

up to 15 metres per hour. How is this movement accomplished? A long standing question is, whether water is 'pushed' or 'pulled' through the plant. Most researchers agree that water is mainly 'pulled' through the plant, and that the driving force for this process is transpiration from the leaves. This is referred to as the **cohesion-tension-transpiration pull model** of water transport. But, what generates this transpirational pull?

Water is transient in plants. Less than 1 per cent of the water reaching the leaves is used in photosynthesis and plant growth. Most of it is lost through the **stomata** in the leaves. This water loss is known as **transpiration**.

You have studied transpiration in an earlier class by enclosing a healthy plant in polythene bag and observing the droplets of water formed inside the bag. You could also study water loss from a leaf using cobalt chloride paper, which turns colour on absorbing water.

11.4 TRANSPIRATION

Transpiration is the evaporative loss of water by plants. It occurs mainly through the **stomata** in the leaves. Besides the loss of water vapour in transpiration, exchange of oxygen and carbon dioxide in the leaf also occurs through pores called stomata (sing. : stoma). Normally stomata are open in the day time and close during the night. The immediate cause of the opening or closing of the stomata is a change in the turgidity of the **guard cells**. The inner wall of each guard cell, towards the pore or **stomatal aperture**, is thick and elastic. When turgidity increases within the two guard cells flanking each stomatal aperture or pore, the thin outer walls bulge out and force the inner walls into a crescent shape. The opening of the stoma is also aided due to the orientation of the microfibrils in the cell walls of the guard cells. Cellulose microfibrils are oriented radially rather than longitudinally making it easier for the stoma to open. When the guard cells lose turgor, due to water loss (or water stress) the elastic inner walls regain their original shape, the guard cells become flaccid and the stoma closes.

Usually the lower surface of a dorsiventral (often dicotyledonous) leaf has a greater number of stomata while in an isobilateral (often monocotyledonous) leaf they are about equal on both surfaces. Transpiration is affected by several external factors: temperature, light, humidity, wind speed. Plant factors that affect transpiration include number and distribution of stomata, per cent of open

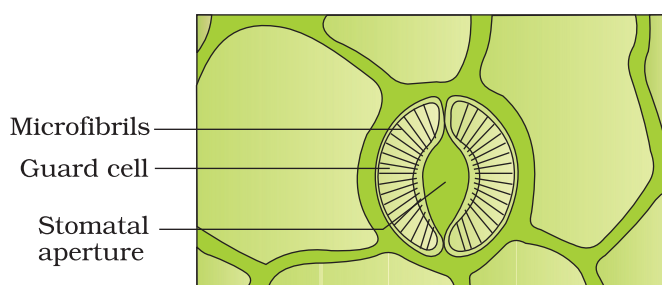


Figure 11.8 A stomatal aperture with guard cells

stomata, water status of the plant, canopy structure etc.

The transpiration driven ascent of xylem sap depends mainly on the following physical properties of water:

- **Cohesion** – mutual attraction between water molecules.
- **Adhesion** – attraction of water molecules to polar surfaces (such as the surface of tracheary elements).
- **Surface Tension** – water molecules are attracted to each other in the liquid phase more than to water in the gas phase.

These properties give water high **tensile strength**, i.e., an ability to resist a pulling force, and high **capillarity**, i.e., the ability to rise in thin tubes. In plants capillarity is aided by the small diameter of the tracheary elements – the **tracheids** and **vessel elements**.

The process of photosynthesis requires water. The system of xylem vessels from the root to the leaf vein can supply the needed water. But what force does a plant use to move water molecules into the leaf parenchyma cells where they are needed? As water evaporates through the stomata, since the thin film of water over the cells is continuous, it results in pulling of water, molecule by molecule, into the leaf from the xylem. Also, because of lower concentration of water vapour in the atmosphere as compared to the substomatal cavity and intercellular spaces, water diffuses into the surrounding air. This creates a 'pull' (Figure 11.9).

Measurements reveal that the forces generated by transpiration can create pressures sufficient to lift a xylem sized column of water over 130 metres high.

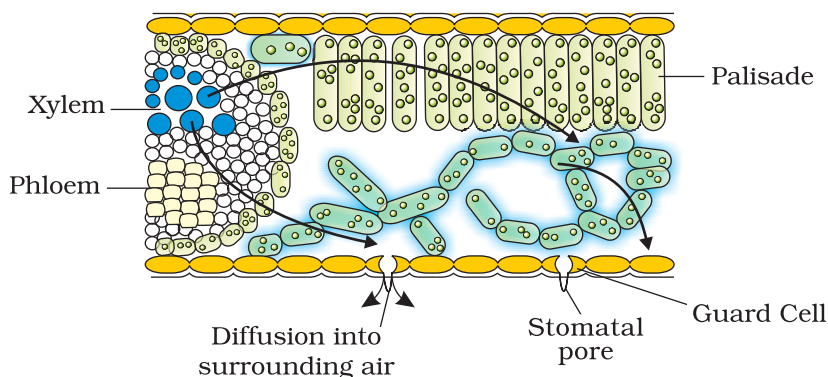


Figure 11.9 Water movement in the leaf. Evaporation from the leaf sets up a pressure gradient between the outside air and the air spaces of the leaf. The gradient is transmitted into the photosynthetic cells and on to the water-filled xylem in the leaf vein.

11.4.1 Transpiration and Photosynthesis – a Compromise

Transpiration has more than one purpose; it

- creates transpiration pull for absorption and transport of plants
- supplies water for photosynthesis
- transports minerals from the soil to all parts of the plant
- cools leaf surfaces, sometimes 10 to 15 degrees, by evaporative cooling
- maintains the shape and structure of the plants by keeping cells turgid

An actively photosynthesising plant has an insatiable need for water. Photosynthesis is limited by available water which can be swiftly depleted by transpiration. The humidity of rainforests is largely due to this vast cycling of water from root to leaf to atmosphere and back to the soil.

The evolution of the C_4 photosynthetic system is probably one of the strategies for maximising the availability of CO_2 while minimising water loss. C_4 plants are twice as efficient as C_3 plants in terms of fixing carbon (making sugar). However, a C_4 plant loses only half as much water as a C_3 plant for the same amount of CO_2 fixed.

11.5 UPTAKE AND TRANSPORT OF MINERAL NUTRIENTS

Plants obtain their carbon and most of their oxygen from CO_2 in the atmosphere. However, their remaining nutritional requirements are obtained from minerals and water for hydrogen in the soil.

11.5.1 Uptake of Mineral Ions

Unlike water, all minerals cannot be passively absorbed by the roots. Two factors account for this: (i) minerals are present in the soil as charged particles (ions) which cannot move across cell membranes and (ii) the concentration of minerals in the soil is usually lower than the concentration of minerals in the root. Therefore, most minerals must enter the root by **active absorption** into the cytoplasm of epidermal cells. This needs energy in the form of ATP. The active uptake of ions is partly responsible for the water potential gradient in roots, and therefore for the uptake of water by osmosis. Some ions also move into the epidermal cells passively.

Ions are absorbed from the soil by both passive and active transport. Specific proteins in the membranes of root hair cells actively pump ions from the soil into the cytoplasm of the epidermal cells. Like all cells, the endodermal cells have many transport proteins embedded in their plasma membrane; they let some solutes cross the membrane, but not others. *Transport proteins of endodermal cells are control points, where a plant adjusts the quantity and types of solutes that reach the xylem.* Note that the root endodermis because of the layer of suberin has the ability to actively transport ions in one direction only.

11.5.2 Translocation of Mineral Ions

After the ions have reached xylem through active or passive uptake, or a combination of the two, their further transport up the stem to all parts of the plant is through the transpiration stream.

The chief sinks for the mineral elements are the growing regions of the plant, such as the apical and lateral meristems, young leaves, developing flowers, fruits and seeds, and the storage organs. Unloading of mineral ions occurs at the fine vein endings through diffusion and active uptake by these cells.

Mineral ions are frequently remobilised, particularly from older, senescing parts. Older dying leaves export much of their mineral content to younger leaves. Similarly, before leaf fall in deciduous plants, minerals are removed to other parts. Elements most readily mobilised are phosphorus, sulphur, nitrogen and potassium. Some elements that are structural components like calcium are not remobilised.

An analysis of the xylem exudates shows that though some of the nitrogen travels as inorganic ions, much of it is carried in the organic form as amino acids and related compounds. Similarly, small amounts of P and S are carried as organic compounds. In addition, small amount of exchange of materials does take place between xylem and phloem. Hence, it is not that we can clearly make a distinction and say categorically that xylem transports only inorganic nutrients while phloem transports only organic materials, as was traditionally believed.

11.6 PHLOEM TRANSPORT: FLOW FROM SOURCE TO SINK

Food, primarily sucrose, is transported by the vascular tissue phloem from a source to a sink. Usually the source is understood to be that part of the plant which synthesises the food, i.e., the leaf, and sink, the part that needs or stores the food. But, the source and sink may be reversed depending on the season, or the plant's needs. Sugar stored in roots may be mobilised to become a source of food in the early spring when the buds of trees, act as sink; they need energy for growth and development of the photosynthetic apparatus. Since the source-sink relationship is variable, the direction of movement in the phloem can be upwards or downwards, i.e., **bi-directional**. This contrasts with that of the xylem where the movement is always **unidirectional**, i.e., upwards. Hence, unlike one-way flow of water in transpiration, food in phloem sap can be transported in any required direction so long as there is a source of sugar and a sink able to use, store or remove the sugar.

Phloem sap is mainly water and sucrose, but other sugars, hormones and amino acids are also transported or **translocated** through phloem.

11.6.1 The Pressure Flow or Mass Flow Hypothesis

The accepted mechanism used for the translocation of sugars from source to sink is called the pressure flow hypothesis. (see Figure 11.10). As glucose is prepared at the source (by photosynthesis) it is converted to sucrose (a disaccharide). The sugar is then moved in the form of sucrose into the companion cells and then into the living phloem sieve tube cells by active transport. This process of loading at the source produces a hypertonic condition in the phloem. Water in the adjacent xylem moves into the phloem by osmosis. As osmotic pressure builds up the phloem sap will move to areas of lower pressure. At the sink osmotic pressure must be reduced. Again active transport is necessary to move the sucrose out of the phloem sap and into the cells which will use the sugar – converting it into energy, starch, or cellulose. As sugars are removed, the osmotic pressure decreases and water moves out of the phloem.

To summarise, the movement of sugars in the phloem begins at the source, where sugars are loaded (actively transported) into a sieve tube. Loading of the phloem sets up a water potential gradient that facilitates the mass movement in the phloem.

Phloem tissue is composed of sieve tube cells, which form long columns with holes in their end walls called sieve plates. Cytoplasmic strands pass through the holes in the sieve plates, so forming continuous filaments. As hydrostatic pressure in the phloem sieve tube increases, pressure flow begins, and the sap moves through the phloem. Meanwhile, at the sink, incoming sugars are actively transported out of the phloem and removed

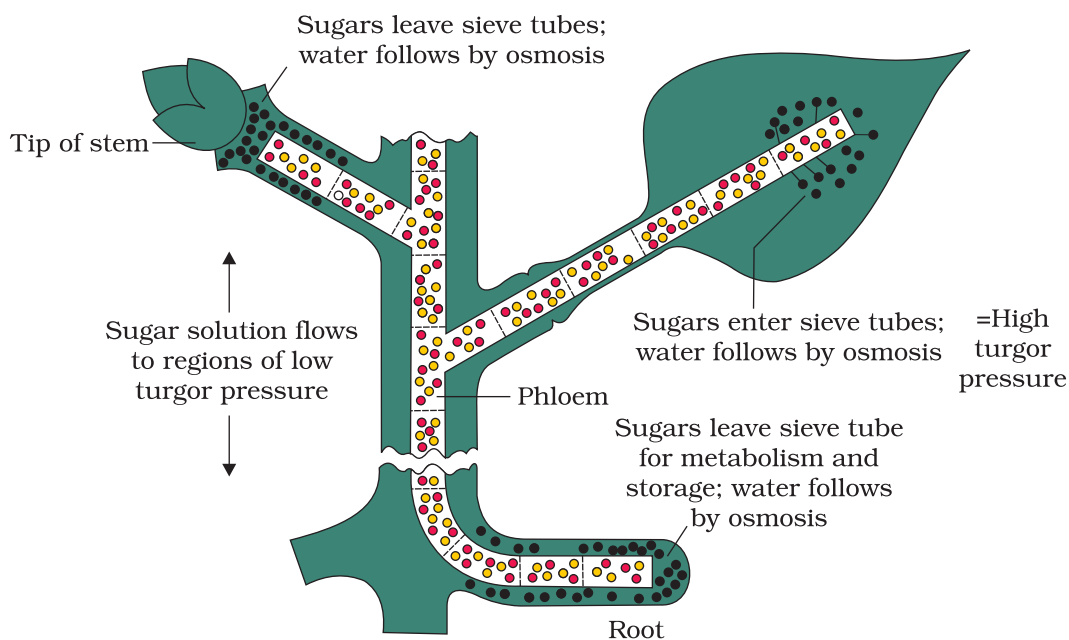


Figure 11.10 Diagrammatic presentation of mechanism of translocation

as complex carbohydrates. The loss of solute produces a high water potential in the phloem, and water passes out, returning eventually to xylem.

A simple experiment, called girdling, was used to identify the tissues through which food is transported. On the trunk of a tree a ring of bark up to a depth of the phloem layer, can be carefully removed. In the absence of downward movement of food the portion of the bark above the ring on the stem becomes swollen after a few weeks. This simple experiment shows that phloem is the tissue responsible for translocation of food; and that transport takes place in one direction, i.e., towards the roots. This experiment can be performed by you easily.

SUMMARY

Plants obtain a variety of inorganic elements (ions) and salts from their surroundings especially from water and soil. The movement of these nutrients from environment into the plant as well as from one plant cell to another plant cell essentially involves movement across a cell membrane. Transport across cell membrane can be through diffusion, facilitated transport or active transport. Water and minerals absorbed by roots are transported by xylem and the organic material synthesised in the leaves is transported to other parts of plant through phloem.

Passive transport (diffusion, osmosis) and active transport are the two modes of nutrient transport across cell membranes in living organisms. In passive transport, nutrients move across the membrane by diffusion, without any use of energy as it is always down the concentration gradient and hence entropy driven. This diffusion of substances depends on their size, solubility in water or organic solvents. Osmosis is the special type of diffusion of water across a semi-permeable membrane which depends on pressure gradient and concentration gradient. In active transport, energy in the form of ATP is utilised to pump molecules against a concentration gradient across membranes. Water potential is the potential energy of water which helps in the movement of water. It is determined by solute potential and pressure potential. The behaviour of the cells depends on the surrounding solution. If the surrounding solution of the cell is hypertonic, it gets plasmolysed. The absorption of water by seeds and drywood takes place by a special type of diffusion called imbibition.

In higher plants, there is a vascular system, xylem and phloem, responsible for translocation. Water minerals and food cannot be moved within the body of a plant by diffusion alone. They are therefore, transported by a mass flow system – movement of substance in bulk from one point to another as a result of pressure differences between the two points.

Water absorbed by root hairs moves deeper into the root by two distinct pathways, i.e., apoplast and symplast. Various ions, and water from soil can be transported upto a small height in stems by root pressure. Transpiration pull model is the most acceptable to explain the transport of water. Transpiration is

the loss of water in the form of vapours from the plant parts through stomata. Temperature, light, humidity, wind speed and number of stomata affect the rate of transpiration. Excess water is also removed through tips of leaves of plants by guttation.

Phloem is responsible for transport of food (primarily) sucrose from the source to the sink. The translocation in phloem is bi-directional; the source-sink relationship is variable. The translocation in phloem is explained by the pressure-flow hypothesis.

EXERCISES

1. What are the factors affecting the rate of diffusion?
2. What are porins? What role do they play in diffusion?
3. Describe the role played by protein pumps during active transport in plants.
4. Explain why pure water has the maximum water potential.
5. Differentiate between the following:
 - (a) Diffusion and Osmosis
 - (b) Transpiration and Evaporation
 - (c) Osmotic Pressure and Osmotic Potential
 - (d) Imbibition and Diffusion
 - (e) Apoplast and Symplast pathways of movement of water in plants.
 - (f) Guttation and Transpiration.
6. Briefly describe water potential. What are the factors affecting it?
7. What happens when a pressure greater than the atmospheric pressure is applied to pure water or a solution?
8.
 - (a) With the help of well-labelled diagrams, describe the process of plasmolysis in plants, giving appropriate examples.
 - (b) Explain what will happen to a plant cell if it is kept in a solution having higher water potential.
9. How is the mycorrhizal association helpful in absorption of water and minerals in plants?
10. What role does root pressure play in water movement in plants?
11. Describe transpiration pull model of water transport in plants. What are the factors influencing transpiration? How is it useful to plants?
12. Discuss the factors responsible for ascent of xylem sap in plants.
13. What essential role does the root endodermis play during mineral absorption in plants?
14. Explain why xylem transport is unidirectional and phloem transport bi-directional.
15. Explain pressure flow hypothesis of translocation of sugars in plants.
16. What causes the opening and closing of guard cells of stomata during transpiration?

CHAPTER 12

MINERAL NUTRITION

12.1 *Methods to Study the Mineral Requirements of Plants*

12.2 *Essential Mineral Elements*

12.3 *Mechanism of Absorption of Elements*

12.4 *Translocation of Solutes*

12.5 *Soil as Reservoir of Essential Elements*

12.6 *Metabolism of Nitrogen*

The basic needs of all living organisms are essentially the same. They require macromolecules, such as carbohydrates, proteins and fats, and water and minerals for their growth and development.

This chapter focusses mainly on inorganic plant nutrition, wherein you will study the methods to identify elements essential to growth and development of plants and the criteria for establishing the essentiality. You will also study the role of the essential elements, their major deficiency symptoms and the mechanism of absorption of these essential elements. The chapter also introduces you briefly to the significance and the mechanism of biological nitrogen fixation.

12.1 METHODS TO STUDY THE MINERAL REQUIREMENTS OF PLANTS

In 1860, Julius von Sachs, a prominent German botanist, demonstrated, for the first time, that plants could be grown to maturity in a defined nutrient solution in complete absence of soil. This technique of growing plants in a nutrient solution is known as **hydroponics**. Since then, a number of improvised methods have been employed to try and determine the mineral nutrients essential for plants. The essence of all these methods involves the culture of plants in a soil-free, defined mineral solution. These methods require purified water and mineral nutrient salts. *Can you explain why this is so essential?*

After a series of experiments in which the roots of the plants were immersed in nutrient solutions and wherein an element was added / removed or given in varied concentration, a mineral solution suitable for

the plant growth was obtained. By this method, essential elements were identified and their deficiency symptoms discovered. Hydroponics has been successfully employed as a technique for the commercial production of vegetables such as tomato, seedless cucumber and lettuce. It must be emphasised that the nutrient solutions must be adequately aerated to obtain the optimum growth. What would happen if solutions were poorly aerated? Diagrammatic views of the hydroponic technique is given in Figures 12.1 and 12.2.

12.2 ESSENTIAL MINERAL ELEMENTS

Most of the minerals present in soil can enter plants through roots. In fact, more than sixty elements of the 105 discovered so far are found in different plants. Some plant species accumulate selenium, some others gold, while some plants growing near nuclear test sites take up radioactive strontium. There are techniques that are able to detect the minerals even at a very low concentration (10^{-8} g/mL). The question is, whether all the diverse mineral elements present in a plant, for example, gold and selenium as mentioned above, are really necessary for plants? How do we decide what is essential for plants and what is not?

12.2.1 Criteria for Essentiality

The criteria for essentiality of an element are given below:

- The element must be absolutely necessary for supporting normal growth and reproduction. In the absence of the element the plants do not complete their life cycle or set the seeds.
- The requirement of the element must be specific and not replaceable by another element. In other words, deficiency of any one element cannot be met by supplying some other element.
- The element must be directly involved in the metabolism of the plant.

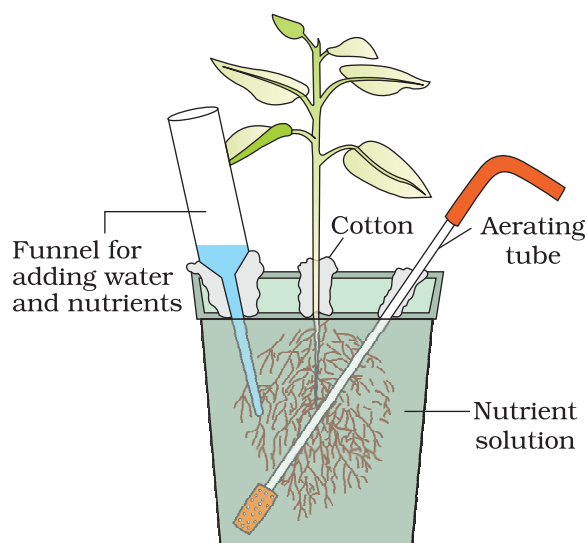


Figure 12.1 Diagram of a typical set-up for nutrient solution culture

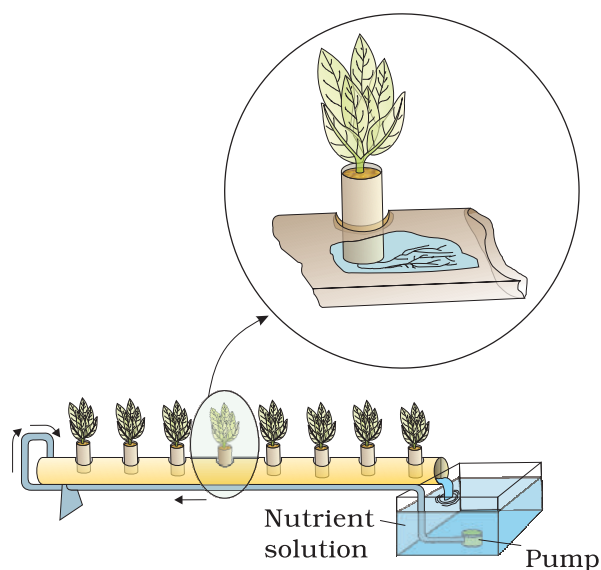


Figure 12.2 Hydroponic plant production. Plants are grown in a tube or trough placed on a slight incline. A pump circulates a nutrient solution from a reservoir to the elevated end of the tube. The solution flows down the tube and returns to the reservoir due to gravity. Inset shows a plant whose roots are continuously bathed in aerated nutrient solution. The arrows indicates the direction of the flow.

Based upon the above criteria only a few elements have been found to be absolutely essential for plant growth and metabolism. These elements are further divided into two broad categories based on their quantitative requirements.

- (i) Macronutrients, and
- (ii) Micronutrients

Macronutrients are generally present in plant tissues in large amounts (in excess of 10 mmole Kg^{-1} of dry matter). The macronutrients include carbon, hydrogen, oxygen, nitrogen, phosphorous, sulphur, potassium, calcium and magnesium. Of these, carbon, hydrogen and oxygen are mainly obtained from CO_2 and H_2O , while the others are absorbed from the soil as mineral nutrition.

Micronutrients or trace elements, are needed in very small amounts (less than 10 mmole Kg^{-1} of dry matter). These include iron, manganese, copper, molybdenum, zinc, boron, chlorine and nickel.

In addition to the 17 essential elements named above, there are some beneficial elements such as sodium, silicon, cobalt and selenium. They are required by higher plants.

Essential elements can also be grouped into four broad categories on the basis of their diverse functions. These categories are:

- (i) Essential elements as components of biomolecules and hence structural elements of cells (e.g., carbon, hydrogen, oxygen and nitrogen).
- (ii) Essential elements that are components of energy-related chemical compounds in plants (e.g., magnesium in chlorophyll and phosphorous in ATP).
- (iii) Essential elements that activate or inhibit enzymes, for example Mg^{2+} is an activator for both ribulose biphosphate carboxylase-oxygenase and phosphoenol pyruvate carboxylase, both of which are critical enzymes in photosynthetic carbon fixation; Zn^{2+} is an activator of alcohol dehydrogenase and Mo of nitrogenase during nitrogen metabolism. *Can you name a few more elements that fall in this category?* For this, you will need to recollect some of the biochemical pathways you have studied earlier.
- (iv) Some essential elements can alter the osmotic potential of a cell. Potassium plays an important role in the opening and closing of stomata. You may recall the role of minerals as solutes in determining the water potential of a cell.

12.2.2 Role of Macro- and Micro-nutrients

Essential elements perform several functions. They participate in various metabolic processes in the plant cells such as permeability of cell

membrane, maintenance of osmotic concentration of cell sap, electron-transport systems, buffering action, enzymatic activity and act as major constituents of macromolecules and co-enzymes.

Various forms and functions of essential nutrient elements are given below.

Nitrogen : This is the essential nutrient element required by plants in the greatest amount. It is absorbed mainly as NO_3^- though some are also taken up as NO_2^- or NH_4^+ . Nitrogen is required by all parts of a plant, particularly the meristematic tissues and the metabolically active cells. Nitrogen is one of the major constituents of proteins, nucleic acids, vitamins and hormones.

Phosphorus: Phosphorus is absorbed by the plants from soil in the form of phosphate ions (either as H_2PO_4^- or HPO_4^{2-}). Phosphorus is a constituent of cell membranes, certain proteins, all nucleic acids and nucleotides, and is required for all phosphorylation reactions.

Potassium: It is absorbed as potassium ion (K^+). In plants, this is required in more abundant quantities in the meristematic tissues, buds, leaves and root tips. Potassium helps to maintain an anion-cation balance in cells and is involved in protein synthesis, opening and closing of stomata, activation of enzymes and in the maintenance of the turgidity of cells.

Calcium: Plant absorbs calcium from the soil in the form of calcium ions (Ca^{2+}). Calcium is required by meristematic and differentiating tissues. During cell division it is used in the synthesis of cell wall, particularly as calcium pectate in the middle lamella. It is also needed during the formation of mitotic spindle. It accumulates in older leaves. It is involved in the normal functioning of the cell membranes. It activates certain enzymes and plays an important role in regulating metabolic activities.

Magnesium: It is absorbed by plants in the form of divalent Mg^{2+} . It activates the enzymes of respiration, photosynthesis and are involved in the synthesis of DNA and RNA. Magnesium is a constituent of the ring structure of chlorophyll and helps to maintain the ribosome structure.

Sulphur: Plants obtain sulphur in the form of sulphate (SO_4^{2-}). Sulphur is present in two amino acids – cysteine and methionine and is the main constituent of several coenzymes, vitamins (thiamine, biotin, Coenzyme A) and ferredoxin.

Iron: Plants obtain iron in the form of ferric ions (Fe^{3+}). It is required in larger amounts in comparison to other micronutrients. It is an important constituent of proteins involved in the transfer of electrons like ferredoxin and cytochromes. It is reversibly oxidised from Fe^{2+} to Fe^{3+} during electron transfer. It activates catalase enzyme, and is essential for the formation of chlorophyll.

Manganese: It is absorbed in the form of manganous ions (Mn^{2+}). It activates many enzymes involved in photosynthesis, respiration and nitrogen metabolism. The best defined function of manganese is in the splitting of water to liberate oxygen during photosynthesis.

Zinc: Plants obtain zinc as Zn^{2+} ions. It activates various enzymes, especially carboxylases. It is also needed in the synthesis of auxin.

Copper: It is absorbed as cupric ions (Cu^{2+}). It is essential for the overall metabolism in plants. Like iron, it is associated with certain enzymes involved in redox reactions and is reversibly oxidised from Cu^+ to Cu^{2+} .

Boron : It is absorbed as BO_3^{3-} or $\text{B}_4\text{O}_7^{2-}$. Boron is required for uptake and utilisation of Ca^{2+} , membrane functioning, pollen germination, cell elongation, cell differentiation and carbohydrate translocation.

Molybdenum : Plants obtain it in the form of molybdate ions (MoO_4^{2-}). It is a component of several enzymes, including nitrogenase and nitrate reductase both of which participate in nitrogen metabolism.

Chlorine : It is absorbed in the form of chloride anion (Cl^-). Along with Na^+ and K^+ , it helps in determining the solute concentration and the anion-cation balance in cells. It is essential for the water-splitting reaction in photosynthesis, a reaction that leads to oxygen evolution.

12.2.3 Deficiency Symptoms of Essential Elements

Whenever the supply of an essential element becomes limited, plant growth is retarded. The concentration of the essential element below which plant growth is retarded is termed as **critical concentration**. The element is said to be deficient when present below the critical concentration.

Since each element has one or more specific structural or functional role in plants, in the absence of any particular element, plants show certain morphological changes. These morphological changes are indicative of certain element deficiencies and are called deficiency symptoms. The deficiency symptoms vary from element to element and they disappear when the deficient mineral nutrient is provided to the plant. However, if deprivation continues, it may eventually lead to the death of the plant. The parts of the plants that show the deficiency symptoms also depend on the mobility of the element in the plant. For elements that are actively mobilised within the plants and exported to young developing tissues, the deficiency symptoms tend to appear first in the older tissues. For example, the deficiency symptoms of nitrogen, potassium and magnesium are visible first in the senescent leaves. In the older leaves, biomolecules containing these elements are broken down, making these elements available for mobilising to younger leaves.

The deficiency symptoms tend to appear first in the young tissues whenever the elements are relatively immobile and are not transported out of the mature organs, for example, elements like sulphur and calcium

are a part of the structural component of the cell and hence are not easily released. This aspect of mineral nutrition of plants is of a great significance and importance to agriculture and horticulture.

The kind of deficiency symptoms shown in plants include chlorosis, necrosis, stunted plant growth, premature fall of leaves and buds, and inhibition of cell division. Chlorosis is the loss of chlorophyll leading to yellowing in leaves. This symptom is caused by the deficiency of elements N, K, Mg, S, Fe, Mn, Zn and Mo. Likewise, necrosis, or death of tissue, particularly leaf tissue, is due to the deficiency of Ca, Mg, Cu, K. Lack or low level of N, K, S, Mo causes an inhibition of cell division. Some elements like N, S, Mo delay flowering if their concentration in plants is low.

You can see from the above that the deficiency of any element can cause multiple symptoms and that the same symptoms may be caused by the deficiency of one of several different elements. Hence, to identify the deficient element, one has to study all the symptoms developed in all the various parts of the plant and compare them with the available standard tables. We must also be aware that different plants also respond differently to the deficiency of the same element.

12.2.4 Toxicity of Micronutrients

The requirement of micronutrients is always in low amounts while their moderate decrease causes the deficiency symptoms and a moderate increase causes toxicity. In other words, there is a narrow range of concentration at which the elements are optimum. Any mineral ion concentration in tissues that reduces the dry weight of tissues by about 10 per cent is considered toxic. Such critical concentrations vary widely among different micronutrients. The toxicity symptoms are difficult to identify. Toxicity levels for any element also vary for different plants. Many a times, excess of an element may inhibit the uptake of another element. For example, the prominent symptom of manganese toxicity is the appearance of brown spots surrounded by chlorotic veins. It is important to know that manganese competes with iron and magnesium for uptake and with magnesium for binding with enzymes. Manganese also inhibits calcium translocation in shoot apex. Therefore, excess of manganese may, in fact, induce deficiencies of iron, magnesium and calcium. Thus, what appears as symptoms of manganese toxicity may actually be the deficiency symptoms of iron, magnesium and calcium. Can this knowledge be of some importance to a farmer? a gardener? or even for you in your kitchen-garden?

12.3 MECHANISM OF ABSORPTION OF ELEMENTS

Much of the studies on mechanism of absorption of elements by plants has been carried out in isolated cells, tissues or organs. These studies

revealed that the process of absorption can be demarcated into two main phases. In the first phase, an initial rapid uptake of ions into the 'free space' or 'outer space' of cells – the apoplast, is passive. In the second phase of uptake, the ions are taken in slowly into the 'inner space' – the symplast of the cells. The passive movement of ions into the apoplast usually occurs through ion-channels, the trans-membrane proteins that function as selective pores. On the other hand, the entry or exit of ions to and from the symplast requires the expenditure of metabolic energy, which is an **active** process. The movement of ions is usually called **flux**; the inward movement into the cells is influx and the outward movement, efflux. You have read the aspects of mineral nutrient uptake and translocation in plants in Chapter 11.

12.4 TRANSLOCATION OF SOLUTES

Mineral salts are translocated through xylem along with the ascending stream of water, which is pulled up through the plant by transpirational pull. Analysis of xylem sap shows the presence of mineral salts in it. Use of radioisotopes of mineral elements also substantiate the view that they are transported through the xylem. You have already discussed the movement of water in xylem in Chapter 11.

12.5 SOIL AS RESERVOIR OF ESSENTIAL ELEMENTS

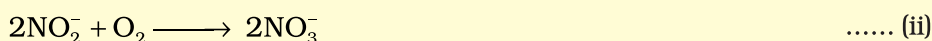
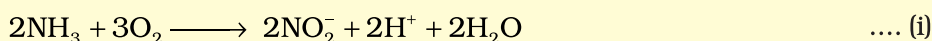
Majority of the nutrients that are essential for the growth and development of plants become available to the roots due to weathering and breakdown of rocks. These processes enrich the soil with dissolved ions and inorganic salts. Since they are derived from the rock minerals, their role in plant nutrition is referred to as mineral nutrition. Soil consists of a wide variety of substances. Soil not only supplies minerals but also harbours nitrogen-fixing bacteria, other microbes, holds water, supplies air to the roots and acts as a matrix that stabilises the plant. Since deficiency of essential minerals affect the crop-yield, there is often a need for supplying them through fertilisers. Both macro-nutrients (N, P, K, S, etc.) and micro-nutrients (Cu, Zn, Fe, Mn, etc.) form components of fertilisers and are applied as per need.

12.6 METABOLISM OF NITROGEN

12.6.1 Nitrogen Cycle

Apart from carbon, hydrogen and oxygen, nitrogen is the most prevalent element in living organisms. Nitrogen is a constituent of amino acids, proteins, hormones, chlorophylls and many of the vitamins. Plants compete with microbes for the limited nitrogen that

is available in soil. Thus, nitrogen is a limiting nutrient for both natural and agricultural eco-systems. Nitrogen exists as two nitrogen atoms joined by a very strong triple covalent bond ($\text{N} \equiv \text{N}$). The process of conversion of nitrogen (N_2) to ammonia is termed as **nitrogen-fixation**. In nature, lightning and ultraviolet radiation provide enough energy to convert nitrogen to nitrogen oxides (NO , NO_2 , N_2O). Industrial combustions, forest fires, automobile exhausts and power-generating stations are also sources of atmospheric nitrogen oxides. Decomposition of organic nitrogen of dead plants and animals into ammonia is called ammonification. Some of this ammonia volatilises and re-enters the atmosphere but most of it is converted into nitrate by soil bacteria in the following steps:



Ammonia is first oxidised to nitrite by the bacteria *Nitrosomonas* and/or *Nitrococcus*. The nitrite is further oxidised to nitrate with the help of the bacterium *Nitrobacter*. These steps are called **nitrification** (Figure 12.3). These nitrifying bacteria are **chemoautotrophs**.

The nitrate thus formed is absorbed by plants and is transported to the leaves. In leaves, it is reduced to form ammonia that finally forms the amine group of amino acids. Nitrate present in the soil is also reduced to nitrogen by the process of denitrification. Denitrification is carried by bacteria *Pseudomonas* and *Thiobacillus*.

12.6.2 Biological Nitrogen Fixation

Very few living organisms can utilise the nitrogen in the form N_2 , available abundantly in the air. Only certain prokaryotic species are capable of fixing nitrogen. Reduction of nitrogen to ammonia by living organisms is

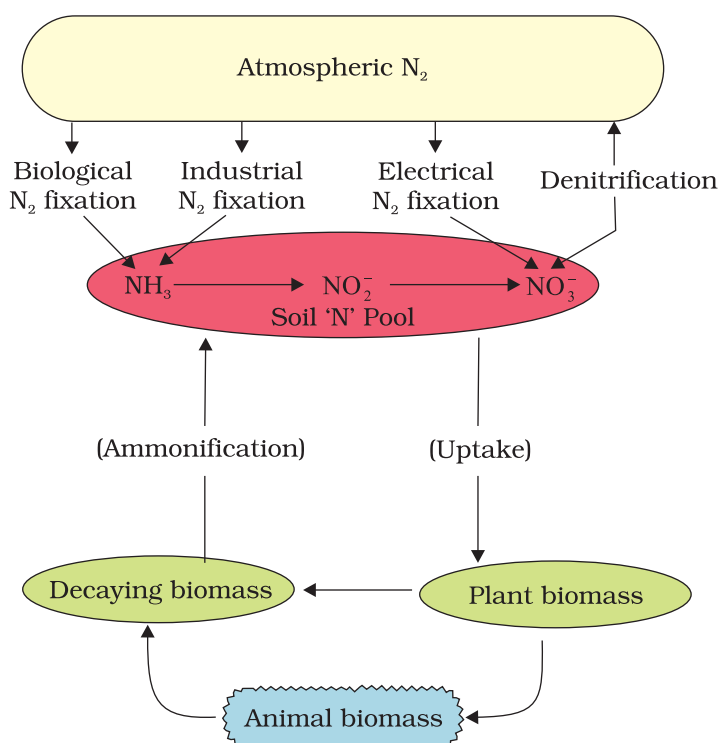
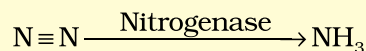


Figure 12.3 The nitrogen cycle showing relationship between the three main nitrogen pools – atmospheric, soil, and biomass

called **biological nitrogen fixation**. The enzyme, nitrogenase which is capable of nitrogen reduction is present exclusively in prokaryotes. Such microbes are called N_2 - fixers.



The nitrogen-fixing microbes could be free-living or symbiotic. Examples of free-living nitrogen-fixing aerobic microbes are *Azotobacter* and *Beijernickia* while *Rhodospirillum* is anaerobic and *Bacillus* free-living. In addition, a number of cyanobacteria such as *Anabaena* and *Nostoc* are also free-living nitrogen-fixers.

Symbiotic biological nitrogen fixation

Several types of symbiotic biological nitrogen fixing associations are known. The most prominent among them is the legume-bacteria relationship. Species of rod-shaped *Rhizobium* has such relationship with the roots of several legumes such as alfalfa, sweet clover, sweet pea, lentils, garden pea, broad bean, clover beans, etc. The most common association on roots is as nodules. These nodules are small outgrowths on the roots. The microbe, *Frankia*, also produces nitrogen-fixing nodules on the roots of non-leguminous plants (e.g., *Alnus*). Both *Rhizobium* and *Frankia* are free-living in soil, but as symbionts, can fix atmospheric nitrogen.

Uproot any one plant of a common pulse, just before flowering. You will see near-spherical outgrowths on the roots. These are nodules. If you cut through them you will notice that the central portion is red or pink. What makes the nodules pink? This is due to the presence of leguminous haemoglobin or leg-haemoglobin.

Nodule Formation

Nodule formation involves a sequence of multiple interactions between *Rhizobium* and roots of the host plant. Principal stages in the nodule formation are summarised as follows:

Rhizobia multiply and colonise the surroundings of roots and get attached to epidermal and root hair cells. The root-hairs curl and the bacteria invade the root-hair. An infection thread is produced carrying the bacteria into the cortex of the root, where they initiate the nodule formation in the cortex of the root. Then the bacteria are released from the thread into the cells which leads to the differentiation of specialised nitrogen fixing cells. The nodule thus formed, establishes a direct vascular connection with the host for exchange of nutrients. These events are depicted in Figure 12.4.

The nodule contains all the necessary biochemical components, such as the enzyme nitrogenase and leghaemoglobin. The enzyme nitrogenase is a Mo-Fe protein and catalyses the conversion of atmospheric nitrogen to ammonia, (Figure 12.5) the first stable product of nitrogen fixation.

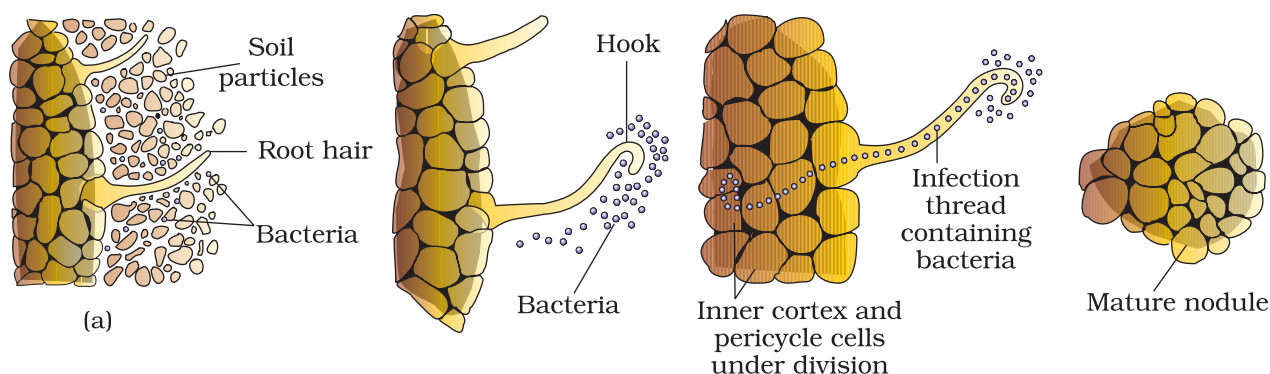
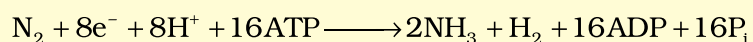


Figure 12.4 Development of root nodules in soyabean : (a) *Rhizobium* bacteria contact a susceptible root hair, divide near it, (b) Successful infection of the root hair causes it to curl, (c) Infected thread carries the bacteria to the inner cortex. The bacteria get modified into rod-shaped bacteroids and cause inner cortical and pericycle cells to divide. Division and growth of cortical and pericycle cells lead to nodule formation, (d) A mature nodule is complete with vascular tissues continuous with those of the root

The reaction is as follows:



The enzyme nitrogenase is highly sensitive to the molecular oxygen; it requires anaerobic conditions. The nodules have adaptations that ensure that the enzyme is protected from oxygen. To protect these enzymes, the nodule contains an oxygen scavenger called leg-haemoglobin. It is interesting to note that these microbes live as aerobes under free-living conditions (where nitrogenase is not operational), but during nitrogen-fixing events, they become anaerobic (thus protecting the nitrogenase enzyme). You must have noticed in the above reaction that the ammonia synthesis by nitrogenase requires a

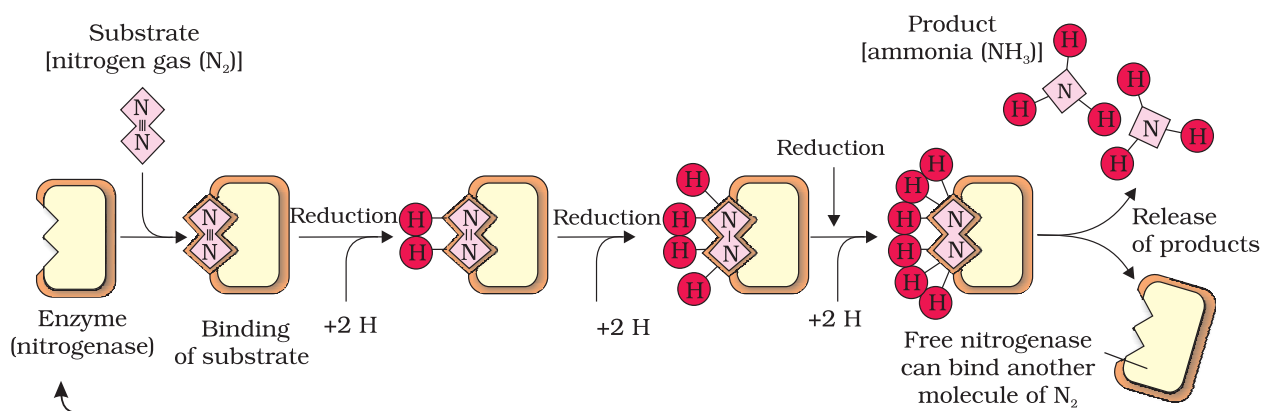
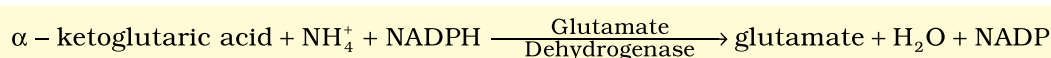


Figure 12.5 Steps of conversion of atmospheric nitrogen to ammonia by nitrogenase enzyme complex found in nitrogen-fixing bacteria

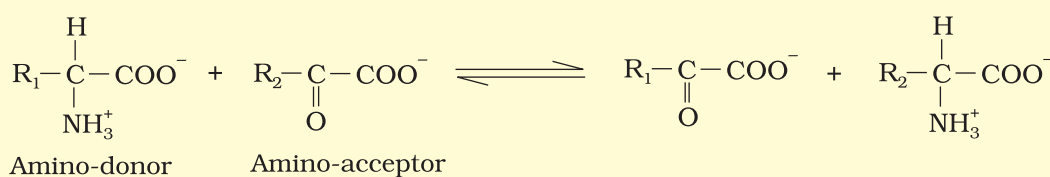
very high input of energy (8 ATP for each NH_3 produced). The energy required, thus, is obtained from the respiration of the host cells.

Fate of ammonia: At physiological pH, the ammonia is protonated to form NH_4^+ (ammonium) ion. While most of the plants can assimilate nitrate as well as ammonium ions, the latter is quite toxic to plants and hence cannot accumulate in them. Let us now see how the NH_4^+ is used to synthesise amino acids in plants. There are two main ways in which this can take place:

(i) Reductive amination : In these processes, ammonia reacts with α -ketoglutaric acid and forms glutamic acid as indicated in the equation given below :



(ii) Transamination : It involves the transfer of amino group from one amino acid to the keto group of a keto acid. Glutamic acid is the main amino acid from which the transfer of NH_2 , the amino group takes place and other amino acids are formed through transamination. The enzyme **transaminase** catalyses all such reactions. For example,



The two most important amides – asparagine and glutamine – found in plants are a structural part of proteins. They are formed from two amino acids, namely aspartic acid and glutamic acid, respectively, by addition of another amino group to each. The hydroxyl part of the acid is replaced by another NH_2 radicle. Since amides contain more nitrogen than the amino acids, they are transported to other parts of the plant via xylem vessels. In addition, along with the transpiration stream the nodules of some plants (e.g., soyabean) export the fixed nitrogen as ureides. These compounds also have a particularly high nitrogen to carbon ratio.

SUMMARY

Plants obtain their inorganic nutrients from air, water and soil. Plants absorb a wide variety of mineral elements. Not all the mineral elements that they absorb are required by plants. Out of the more than 105 elements discovered so far, less than 21 are essential and beneficial for normal plant growth and development. The elements required in large quantities are called macronutrients while those required in less quantities or in trace are termed as micronutrients. These elements are either essential constituents of proteins, carbohydrates, fats, nucleic acid etc.,

and/or take part in various metabolic processes. Deficiency of each of these essential elements may lead to symptoms called deficiency symptoms. Chlorosis, necrosis, stunted growth, impaired cell division, etc., are some prominent deficiency symptoms. Plants absorb minerals through roots by either passive or active processes. They are carried to all parts of the organism through xylem along with water transport.

Nitrogen is very essential for the sustenance of life. Plants cannot use atmospheric nitrogen directly. But some of the plants in association with N_2 -fixing bacteria, especially roots of legumes, can fix this atmospheric nitrogen into biologically usable forms. Nitrogen fixation requires a strong reducing agent and energy in the form of ATP. N_2 -fixation is accomplished with the help of nitrogen-fixing microbes, mainly *Rhizobium*. The enzyme nitrogenase which plays an important role in biological N_2 fixation is very sensitive to oxygen. Most of the processes take place in anaerobic environment. The energy, ATP, required is provided by the respiration of the host cells. Ammonia produced following N_2 fixation is incorporated into amino acids as the amino group.

EXERCISES

1. 'All elements that are present in a plant need not be essential to its survival'. Comment.
2. Why is purification of water and nutrient salts so important in studies involving mineral nutrition using hydroponics?
3. Explain with examples: macronutrients, micronutrients, beneficial nutrients, toxic elements and essential elements.
4. Name at least five different deficiency symptoms in plants. Describe them and correlate them with the concerned mineral deficiency.
5. If a plant shows a symptom which could develop due to deficiency of more than one nutrient, how would you find out experimentally, the real deficient mineral element?
6. Why is that in certain plants deficiency symptoms appear first in younger parts of the plant while in others they do so in mature organs?
7. How are the minerals absorbed by the plants?
8. What are the conditions necessary for fixation of atmospheric nitrogen by *Rhizobium*. What is their role in N_2 -fixation?
9. What are the steps involved in formation of a root nodule?
10. Which of the following statements are true? If false, correct them:
 - (a) Boron deficiency leads to stout axis.
 - (b) Every mineral element that is present in a cell is needed by the cell.
 - (c) Nitrogen as a nutrient element, is highly immobile in the plants.
 - (d) It is very easy to establish the essentiality of micronutrients because they are required only in trace quantities.

CHAPTER 13

PHOTOSYNTHESIS IN HIGHER PLANTS

- 13.1 *What do we Know?*
- 13.2 *Early Experiments*
- 13.3 *Where does Photosynthesis take place?*
- 13.4 *How many Pigments are involved in Photosynthesis?*
- 13.5 *What is Light Reaction?*
- 13.6 *The Electron Transport*
- 13.7 *Where are the ATP and NADPH Used?*
- 13.8 *The C₄ Pathway*
- 13.9 *Photorespiration*
- 13.10 *Factors affecting Photosynthesis*

All animals including human beings depend on plants for their food. Have you ever wondered from where plants get their food? Green plants, in fact, have to make or rather synthesise the food they need and all other organisms depend on them for their needs. Green plants carry out 'photosynthesis', a physico-chemical process by which they use light energy to drive the synthesis of organic compounds. Ultimately, all living forms on earth depend on sunlight for energy. The use of energy from sunlight by plants doing photosynthesis is the basis of life on earth. Photosynthesis is important due to two reasons: it is the primary source of all food on earth. It is also responsible for the release of oxygen into the atmosphere by green plants. *Have you ever thought what would happen if there were no oxygen to breath?* This chapter focusses on the structure of the photosynthetic machinery and the various reactions that transform light energy into chemical energy.

13.1 WHAT DO WE KNOW?

Let us try to find out what we already know about photosynthesis. Some simple experiments you may have done in the earlier classes have shown that chlorophyll (green pigment of the leaf), light and CO₂ are required for photosynthesis to occur.

You may have carried out the experiment to look for starch formation in two leaves – a variegated leaf or a leaf that was partially covered with black paper, and one that was exposed to light. On testing these leaves for starch it was clear that photosynthesis occurred only in the green parts of the leaves in the presence of light.

Another experiment you may have carried out is the half-leaf experiment, where a part of a leaf is enclosed in a test tube containing some KOH soaked cotton (which absorbs CO_2), while the other half is exposed to air. The setup is then placed in light for some time. On testing for starch later in the two halves of the leaf, you must have found that the exposed part of the leaf tested positive for starch while the portion that was in the tube, tested negative. This showed that CO_2 was required for photosynthesis. *Can you explain how this conclusion could be drawn?*

13.2 EARLY EXPERIMENTS

It is interesting to learn about those simple experiments that led to a gradual development in our understanding of photosynthesis.

Joseph Priestley (1733-1804) in 1770 performed a series of experiments that revealed the essential role of air in the growth of green plants. Priestley, you may recall, discovered oxygen in 1774. Priestley observed that a candle burning in a closed space – a bell jar, soon gets extinguished (Figure 13.1 a, b, c, d). Similarly, a mouse would soon suffocate in a closed space. He concluded that a burning candle or an animal that breathe the air, both somehow, damage the air. But when he placed a mint plant in the same bell jar, he found that the mouse stayed alive and the candle continued to burn. Priestley hypothesised as follows: Plants restore to the air whatever breathing animals and burning candles remove.

Can you imagine how Priestley would have conducted the experiment using a candle and a plant? Remember, he would need to rekindle the candle to test whether it burns after a few days. *How many different ways can you think of to light the candle without disturbing the set-up?*

Using a similar setup as the one used by Priestley, but by placing it once in the dark and once in the sunlight, Jan Ingenhousz (1730-1799) showed that sunlight is essential to the plant process that somehow purifies the air fouled by burning candles or breathing animals. Ingenhousz in an elegant experiment with an aquatic plant showed that in bright sunlight, small bubbles were formed around the green parts while in the dark they did not. Later he identified these bubbles to be of oxygen. Hence he showed that it is only the green part of the plants that could release oxygen.

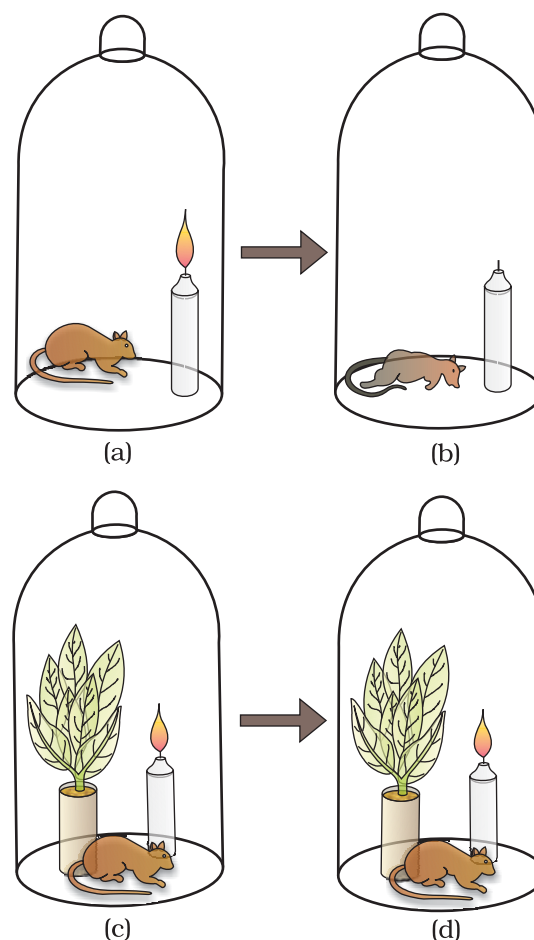
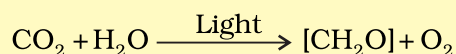


Figure 13.1 Priestley's experiment

It was not until about 1854 that Julius von Sachs provided evidence for production of glucose when plants grow. Glucose is usually stored as starch. His later studies showed that the green substance in plants (chlorophyll as we know it now) is located in special bodies (later called chloroplasts) within plant cells. He found that the green parts in plants is where glucose is made, and that the glucose is usually stored as starch.

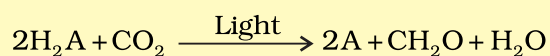
Now consider the interesting experiments done by T.W Engelmann (1843 – 1909). Using a prism he split light into its spectral components and then illuminated a green alga, *Cladophora*, placed in a suspension of aerobic bacteria. The bacteria were used to detect the sites of O₂ evolution. He observed that the bacteria accumulated mainly in the region of blue and red light of the split spectrum. A first action spectrum of photosynthesis was thus described. It resembles roughly the absorption spectra of chlorophyll *a* and *b* (discussed in section 13.4).

By the middle of the nineteenth century the key features of plant photosynthesis were known, namely, that plants could use light energy to make carbohydrates from CO₂ and water. The empirical equation representing the total process of photosynthesis for oxygen evolving organisms was then understood as:

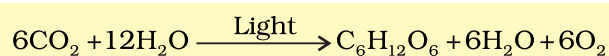


where [CH₂O] represented a carbohydrate (e.g., glucose, a six-carbon sugar).

A milestone contribution to the understanding of photosynthesis was that made by a microbiologist, Cornelius van Niel (1897-1985), who, based on his studies of purple and green bacteria, demonstrated that photosynthesis is essentially a light-dependent reaction in which hydrogen from a suitable oxidisable compound reduces carbon dioxide to carbohydrates. This can be expressed by:



In green plants H₂O is the hydrogen donor and is oxidised to O₂. Some organisms do not release O₂ during photosynthesis. When H₂S, instead is the hydrogen donor for purple and green sulphur bacteria, the 'oxidation' product is sulphur or sulphate depending on the organism and not O₂. Hence, he inferred that the O₂ evolved by the green plant comes from H₂O, not from carbon dioxide. This was later proved by using radioisotopic techniques. The correct equation, that would represent the overall process of photosynthesis is therefore:



where C₆ H₁₂ O₆ represents glucose. The O₂ released is from water; this was proved using radio isotope techniques. Note that this is not a single

reaction but description of a multistep process called photosynthesis. *Can you explain why twelve molecules of water as substrate are used in the equation given above?*

13.3 WHERE DOES PHOTOSYNTHESIS TAKE PLACE?

You would of course answer: in 'the green leaf' or you may add, 'in the chloroplasts' based on what you earlier read in Chapter 8. You are definitely right. Photosynthesis does take place in the green leaves of plants but it does so also in other green parts of the plants. *Can you name some other parts where you think photosynthesis may occur?*

You would recollect from previous unit that the mesophyll cells in the leaves, have a large number of chloroplasts. Usually the chloroplasts align themselves along the walls of the mesophyll cells, such that they get the optimum quantity of the incident light. *When do you think the chloroplasts will be aligned with their flat surfaces parallel to the walls? When would they be perpendicular to the incident light?*

You have studied the structure of chloroplast in Chapter 8. Within the chloroplast there is the membranous system consisting of grana, the stroma lamellae, and the fluid stroma (Figure 13.2). There is a clear division of labour within the chloroplast. The membrane system is responsible for trapping the light energy and also for the synthesis of ATP and NADPH. In stroma, enzymatic reactions incorporate CO_2 into the plant leading to the synthesis of sugar, which in turn forms starch. The former set of reactions, since they are directly light driven are called **light reactions**. The latter are not directly light driven but are dependent on the products of light reactions (ATP and NADPH). Hence, to distinguish the latter they are called, by convention, as **dark reactions**. However, this should not be construed to mean that they occur in darkness or that they are not light-dependent.

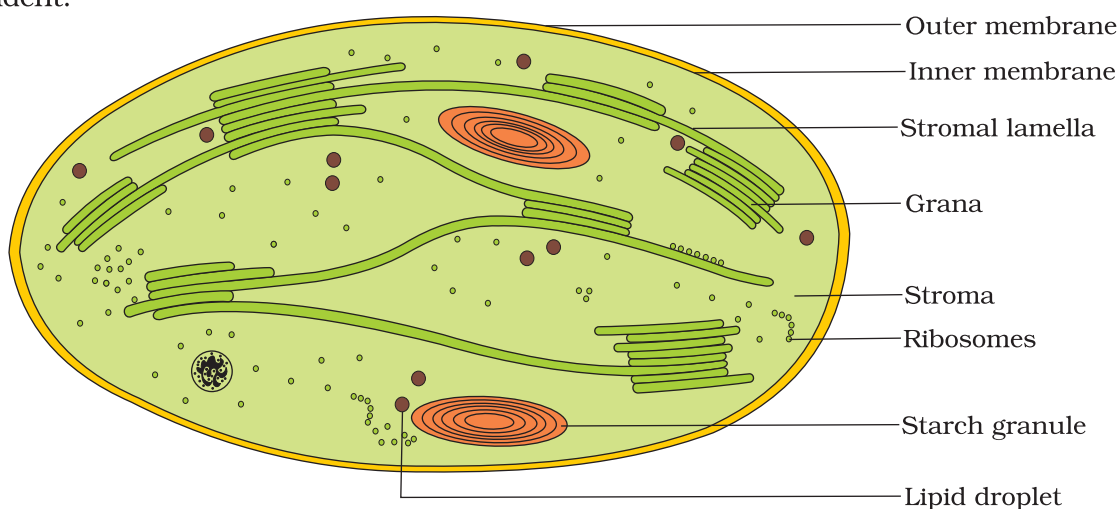


Figure 13.2 Diagrammatic representation of an electron micrograph of a section of chloroplast

13.4 HOW MANY PIGMENTS ARE INVOLVED IN PHOTOSYNTHESIS?

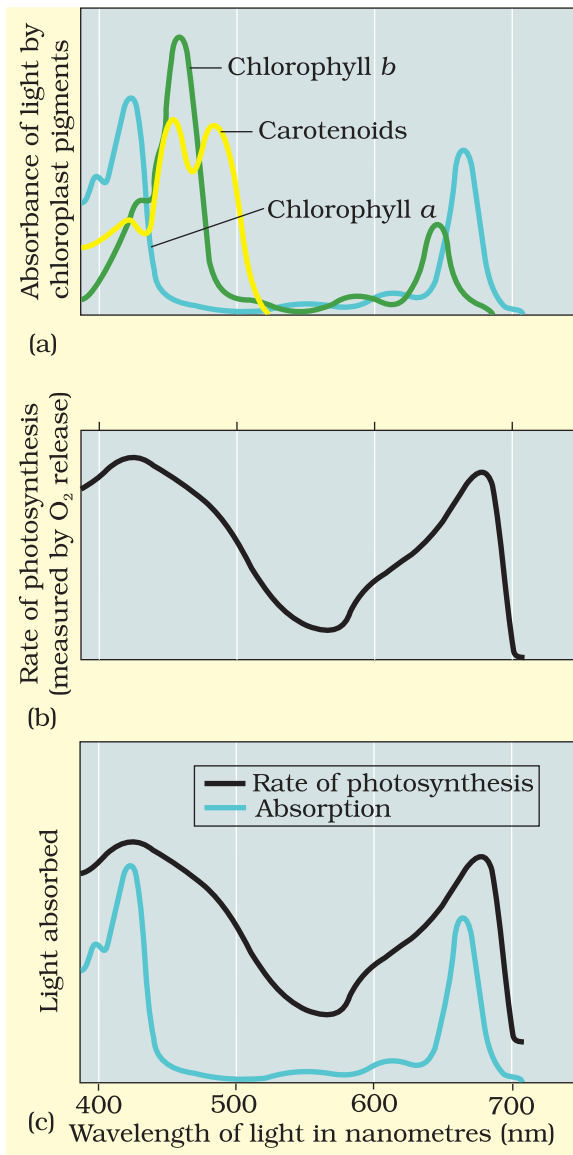


Figure 13.3a Graph showing the absorption spectrum of chlorophyll *a*, *b* and the carotenoids

Figure 13.3b Graph showing action spectrum of photosynthesis

Figure 13.3c Graph showing action spectrum of photosynthesis superimposed on absorption spectrum of chlorophyll *a*

Looking at plants have you ever wondered why and how there are so many shades of green in their leaves – even in the same plant? We can look for an answer to this question by trying to separate the leaf pigments of any green plant through paper chromatography. A chromatographic separation of the leaf pigments shows that the colour that we see in leaves is not due to a single pigment but due to four pigments: **Chlorophyll *a*** (bright or blue green in the chromatogram), **chlorophyll *b*** (yellow green), **xanthophylls** (yellow) and **carotenoids** (yellow to yellow-orange). Let us now see what roles various pigments play in photosynthesis.

Pigments are substances that have an ability to absorb light, at specific wavelengths. *Can you guess which is the most abundant plant pigment in the world?* Let us study the graph showing the ability of chlorophyll *a* pigment to absorb lights of different wavelengths (Figure 13.3 a). Of course, you are familiar with the wavelength of the visible spectrum of light as well as the VIBGYOR.

*From Figure 13.3a can you determine the wavelength (colour of light) at which chlorophyll *a* shows the maximum absorption? Does it show another absorption peak at any other wavelengths too? If yes, which one?*

Now look at Figure 13.3b showing the wavelengths at which maximum photosynthesis occurs in a plant. Can you see that the wavelengths at which there is maximum absorption by chlorophyll *a*, i.e., in the blue and the red regions, also shows higher rate of photosynthesis. Hence, we can conclude that chlorophyll *a* is the chief pigment associated with photosynthesis. *But by looking at Figure 13.3c can you say that there is a complete one-to-one overlap between the absorption spectrum of chlorophyll *a* and the action spectrum of photosynthesis?*

These graphs, together, show that most of the photosynthesis takes place in the blue and red regions of the spectrum; some photosynthesis does take place at the other wavelengths of the visible spectrum. Let us see how this happens. Though chlorophyll is the major pigment responsible for trapping light, other thylakoid pigments like chlorophyll *b*, xanthophylls and carotenoids, which are called accessory pigments, also absorb light and transfer the energy to chlorophyll *a*. Indeed, they not only enable a wider range of wavelength of incoming light to be utilised for photosynthesis but also protect chlorophyll *a* from photo-oxidation.

13.5 WHAT IS LIGHT REACTION?

Light reactions or the 'Photochemical' phase include light absorption, water splitting, oxygen release, and the formation of high-energy chemical intermediates, ATP and NADPH. Several complexes are involved in the process. The pigments are organised into two discrete photochemical **light harvesting complexes (LHC)** within the **Photosystem I (PS I)** and **Photosystem II (PS II)**. These are named in the sequence of their discovery, and not in the sequence in which they function during the light reaction. The LHC are made up of hundreds of pigment molecules bound to proteins. Each photosystem has all the pigments (except one molecule of chlorophyll *a*) forming a light harvesting system also called **antennae** (Figure 13.4). These pigments help to make photosynthesis more efficient by absorbing different wavelengths of light. The single chlorophyll *a* molecule forms the **reaction centre**. The reaction centre is different in both the photosystems. In PS I the reaction centre chlorophyll *a* has an absorption peak at 700 nm, hence is called **P700**, while in PS II it has absorption maxima at 680 nm, and is called **P680**.

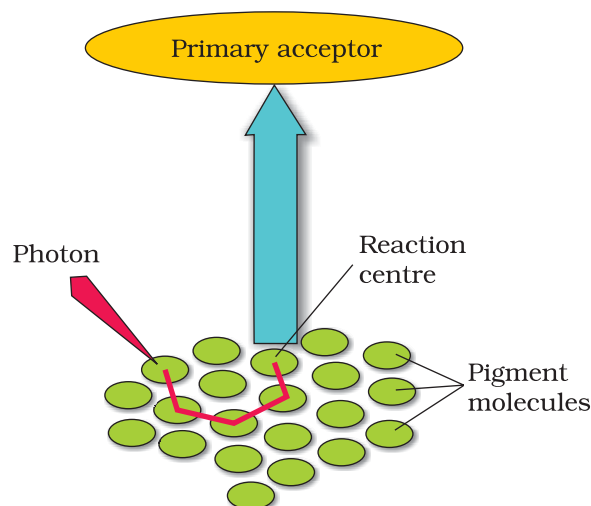


Figure 13.4 The light harvesting complex

13.6 THE ELECTRON TRANSPORT

In photosystem II the reaction centre chlorophyll *a* absorbs 680 nm wavelength of red light causing electrons to become excited and jump into an orbit farther from the atomic nucleus. These electrons are picked up by an electron acceptor which passes them to an **electrons transport**