.1: Changes in ion concentration in the external nutrient solution and in the root sap of maize and bean (expressed in mM). The data are recorded after 4 days.

Ion	After 4 days				
	External Concentration			Concentration in the root sap	
	Initial Concentration	Maize	Bean	Maize	Bean
Potassium	2.00	0.14	0.67	160	84
Nitrate	2.00	0.13	0.07	38	35
Calcium	1.00	0.94	0.59	3	10
Sulphate	0.67	0.61	0.81	14	6
Sodium	0.32	0.51	0.58	0.6	6
Phosphate	0.25	0.06	0.09	6	12

Lyotropic series is a classification of ions in order of their ability to salt out or salt in proteins. First worked out by Franz Hofmeister, the series is also called as Hofmeister series. The series are arranged in an ascending or descending order of their influence on the properties of the solvent and on the degree of the chemical reactions and the physicochemical processes occurring there.

The binding affinity of different cations has been classified according to the lyotropic series $\text{Al}^{3+} > \text{H}^+ > \text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^+ = \text{NH}_4^+ > \text{Na}^+$ which indicates that the monovalent cations will be less strongly retained than the divalent ones and the trivalent ions will have the highest binding affinity. Since cation adsorption is a reversible phenomenon and any ions with a lower affinity (say Ca^{2+}) can be replaced with that a higher affinity (say H^+), this ion exchange capacity or ease of removal follows a pattern reverse of the lyotropic series. On that account, sodium ions are exchanged most readily while aluminium ions show least exchangeability.

The colloidal fraction with its adsorbed ions represents the main nutrient reservoir. However, it is the ions that are present in the soil solution which constitute the immediate source of mineral nutrients to the plants. We can view the entire soil system as a dynamic one where weathering of rocks particles are releasing ions that continuously replenish the colloidal reservoir. These exchangeable ions held by the colloidal system replace/replenish the free ions in the soil solution which have been taken up/consumed by the roots.

A careful study of the above Table 4.1 shows the following:

- The concentration of potassium, phosphate and nitrate declined significantly in the bathing medium within four days.
- The concentration of sodium and sulphate, however, increased indicating that water was absorbed faster than either of the two ions, or possibly they leach out.
- The rate of uptake especially of potassium and calcium, differed between the two plant species, maize, and bean.
- The ion concentration (particularly of K^+ , NO_3^- and SO_4^{2-}) in the root was considerably higher than in the nutrient solution used for the experiment.

These results show certain characteristics of nutrient uptake.

1. **Selectivity:** Certain mineral elements are taken up preferentially while the others are discriminated against or nearly excluded.
2. **Accumulation:** The concentration of mineral elements can be much higher in the plant sap than in external solution. This means the uptake is against concentration gradient.
3. **Genotype:** The nutrient uptake capacity of various plant species differs due to their different genetic makeup.

You know that chemically Na^+ resembles K^+ very closely but the rate of absorption of K^+ is not influenced by similar concentration of Na^+ ions in the medium. The process of K^+ absorption is, therefore, selective, and uninfluenced by a related ion. Similarly, several other monovalent and less related divalent ions also have no effect on K^+ uptake. Likewise, absorption of Cl^- is unaffected by related halides, fluoride and iodide, as well as other anions like NO_3^- , $\text{H}_2\text{PO}_4^{2-}$ or SO_4^{2-} . However, interestingly, Ca^{2+} is an absolute requirement for this selectivity. For example, in the absence of Ca^{2+} , the absorption of K^+ is inhibited by Na^+ .

Even though the ion uptake mechanism is highly specific, yet it can often 'fooled' by similar ions. For example, it has been seen that absorption of K^+ can be competitively inhibited by rubidium (Rb^+). Similarly, competitive inhibition of Cl^- by Br^- , Ca^{2+} , Sr^{2+} by Mg^{2+} , and sulphate by selenate (SeO_4^{2-}) has also been observed.

The selectivity and the rate of uptake of the nutrients and metabolites are influenced by temperature, O_2 , poisons, carbohydrate content of the tissues and light. Such effects are like enzyme-mediated reactions and indicate that proteins are involved in solute uptake. You will study about transport proteins and mechanism of ion uptake later in this unit.

4.2.3 Movement of Nutrients in the Root

In the previous Units 1 and 2, we discussed about the two main routes – apoplast and symplast – by which water and dissolved solutes are conducted across the root interior into xylem vessels and tracheids. The cell wall spaces and intercellular spaces in the root's epidermis and cortex are virtually continuous with the external soil solution.

As was mentioned in Unit 2, ions can move up into the root hair as well as epidermal cells. An ion that is absorbed by an epidermal cell and moving towards the xylem in the symplast must cross the epidermis, several cortical cells, the endodermis and the pericycle. The movement would involve transport directly through each of the two primary walls, middle lamella, and plasma membranes between the cytosol of adjacent cells. Alternatively, the solute can move through plasmodesmata without crossing the plasma membrane or diffusing through the cell walls.

The nutrient movement along the apoplast is prevented at root endodermal cells because these cells are lined with Casparian strips. Therefore, the water

and dissolved substances must enter the cell and pass through them via the symplastic route. Thus, all minerals must pass through the cytoplasm to reach the xylem.

With this background we are now ready to trace the path of a solute (or nutrient) entering the root from the surrounding soil solution.

If the cell full of ions is returned to a lower-salt solution, some of the absorbed ions will diffuse out into the external medium. This process of free diffusion of solutes follows the simple laws of diffusion. This free diffusion means free movement of ions in and out of the tissue. This part of the cell wall tissue is referred to as **Outer Space**.

4.2.4 Free Space

The first step in mineral uptake is the diffusion of ions through the cell wall into the apparent free space.

You have already studied that the primary cell wall of the plant cell consists of mainly cellulose microfibrils which are embedded in an amorphous matrix of two polysaccharides, hemicelluloses and pectic substances. Hemicelluloses are made of sugars other than glucose (e.g. xyloglucans) while pectic substances are partly made from polygalacturonic acids. These acids have weak carboxylic acid groups ($-\text{COOH}$) that ionize and give rise to negative charges ($-\text{COO}^-$) on which hydrogen ions are loosely held. When positively charged ions such as K^+ , Mg^{+2} , Ca^{2+} pass through the plant cell wall they displace hydrogen ions of the carboxyl groups and are held there by the weak inter-ionic attractive forces. The negative charges of the COO^- group cell wall are termed **cation adsorption sites** or **cation exchange sites** or **Donnan Free Space**. A cation such as Ca^{2+} with a relatively high adsorptive capacity will displace ions with a lesser adsorptive affinity (e.g. K^+).

The cellulose microfibrils are not very tightly packed; as a result, they leave small pores between them. The pores are large enough to allow free movement of water and dissolved substances. The diameter of these pores is in the range of 5.0 nm whereas, the dimensions of hydrated ions such as K^+ and Ca^{2+} are smaller (Table 4.2). So, the pores do not restrict the movement of ions. Water and dissolved nutrient molecules, ions, and metabolites of the size of glucose, sucrose, and amino acids diffuse readily across primary cell walls.

Table 4.2: Relative sizes of pores and molecules

Comparative Cell Wall of Maize	100-200
Pores of Cell Wall	> 5.0
Sucrose	1.0
Hydrated ions	
K^+	0.66
Ca^{2+}	0.82

The intercellular spaces, the negatively charged regions (Donnan Free Space) in the amorphous matrix and the pores in the cellulose microfibrils are readily accessible to water and dissolved ions. The fraction of the volume of the plant

tissue readily accessible to diffusion of an external solute dissolved in water is termed '**Free space**'. The free space in the root is bound by the plasma membrane of the epidermal and cortical cells and the casparian strip of the endodermis.

Any substance which can easily pass through the free space reaches the external surface of the protoplasm. Here, it encounters the plasma membrane which is an effective barrier to its movement further inward. Nevertheless, the plasma membrane does not act like a passive barrier, it can allow the passage of some substances into the cell interior while selectively inhibiting the passage of certain others. In the following section, we will discuss the transport of nutrients across the plasma membrane.

Apparent free space (AFS) is not separated from the environment by any membrane and ions can enter it by free diffusion. This space is negatively charged like the colloids and can also hold some cations by electrostatic attraction. Thus, this space denotes that portion which accommodates ions by free diffusion and where some ions are held electrostatically. The AFS is estimated around 10-25 percent of the root volume. The AFS consists of cell walls and the intercellular spaces, areas considered to be equivalent to the apoplast of the root epidermal and cortical cells which the ions can enter without crossing the membranes.

SAQ 1

- a) State whether the following statements are true/false.
- i) The apparent free space (AFS) is negatively charged. []
 - ii) The colloidal particles of soil provide a small surface area for interaction of mineral elements. []
 - iii) The exchange capacity of mineral ions is independent of pH of soil. []
 - iv) Passive diffusion is unaffected by use of metabolic inhibitors. []
 - v) Sodium ions are the most readily exchanged cations. []
- b) Tick mark the correct alternative from the words given in each parenthesis.
- i) The clay micelles have (negative/positive) charge. []
 - ii) Cytoplasmic strands connecting the adjacent cells are (plasmodesmata/casparian strip). []
 - iii) The ion exchange capacity is affected by (temperature/pH) of the soil. []
 - iv) The apoplastic pathway is broken at the (casparian strip/plasmodesmata) of the endodermal cells. []
 - v) The fixed negative charges formed by the weakly acidic carboxyl group of polygalacturonic acids form (cation exchange sites/anion exchange sites). []
 - vi) K^+ absorption can be competitively inhibited by (sodium/rubidium). []
-

4.3 TRANSPORT OF IONS ACROSS PLASMA MEMBRANE

After entering the free space, the mineral nutrients must now cross the plasma membranes before entering the root and begin long distance transport to the shoot. Transport across the root membranes follows certain rules based on our current knowledge of membrane composition, architecture, and function. Uptake of ions may be **passive** (occurring due to physical forces, independent of temperature, and unaffected by the application of metabolic inhibitors) or **active** (possible with expenditure of metabolic energy). Passive uptake of ions may also be mediated by transport proteins which facilitate the passage of selected ions and molecules. This includes three main types, viz., channels, carriers, and pumps. **Primary active transport** requires ATP to undertake a transport against the concentration gradient with the help of electrogenic pumps. **Secondary active transport**, on the other hand uses stored free energy (in the form of H^+ gradient) and indirectly driven by pumps like the proton motive force.

In this section, we will study the transport of solutes, particularly inorganic ions across the plasma membrane, their entry into the xylem elements and long-distance transport from root to shoot. The transport of substances across the membrane follow three basic modes, viz., simple diffusion, facilitated diffusion, and active transport (diagrammatically depicted in Fig. 4.4).

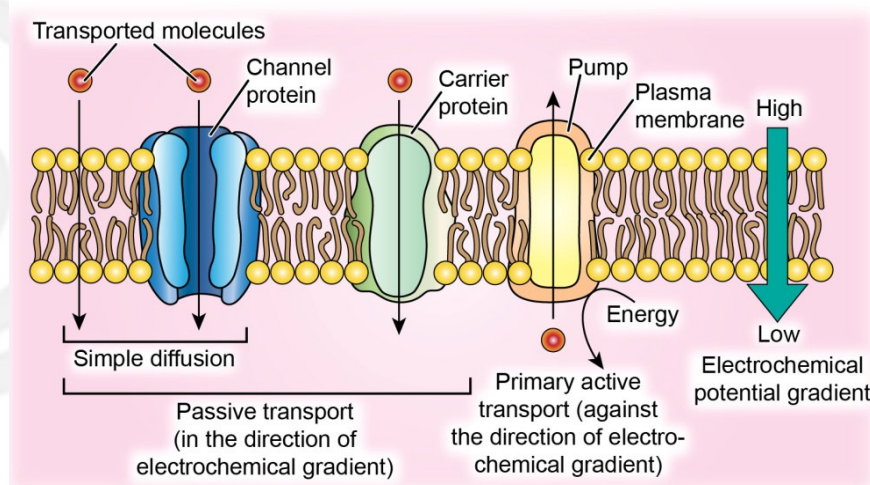


Fig. 4.4: Different routes for transportation of molecules across the plasma membrane (From Taiz *et al.*).

4.3.1 Simple Diffusion

Simple diffusion is one of the means for transport of ions across the plant cell membranes (Fig. 4.5). An artificial or synthetic lipid membrane made in the laboratory allows the non-polar solute molecules to pass through it more readily than the polar molecules. Over a period, these molecules can diffuse across the lipid bilayer down its concentration gradient by virtue of their own kinetic energy. However, the rate at which a molecule diffuses across a lipid bilayer depends on the size of the molecule and its relative solubility in the lipid. Non-ionic hydrophilic substances are generally taken up as inverse function of their size, whereas hydrophobic substances are transported as a function of their lipid solubility.

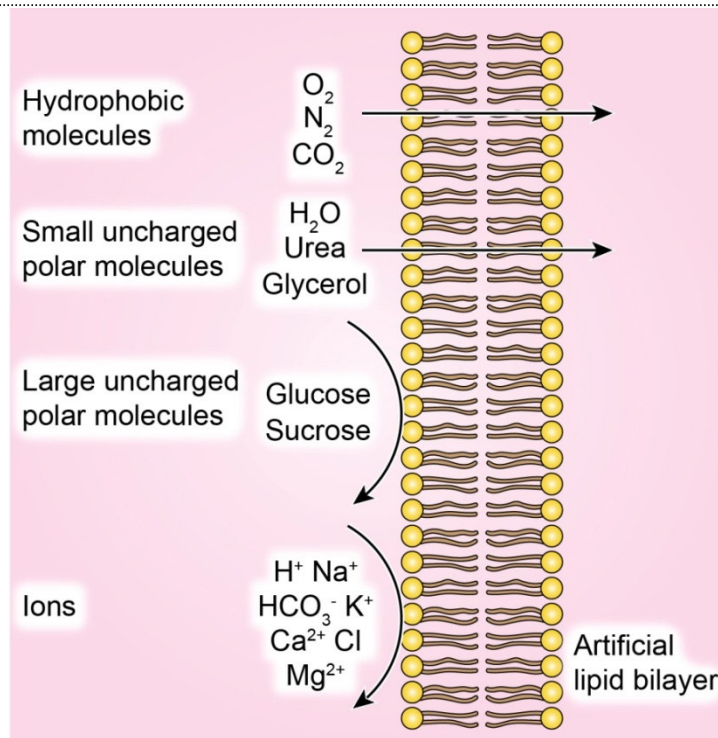


Fig. 4.5: The relative permeability of an artificial lipid bilayer to different class of molecules.

Small non-polar and hydrophobic molecules like O_2 and N_2 readily dissolve in lipids and therefore, rapidly diffuse across the bilayer. Ethanol (46 Da), carbon dioxide (44 Da) and Urea (60 Da) cross the bilayer rapidly while the larger glucose molecules (180 Da) do not. Interestingly, the water molecules (18 Da), even though polar and relatively insoluble in lipids diffuse very rapidly across the lipid bilayer through special water-selective channels or **aquaporins**.

Membrane lipid bilayers are relatively impermeable to charged ions, despite their small size. This is because the charge and large hydration shell around them prevents them from entering the hydrocarbon phase of the bilayer. Lipid bilayers are 10^9 times more permeable to water than to small ions as Na^+ or K^+ (Fig. 4.6). K^+ is more permeable than most ions whose permeability coefficient is arbitrarily set at 1.0. It is taken as a standard in solution to which the permeability of their ions is compared.

However, some solutes like O_2 , CO_2 and NH_3 are non-polar and readily diffuse through the lipid bilayer of the membrane. The ability of lipid bilayer to discriminate between low and high molecular weight hydrophilic materials, permitting the former to diffuse across but not the latter is due to the presence of pores in the bilayers. These pores, called “**statistical pores**” are formed randomly because of thermal movement of acyl phospholipid chains. Since these pores are only transient, they cannot be viewed even under the transmission electron microscope.

Transport by diffusion is a passive process and does not require a direct input of ATP. The driving force for diffusion comes from the concentration or electrochemical gradient of the solute being transported and obeys Fick's Law, which states that the rate at which molecules in a solution diffuse from one region to another is a function of their concentration difference. In other words, the rate of movement of a substance is directly proportional to the concentration gradient. As a result, solutes do not get accumulated against this gradient.

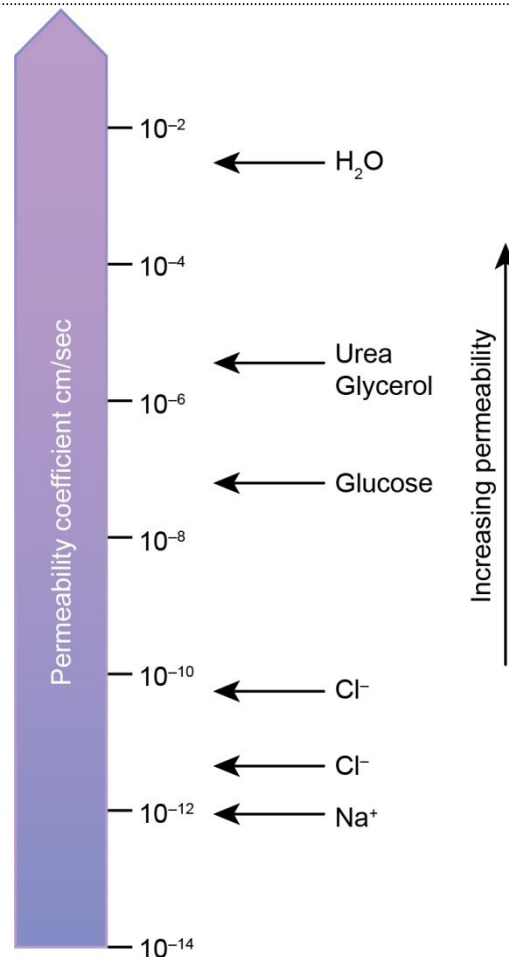


Fig. 4.6: Permeability coefficients cm s^{-1} for the passage of various molecules through artificial lipid bilayers.

4.3.2 Membrane Transport Proteins

Despite having some similarities, biological membranes differ on many counts with the artificial membranes. It has been recognized since long that certain ions enter the cells much rapidly than the lipid bilayer would theoretically allow them to do so. Even though the lipid bilayers do not permit the entry of polar molecules such as ions, sugars, amino acids, nucleotides and cell metabolites, these substances do pass through the biological membranes.

For this purpose, the biological membranes contain number of proteins, called **transport proteins**. Many of these specialized proteins facilitate or mediate diffusion of ions or charges solutes, which is otherwise not possible through a pure lipid bilayer. The solubility problem is overcome and this rapid, assisted diffusion of solutes across the membranes has been termed **facilitated diffusion**.

Since the concentration gradient (for uncharged solutes) or the electrochemical gradient (for charged solutes and ions) determines the direction of transport, i.e., it operates along a concentration gradient, it is also called **passive-mediated transport**.

There are three classes of membrane transport proteins, viz., **channels** (pores-simple and gated), **carriers** (transporters) and **pumps** (for pumping solutes against their concentration gradient or electrochemical potential by directly using ATP).

Channels

We can visualize channel proteins as charged-lined, water-filled channels of transmembrane proteins which extend across the membrane and act as selective pores. They allow specific ions or molecules to permeate through the channels. The transport specificity of these channels is determined by the size of the pore, the nature and density of the surface charges present on their inner lining. The channels are named/ identified based on the type and the hydrated size of ion species they allow. Only passive transport takes place through these channels and by virtue of the electric charge and pore size only water and ions can pass through. Large quantities of water and ions (10^8 ions per second) are transported in large through the channels.

The channel pores do not remain open all the time and many of them have proteins which act as gates which open and close in response to external signals. Such channels are called **gated channels** where a change in the three-dimensional conformation of the protein results in opening or closure of the pores. The “gates” may open in response to either:

- i) membrane potentials of magnitude. Such channels are called **electrically gated channels**; or
- ii) modulating signals like hormones, light or even phosphorylation.

Many channel proteins are believed to be inducible as they are synthesized only in the presence of sufficient solute. The presence of K^+ , Cl^- , and Ca^{2+} channels has been firmly established whereas there are indications of the presence of many more for other inorganic and organic ions. K^+ channels have been established in the guard cells (see Fig. 4.7). You have already read about the mechanism of stomatal opening and closure in Unit 2. It is now believed that a variety of different channels may exist for a common ion, but operative under different voltage ranges, or induced by different signals. This means that the permeability of a membrane will be variable from time to time depending upon the activity of these ion channels.

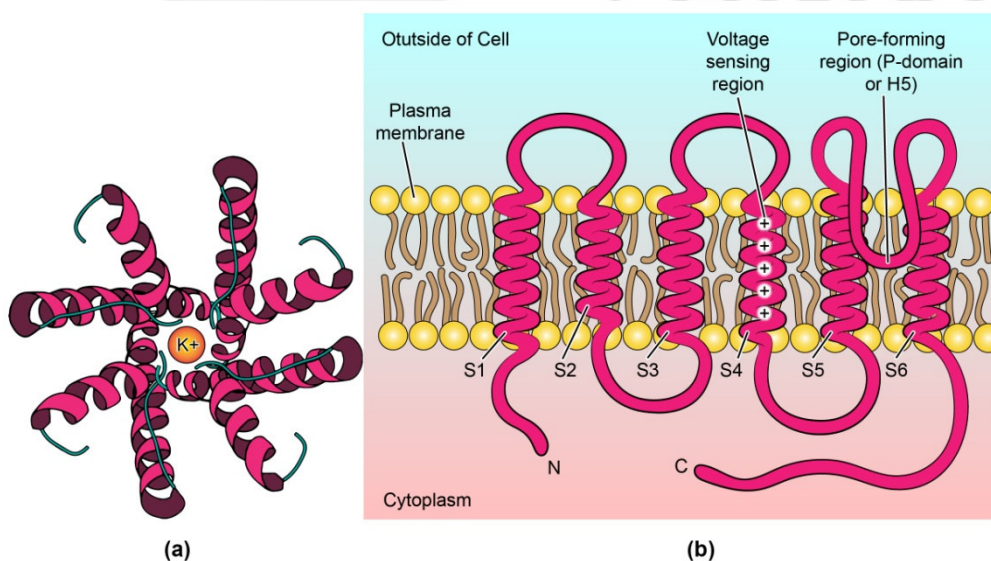


Fig. 4.7: a) A K^+ -channel showing a polypeptide chain of subunits; b) The pore-forming region is in the form of a loop between helices 5 and 6 (From Taiz *et al*).

Selective accumulation of ions by roots

Roots are capable of accumulating selective nutrients/ions. This is on account of the presence of specific transport proteins which are selective, and, in many cases, there is active transport. As a result, many a times the concentration of certain selected ions becomes more than that in the surrounding medium. The difference in the ion concentration within and outside the cell is called **accumulation ratio**. For example, K^+ concentration within the maize root cells may be 1000 times more than the concentration outside the cell. On the other hand, Na^+ is present in very low concentrations in plant cells (as against the animal cells). More so, Na^+ gets actively expelled from most plants' cells. As a result, the plant cells do not accumulate much Na^+ .

Carriers (Transporters)

Ionophores are organic molecules which act as carriers of specific ions. They increase the permeability of membranes to these specific ions, make them diffuse through the membrane and finally releasing them to the other side of the membrane. The free ionophore once again returns to its original site of the membrane to bind more such ions (Fig. 4.8). Such carriers are necessarily soluble in non-polar solvents.

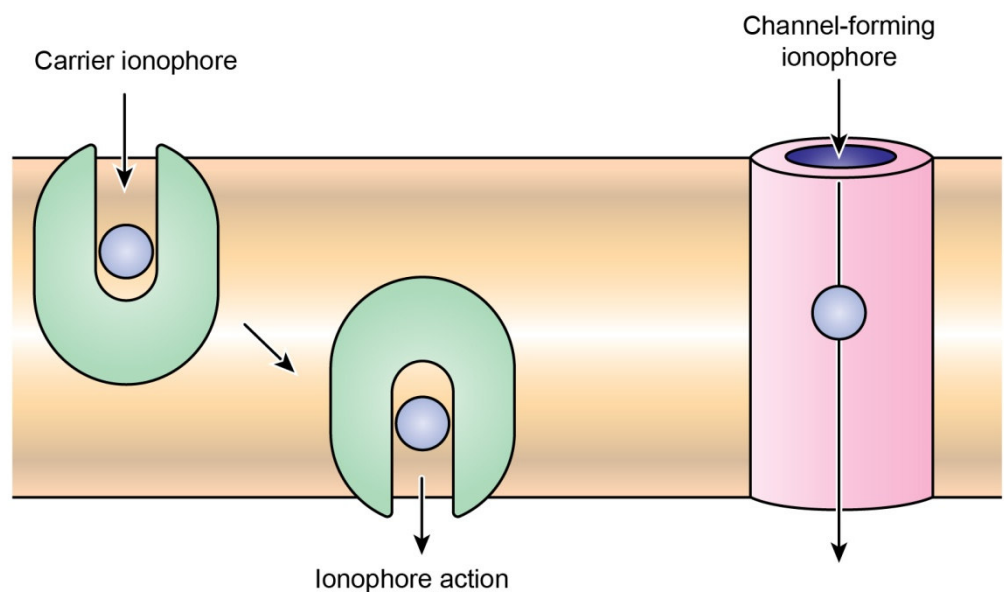


Fig. 4.8: Transportation of ions by carrier ionophores.

Carrier proteins exhibit a high degree of specificity for a particular ion or organic metabolite (Fig. 4.9). Unlike the channels, their pores do not extend completely across the membrane. Their binding with a particular ion/molecule is reminiscent of an enzyme-substrate complex and the binding of the solute particles to the carriers induces a conformational change (a modification of the tertiary structure and shape) in the enzyme. They, however, do not induce any chemical modification of the bound ions' metabolites. Since the carrier-mediated transport involves conformational changes in the protein and its travel through to the other side the bilayer, the rate of transport is much slower than that through channels (as discussed above). To explain, whereas 100-1000 molecules ions per second could be transported by carriers, the figure could reach a few million at the same time by channels.

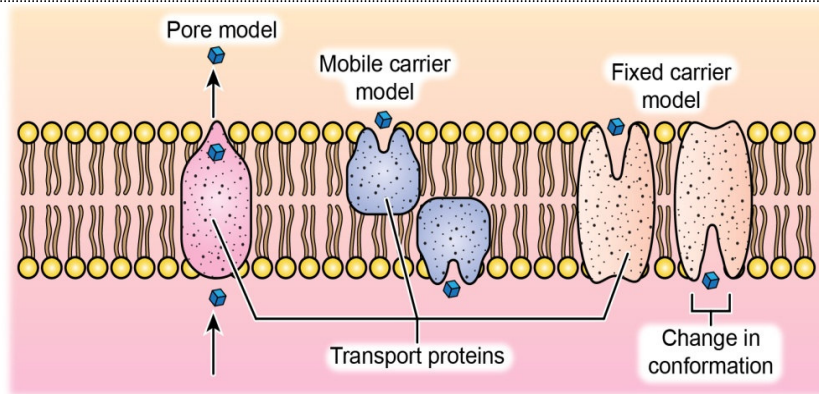


Fig. 4.9: Three different possible modes of transport of ions and other molecules across the plasma membrane.

Unlike the channel-mediated transport, movement of ions/metabolites through the carriers can either be passive (facilitated diffusion) when operative along/down an electrochemical potential without the expenditure of ATP, or by secondary active transport.

Studies on plants cell membrane suggest that inorganic ions can permeate through aqueous protein channels called **permeases**. The permeases are ion specific because the permeability of different ions varies. Most membranes are more permeable to K^+ than to other ions.

Ion uptake through specific carrier proteins can be fooled by similar ions. It seems probable that certain proteins transport more than one ion at the same site and so the ions compete with each other. For example, K^+ , Cs^+ , Rb^+ are apparently transported at the same site, and Na^+ and Li^+ both are transported at the other site. But ions transported at different sites do not compete with each other; for instance, K^+ does not compete with Na^+ .

Ion transport in plants also takes place through **ionophores** (see Fig 4.8). As you know ionophores are small polypeptides and proteins that shield the charge of ions from hydrophobic environment of the membrane. Ionophores have been isolated from bacteria and fungi. When they are added to the artificial lipid bilayer, they increase the rate of diffusion of specific ions by as much as one million-fold.

There is much indirect evidence for mobile carrier proteins in plant membranes. So far only sucrose carrier is identified. The sucrose carriers help in loading of sucrose into the phloem.

Secondary active transport is an active transport which does not utilize ATP but instead uses energy stored in the proton motive force (pmf) or other ion gradients and operates by symport or antiport.

Driving Force of Protein-mediated transport

Let us now find out what is the driving force involved in protein mediated transport. Many membrane transport proteins allow specific solutes to move across the lipid bilayer. If the transported molecule is uncharged, then the difference in its concentration on the two sides of the membrane, that is its concentration gradient determines the direction of transport. However, if the solute to be transported carries a net charge (either negative or positive), then both its concentration gradient and the total electrical gradient across the membrane influence its transport.

In fact, all plasma membranes have electric potentials (transmembrane potential) across them with inside of the cell more negative compared to the outside. This is due to active transport of ions particularly H^+ ions out of the cell. This potential difference allows the entry of positively charged ions into the cell but opposes the entry of negatively charged ions.

4.3.3 Pumps and Active Transport

Nernst equation

For example, the Nernst equation for a univalent cation at $25^\circ C$ can be expressed as :

$$\Delta E1 = 59 \text{ mV} \log \frac{C_1^0}{C_1^2}$$

which suggests that Nernst potential of 59 mV ($C_0/C_1 = 10/1$; as $\log 10 = 1$) corresponds to a ten fold difference in concentration. In other words, for passive diffusion of an ion to occur, a membrane potential of 59 mV would be sufficient to maintain a ten fold concentration gradient of that ion. Membrane potential is exhibited by all cells due to the asymmetry of distribution of ions at the two faces of a membrane.

Many ions/metabolites tend to accumulate inside the cell as their uptake is very rapid and is even against the predicted concentrations as calculated from **Nernst equation**. Nernst equation is an equation that relates the reduction potential of an electrochemical reaction. These transport processes continue solute intake even against the established concentration gradients. Such a transport process is called **Active Transport**. This is also a unidirectional transport mediated by carrier proteins. Moreover, it does not occur spontaneously. Active transport also occurs in the reverse direction, i.e., from inside to the outside, to maintain the internal solute concentration. Active transport can be visualized as a carrier protein mediated process occurring against a concentration gradient and requiring an input of energy. The source of metabolic energy source may not always be ATP although it is usually so.

When the protein carrier utilizes directly energy derived from ATP-hydrolysis, oxidation-reduction reactions or the absorption of light, it is called as **primary active transport** and the membrane proteins carrying out this uphill transport are called **Pumps**. These pumps transport ions like H^+ or Ca^{2+} but some of them also transport large organic molecules.

Thus, the presence of many anions like NO_3^- , Cl^- , $H_2PO_4^-$, and SO_4^{2-} in higher concentrations indicates that they are taken up actively. On the other hand the internal concentrations of cations like NO^+ , Mg^{2+} , and Ca^{2+} is lower than the predicted values based on Nernst equation suggesting that they can enter the cell by diffusion but will have to be exported (transported out) by active means. Thus, the Nernst equation helps us to distinguish between active and passive transport. You will find that the Fig. 4.10 summarizes the control of ion concentrations by passive and active processes occurring in the plant cell cytosol and vacuole.

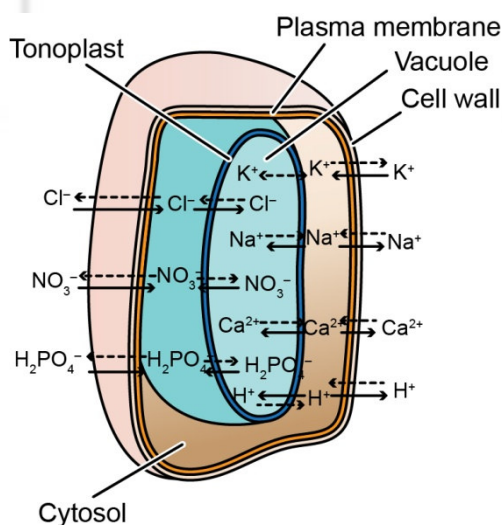


Fig. 4.10: Control of ionic concentrations in the cytosol and vacuole by active (solid arrows $\longrightarrow$) and passive (dashed arrows $\cdots\longrightarrow$) transport processes (From Taiz *et al*).

It is quite clear from the above that:

- Sodium ions are pumped actively out of the cytosol into the extracellular space and into the vacuole.
- Potassium ions are capable of active as well as passive transport under different conditions. Both the vacuole and the cytosol accumulate K^+ passively. Active uptake of K^+ takes place when the concentration of K^+ outside the cell is low.
- To maintain the cytosolic pH near 7.0, the excess protons (H^+) are pumped out of the cytosol actively. This is perhaps the reason that both the extracellular medium as well the vacuole are nearly 1-2 units more acidic than the cytosol.
- Ca^{2+} leaves both the vacuoles and cytosol by active transport.
- Anions enter the cell by active mechanism.

Since K^+ is present in highest concentrations in plant cells and there is a simultaneous transport of many ions across a membrane, the diffusion potential can be more accurately calculated by the **Goldman equation** (modified from Nernst equation).

The primary active transport involves the utilization of energy from ATP hydrolysis, or an oxidation reduction. Membrane proteins carrying out such a transport are called pumps. Most pumps transport H^+ and Ca^{2+} . Also, the plasma membrane $H^+-ATPase$ generates the gradient of electrochemical potential of H^+ across the plasma membrane.

Together, the proton gradient and the normal membrane potential (i.e., the gradient of electrochemical potential for H^+) are called as the **proton motive force (PMF)**.

The PMF tends to move protons back across the membrane and is the primary source of energy for many plant activities including ATP synthesis in the mitochondria and chloroplasts. This electrogenic H^+ transport-generated proton motive force acts as a driving force for transporting various solutes actively (e.g., anions, cations, amino acids, and sugars) against their gradient of electrochemical potential. This is achieved by coupling the uphill transport of one solute to the downhill transport of another (Fig. 4.11).

Goldman Equation

It is an equation that helps to predict the diffusion potential across a membrane. It is a function of the concentrations and permeabilities of all those ions which pass by various transmembrane mechanisms. Goldman equation is a modified version of Nernst equation.

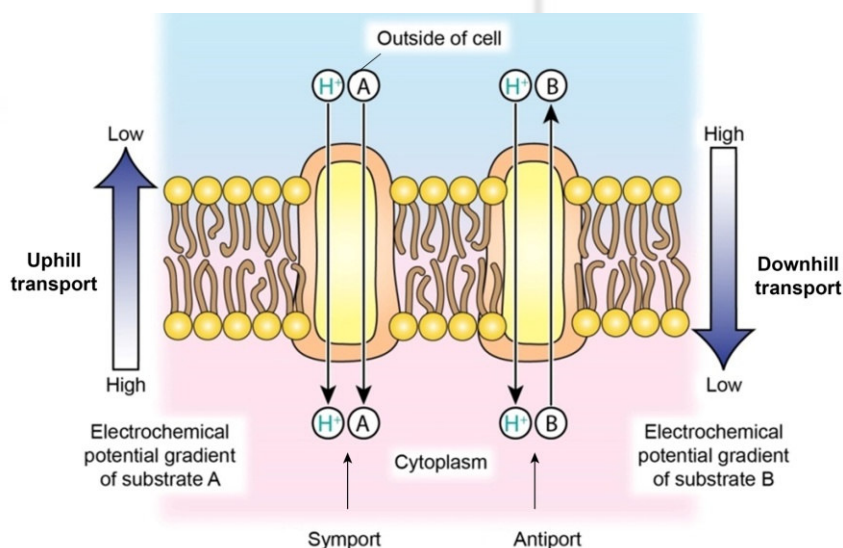


Fig. 4.11: Secondary active transport (Symport and Antiport) coupled to the force created by the proton gradient (From Taiz *et al*).

The equation explains the difference in electrical potential between the two compartments at equilibrium for a particular ion.

It means that at equilibrium, the difference in concentration of an ion between two compartments is balanced by the voltage difference between the compartments.

This type of *ATPase* – proton pump carrier – mediated transport is called **secondary active transport** that is driven indirectly by pumps. No doubt, the ultimate energy for the electrogenic pump is derived from ATP (formed by oxidative phosphorylation in the mitochondria during ETC) (See Fig. 4.12). Secondary active transport utilizes the electrogenic H^+ transport generated proton motive force.

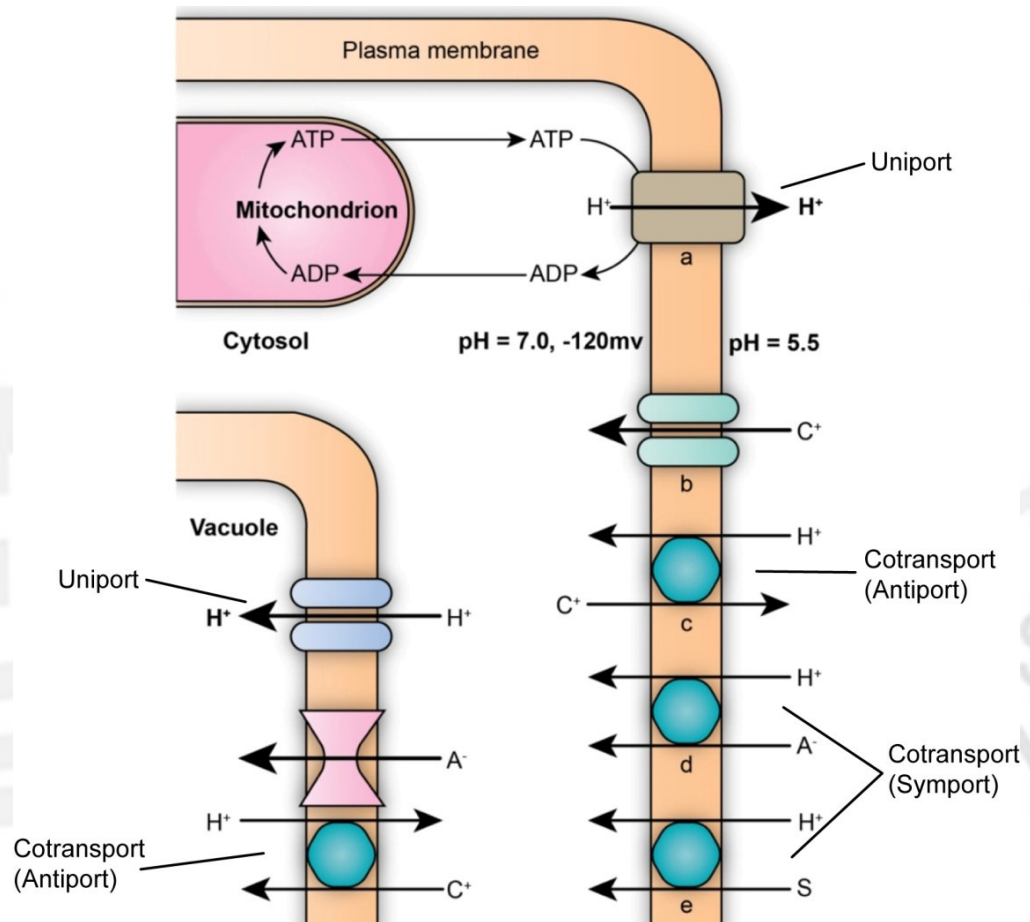


Fig. 4.12: Activity of membrane ATPase-proton pump related to solute exchange (From Hopkins & Hüner).

The proton pump can either utilize energy of ATP to establish a proton gradient and a potential difference at the membrane (inside face being more negative). The ion channels may get activated by energy derived from the proton gradient of antiport and symport channels are driven.

For the *ATPase* proton pump mediated transport to be effective, the proton-translocating protein is thought to possess its ATP binding site on the cytosolic side. Hydrolysis of ATP forces the translocation of one or more protons from the cytosol to the surrounding apoplastic cell wall space. The transport is said to be **uniport** when the *ATPase*-proton pump translocates a single type of ion in just one direction.

However, the secondary active transport can couple the transport of other solutes across the membrane by several other ways. Specific carrier proteins are present in the membranes which help in return of protons to the cytosol along with other solute molecules simultaneously. This is called **co-transport**. The same carrier protein couples the flux of protons. If the other substances move in the same direction as the H^+ , it is called **symport** (mediated by symporter proteins). For example, sugars are symported as uncharged solutes powered by the proton gradient. If the second substance moves in the opposite direction (uphill transport) from the proton (Downhill transport) this cotransport is called **antiport** (mediated by antiporter proteins).

The plasma membrane H^+ -ATPase is believed to contain 10 transmembrane segments and a regulatory domain. The regulatory domain is also auto inhibitory (Fig. 4.13). An enzyme that promotes hydrolysis of ATP in the presence of K^+ has also been isolated from plants. It is suggested that the enzyme binds K^+ ion at specific site and changes conformation on binding ATP. After hydrolysis of ATP it returns to its original conformation and the ion is released to the other side of the membrane. A working model depicting the active transport in plant cell with H^+ translocating ATPase located in plasma membrane and tonoplast is shown in Fig. 4.13.

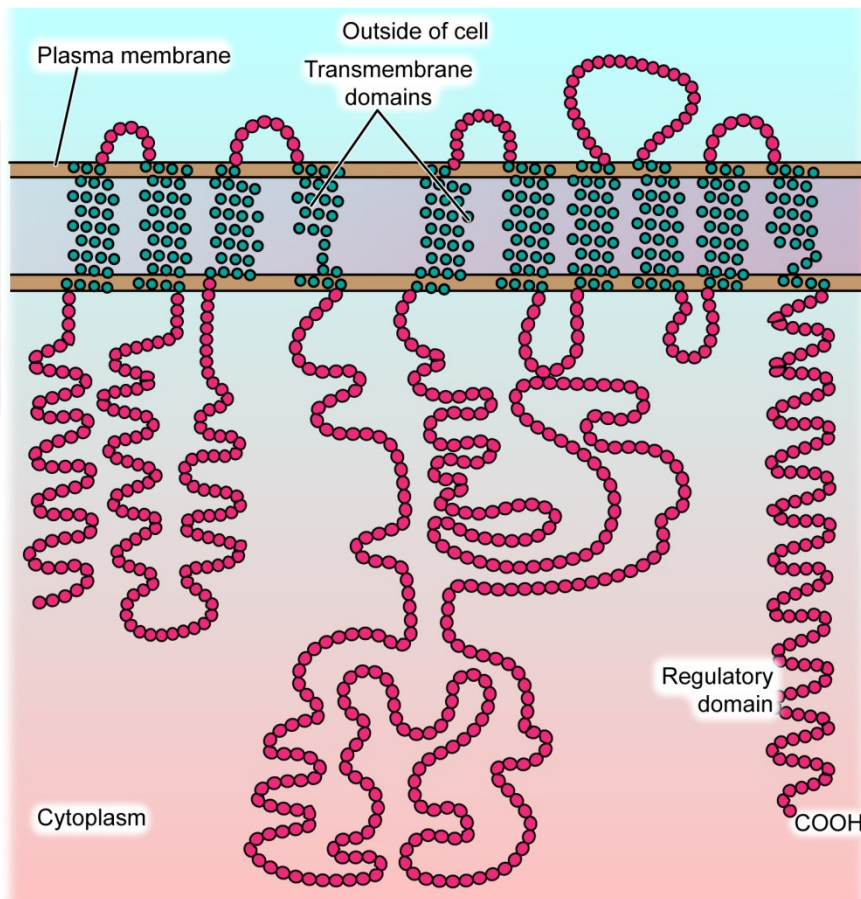


Fig. 4.13: Two-dimensional representation of the plasma membrane H^+ -ATPase (From Taiz et al).

A study on the rate of uptake of K^+ using radioactive isotope shows that uptake resembles an enzyme-substrate reaction curve and carriers involved behave like specialized membrane-bound enzymes. At higher concentration, the carrier site for ions gets saturated, hence the uptake does not increase further with increase in concentration of ions in the solution. The binding sites of the carrier can also be inhibited by competitive inhibitors.

There exist many types of transport proteins in the plasma membrane and the tonoplast which act as symporters and antiporters, alongwith ion channels (Fig.4.14).

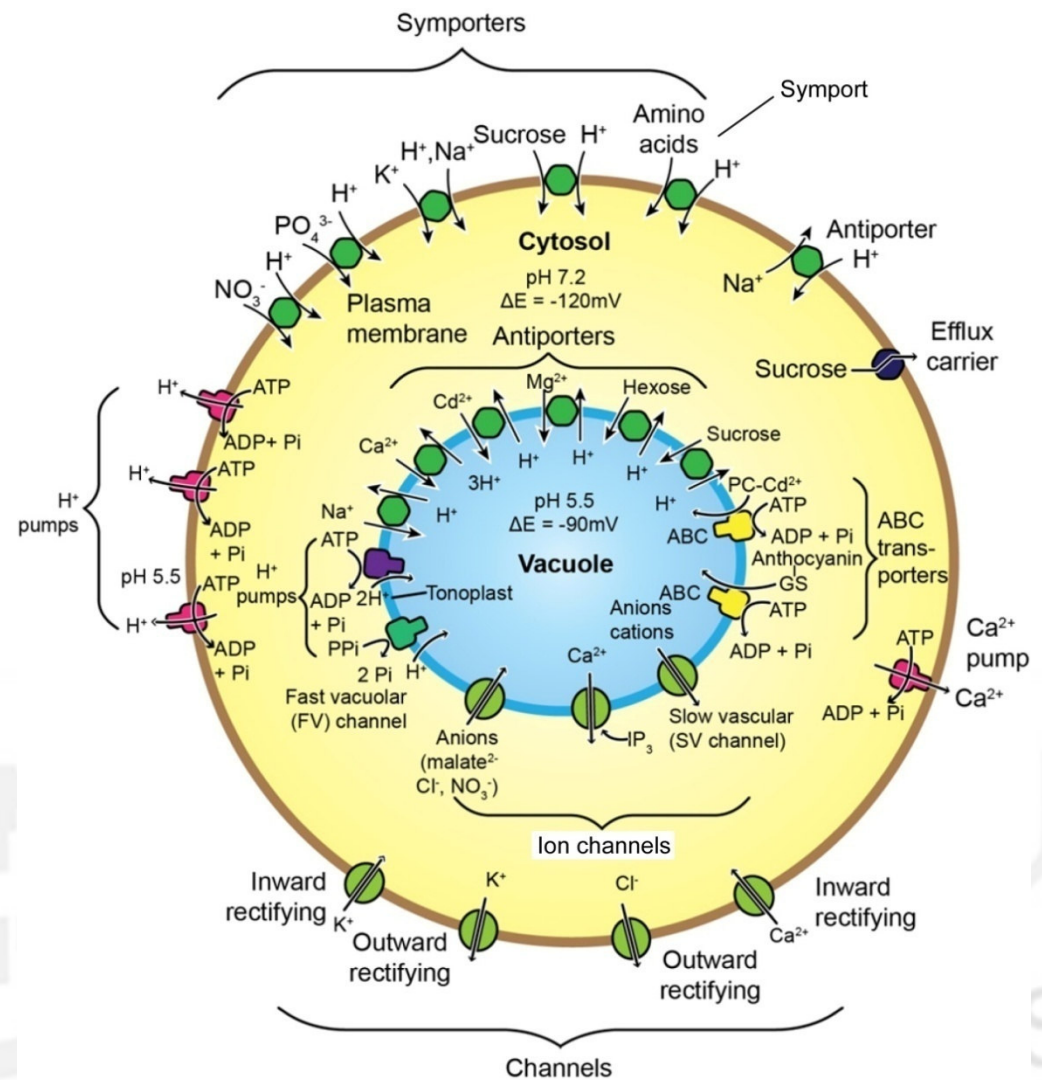


Fig. 4.14: An overview of different antiports, symports and channels operative in a plant cell (From Taiz *et al*).

SAQ 2

- In the following statements fill in the blank spaces with appropriate words:
 - The driving force for diffusion is
 - The proton pump in plant is an ATP hydrolyzing enzyme called
 - When proton pump works, protons are transported from to of the cell.
- In the following statements tick mark the correct word given in the parenthesis.
 - Most membranes are permeable to (K^+/Na^+).
 - When ionophores are added to the artificial lipid bilayer the permeability of the membrane (decrease/increases).

- iii) Transport of the proton across the membrane develops an electric potential gradient which is (negative/positive) outside of the cell.
 - iv) The net result of the operation of the proton pump is that the pH of the medium outside the plasma membrane becomes (acidic/alkaline).
 - v) An element which is preferentially translocated by the apoplastic pathway is (potassium/calcium).
- c) Which of the following statements are true or false?
- i) The gated channels may open and close in response to modulating signals. []
 - ii) Water molecules can pass very rapidly through the membranes by carrier proteins. []
 - iii) The primary active transport utilizes ATP directly. []
 - iv) Facilitated diffusion of solutes operates along the electrochemical gradient across the membrane. []
 - v) Proton-motive force drives the secondary active transport. []

4.4 RADIAL MOVEMENT OF IONS IN ROOTS

Once inside the root hair, the mineral ions must reach the xylem before they could be carried to various parts of the shoot along the transpiration stream. Movement of ion transport across the root has adjusted itself according to the anatomical characteristics of roots themselves. The movement of ions from the surface of roots to the tracheary elements of xylem is referred to as the **radial movement of ions**.

To begin with, the mineral ions enter the cell wall of roots/root hair by diffusion into the apparent free spaces. Thereafter, they follow symplastic and apoplastic routes. The nutrients move along the cell wall and intercellular spaces of the epidermal and cortical cells, some nutrients are absorbed by these cells and enter symplastic pathway in cytoplasm through plasmodesmata. Rest of the ions are screened by membranes of the endodermal cells which control the rates of absorption and type of solute absorbed. Some of these solutes are transported into the vacuole, where they contribute greatly to negative osmotic potential of root facilitating water uptake, turgor pressure and growth of roots through soil.

Although there is a continuous phase of apoplast from the root surface through the cortex, the ions necessarily enter the symplast at the level of endodermis because of the presence of casparian strips, before entering the stele. So, there is no further entry of water and minerals endodermis onwards via the apoplast. The casparian bands also prevent the ions from diffusing back from xylem by the apoplastic route, thereby helping to maintain a higher ion concentration of ions in the xylem as compared to the one in the soil water.

The preferential use of apoplastic or symplastic pathway by different ions is not clearly known. Experiments with radioisotopes of ^{86}Rb and ^{36}Cl on the water plant *Vallisneria* show that these ions take only symplastic pathway probably through plasmodesmata. So far, it has been technically difficult to ascertain the role of plasmodesmata, however, their presence in regions where transport is active indirectly indicates that ions move through them in symplastic routes.

Studies using radioisotopes of ^{45}Ca in barley seedlings show that it is preferentially transported along apoplastic pathway and its concentration in the cytoplasm remains minimum because it can precipitate both organic and inorganic phosphates within to cells. Mg^{2+} also moves slowly through apoplastic pathway.

Regardless of the pathway, the radial movement through symplasm brings the mineral elements and other solutes to the stele where they are released into the xylem. Earlier it was believed that the ions were being passively “leaked”. However, this transfer is now shown to be an energy-requiring process in which xylem parenchyma cells play an important role. Delivery of ions into the xylem vessels is against a concentration gradient and is sensitive to uncouplers of ATP formation like carbonyl- cyanide-m-chlorophenylhydrazone (CCCP) and also to an inhibitor of protein synthesis viz., cycloheximide, thereby suggesting the existence of mechanisms that exercise metabolic control over flux of ions from xylem parenchyma to the tracheary elements of xylem via carriers, ion channels and the plasma membrane H^+ -ATPases. The mechanism is like that described above for loading of nutrients into the cells and involves carrier proteins.

4.5 LONG DISTANCE TRANSPORT – XYLEM LOADING AND UNLOADING

Once in the xylem vessels, the transport of the mineral elements from the root to the shoot is driven by the gradient of hydrostatic pressure (root pressure) and by the gradient of water potential. The gradient of water potential between roots and shoot is usually quite steep during the day when the stomata are open. It follows the pattern: atmosphere < leaf cells < xylem sap < root cells < external solution (soil). Transport in xylem vessels is mainly unidirectional. An increase in the transpiration rate enhances both the uptake and the translocation of mineral element in the xylem.

The lateral transport of ions from root xylem to leaves probably takes place via xylem transfer cells (Fig. 4.15) which have two special features:

- i) the cell wall of these cells facing xylem is elaborately corrugated and invaginated for providing large surface area for absorption; and
- ii) the cells contain many mitochondria that are located close to the convoluted and corrugated wall to supply ATP for the active transport that takes place across these walls.

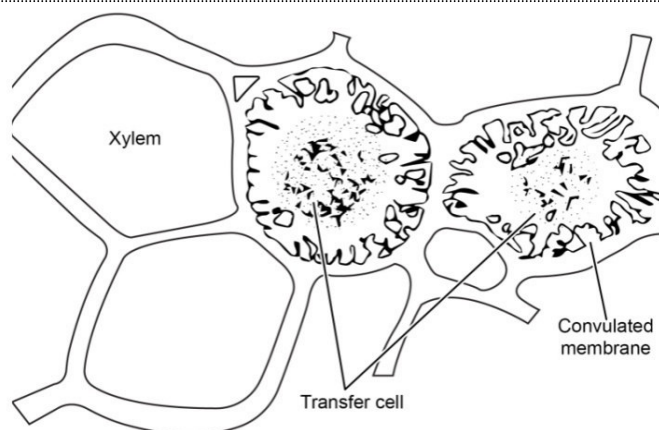


Fig. 4.15: Xylem transfer cells.

The transfer cells are also present at places where large quantities of ions or organic solutes are moved in and out of conducting cells or storage tissue. You will study more about transfer cells in Unit 8 on phloem transport.

Mineral elements which are phloem mobile can be re-translocated from the shoot to the root via phloem, though the main transported compound in the phloem is sucrose and other organic compounds. The transport in phloem is bidirectional. The direction of transport is determined by the nutritional requirements of various plant organs or tissues.

SAQ 3

Match the contents of column A with those of B.

Column A	Column B
a) Symplast	1. amplification of surface area
b) CCCP	2. endodermis
c) Xylem transfer cells	3. Diffusion
d) Entry into apparent free space	4. Retranslocation
e) Casparian strip	5. ATP uncoupler
f) Phloem	6. Plasmodesmata

4.6 SUMMARY

In this Unit you have studied that:

- A complex interaction takes place between soil and plant roots which results in uptake of nutrient ions by plants.
- The charged colloidal soil surfaces help in making available the nutrient ions (mostly negatively charged) for transport.
- The uptake of ions by roots from the soil is influenced by ion exchange capacity of the soil, pH and the presence of different anions and cations.

- Once the mineral ions enter the apparent free space by diffusion, they move rapidly towards the root interior either through the intercellular space in the root epidermis and cortex (apoplast) or across the selectively permeable membrane of the epidermis or cortex (symplast).
- The electrochemical gradient integrates (combines) the concentration gradients and electrical potential gradients and is the main driving force behind transport across biological membranes. In any case, all minerals eventually must cross the cell membrane and pass the cytoplasm of the endodermis cells (due to the presence of Casparian bands), if they are to reach the xylem vessels which carry them upward along with water and transports them throughout the plant body.
- Solutes cross the membrane by simple diffusion, facilitated diffusion or active transport. Transportation across the membranes is mediated by specialized proteins – channels, carriers, and pumps. The movement of ions against the electrochemical gradient requires metabolic energy in the form of ATP. Electrochemical gradient is also maintained by proton pumps which operate constantly and generate transmembrane potential across the membrane and facilitate transport of cations, anions, amino acids, and sugars.
- Secondary active transport uses energy from the proton motive force to transport solutes across the membrane by symport or antiport.
- Water flux across the membranes is facilitated through aquaporins.
- The ions are loaded into the xylem from the root symplast from where the long-distance transport of ions from root to shoot is driven by the gradient of hydrostatic pressure and water potential in the transpiration stream.

4.7 TERMINAL QUESTIONS

1. a) The uptake of minerals is very specific. Explain the mechanism that ensures this specificity.
b) How is the uptake of ions in some cases fooled by similar ones?
2. List three factors that influence the uptake of ions by the roots from soil.
3. Why cannot potassium and sodium ions cross the artificial lipid bilayer?
4. While studying uptake of K^+ by plant membrane it was observed that uptake was affected by chemicals that inhibit respiration. What inference can be drawn from this observation?
5. Describe the concept of apparent free space and its role in uptake of nutrient ions.
6. What is the role of pH in ion exchange capacity of ions?
7. Distinguish between:
 - i) Active and passive ion uptake
 - ii) Carriers and channels

- iii) Simple and facilitated diffusion
 - iv) Primary and secondary active transport
8. Write short notes on:
- i) Carrier proteins
 - ii) Nernst Equation
 - iii) Electrogenic pumps
 - iv) Radial movement of ions in roots

4.8 ANSWERS

Self-Assessment Questions

1. a) i) True; ii) False; iii) False; iv) True; v) True.
- b) i) negative
- ii) plasmodesmata
- iii) pH
- iv) casparian strip
- v) cation exchange sites
- vi) rubidium
2. a) i) concentration gradient;
- ii) *ATPase*
- iii) cytoplasm, outside
- b) i) K^+
- ii) increases
- iii) positive
- iv) acidic
- v) calcium
- c) i) True; ii) False; iii) True; iv) True; v) True.
3. a) Plasmodesmata
- b) ATP uncoupler
- c) amplification of surface area
- d) Diffusion
- e) endodermis
- f) Retranslocation

Terminal Questions

1.
 - a) The specificity of transport of certain ions is due to carrier proteins which behave more like enzymes and the transport is like enzyme mediated reactions. The carrier protein has a specific site for binding an ion that is to be transported.
 - b) Uptake of ions is fooled when two or more than two ions can bind at the same site of carrier proteins. They must be structurally analogous to compete for the same site.
2.
 - i) Ion exchange capacity of soil; ii) soil pH; and iii) Presence of different anions and cations.
3. Although both K^+ and Na^+ ions are small, the charge and the hydrations shell around them prevents them from entering the hydrophobic hydrocarbon phase of the lipid bilayer.
4. Inhibition of K^+ uptake by respiratory inhibitors shows that K^+ transport is linked to, and dependent on ATP produced from respiration.
5. Refer to Subsection 4.2.4.
6. Refer to Subsection 4.2.1.
7.
 - i) Refer to Section 4.3.
 - ii) Refer to Subsections 4.3.2 and 4.3.3.
 - iii) Refer to Subsections 4.3.1 and 4.3.2.
 - iv) Refer to Subsection 4.3.3.
8.
 - i) Refer to Subsection 4.3.2.
 - ii) Refer to Subsection 4.3.2.
 - iii) Refer to Subsection 4.3.3.
 - iv) Refer to Section 4.4.