# Chapter 10
# Cell Cycle and Cell Division



Are you aware that all organisms, even the largest, start their life from a single cell? You may wonder how a single cell then goes on to form such large organisms. Growth and reproduction are characteristics of cells, indeed of all living organisms. All cells reproduce by dividing into two, with each parental cell giving rise to two daughter cells each time they divide. These newly formed daughter cells can themselves grow and divide, giving rise to a new cell population that is formed by the growth and division of a single parental cell and its progeny. In other words, such cycles of growth and division allow a single cell to form a structure consisting of millions of cells.

## 10.1 Cell Cycle

Cell division is a very important process in all living organisms. During the division of a cell, DNA replication and cell growth also take place. All these processes, i.e., cell division, DNA replication, and cell growth, hence, have to take place in a coordinated way to ensure correct division and formation of progeny cells containing intact genomes. The sequence of events by which a cell duplicates its genome, synthesises the other constituents of the cell and eventually divides into two daughter cells is termed **cell cycle.** Although cell growth (in terms of cytoplasmic increase) is a continuous process, DNA synthesis occurs only during one specific stage in the cell cycle. The replicated chromosomes (DNA) are then distributed to daughter nuclei by a complex series of events during cell division. These events are themselves under genetic control.

### 10.1.1 Phases of Cell Cycle

A typical eukaryotic cell cycle is illustrated by human cells in culture. These cells divide once in approximately every 24 hours (Figure 10.1). However, this duration of cell cycle can vary from organism to organism and also from cell type to cell type. Yeast for example, can progress through the cell cycle in only about 90 minutes.

The cell cycle is divided into two basic phases:

- **Interphase**
- **M Phase (Mitosis phase)**

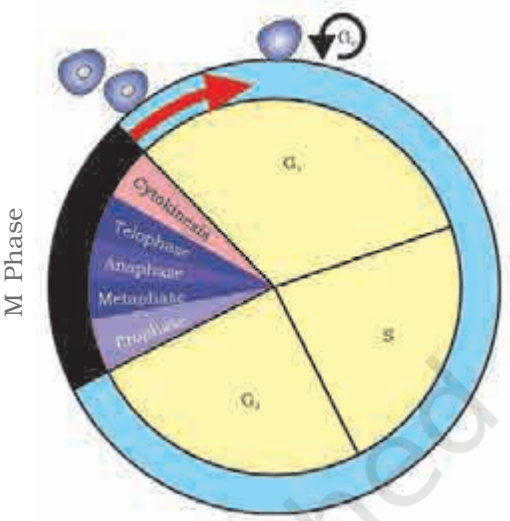



**Figure 10.1** A diagrammatic view of cell cycle indicating formation of two cells from one cell

The M Phase represents the phase when the actual cell division or mitosis occurs and the interphase represents the phase between two successive M phases. It is significant to note that in the 24 hour average duration of cell cycle of a human cell, cell division proper lasts for only about an hour. The interphase lasts more than 95% of the duration of cell cycle.

The M Phase starts with the nuclear division, corresponding to the separation of daughter chromosomes **(karyokinesis)** and usually ends with division of cytoplasm **(cytokinesis).** The interphase, though called the resting phase, is the time during which the cell is preparing for division by undergoing both cell growth and DNA replication in an orderly manner. The interphase is divided into three further phases:

- **$G_1$ phase (Gap 1)**
- **S phase (Synthesis)**
- **$G_2$ phase (Gap 2)**

$G_1$ phase corresponds to the interval between mitosis and initiation of DNA replication. During $G_1$ phase the cell is metabolically active and continuously grows but does not replicate its DNA. S or **synthesis** phase marks the period during which DNA synthesis or replication takes place. During this time the amount of DNA per cell doubles. If the initial amount of DNA is denoted as 2C then it increases to 4C. However, there is no increase in the chromosome number; if the cell had diploid or 2n number of chromosomes at $G_1$, even after S phase the number of chromosomes remains the same, i.e., 2n.

In animal cells, during the S phase, DNA replication begins in the nucleus, and the centriole duplicates in the cytoplasm. During the $G_2$ phase, proteins are synthesised in preparation for mitosis while cell growth continues.

How do plants and animals continue to grow all their lives? Do all cells in a plant divide all the time? Do you think all cells continue to divide in all plants and animals? Can you tell the name and the location of tissues having cells that divide all their life in higher plants? Do animals have similar meristematic tissues?

You have studied mitosis in onion root tip cells. It has 16 chromosomes in each cell. Can you tell how many chromosomes will the cell have at $G_1$ phase, after S phase, and after M phase? Also, what will be the DNA content of the cells at $G_1$, after S and at $G_2$, if the content after M phase is 2C?

Some cells in the adult animals do not appear to exhibit division (e.g., heart cells) and many other cells divide only occasionally, as needed to replace cells that have been lost because of injury or cell death. These cells that do not divide further exit $G_1$ phase to enter an inactive stage called ***quiescent stage ($G_0$)*** of the cell cycle. Cells in this stage remain metabolically active but no longer proliferate unless called on to do so depending on the requirement of the organism.

In animals, mitotic cell division is only seen in the diploid somatic cells. Against this, the plants can show mitotic divisions in both haploid and diploid cells. From your recollection of examples of alternation of generations in plants (Chapter 3) identify plant species and stages at which mitosis is seen in haploid cells.

## 10.2 M PHASE

This is the most dramatic period of the cell cycle, involving a major reorganisation of virtually all components of the cell. Since the number of chromosomes in the parent and progeny cells is the same, it is also called as ***equational division.*** Though for convenience mitosis has been divided into four stages of nuclear division (karyokinesis), it is very essential to understand that cell division is a progressive process and very clear-cut lines cannot be drawn between various stages. Karyokinesis involves following four stages:

- ***Prophase***
- ***Metaphase***
- ***Anaphase***
- ***Telophase***

### 10.2.1 Prophase

Prophase which is the first stage of karyokinesis of mitosis follows the S and $G_2$ phases of interphase. In the S and $G_2$ phases the new DNA molecules formed are not distinct but intertwined. Prophase is marked by the initiation of condensation of chromosomal material. The chromosomal material becomes untangled during the process of chromatin condensation (Figure 10.2 a). The centrosome, which had undergone duplication during S phase of interphase, now begins to move towards opposite poles of the cell. The completion of prophase can thus be marked by the following characteristic events:

- Chromosomal material condenses to form compact mitotic chromosomes. Chromosomes are seen to be composed of two chattached together at the centromere.
- Centrosome which had undergone duplication during interphase, begins to move towards opposite poles of the cell. Each centrosome radiates out microtubules called asters. The two asters together with spindle fibres forms mitotic apparatus.

Cells at the end of prophase, when viewed under the microscope, do not show golgi complexes, endoplasmic reticulum, nucleolus and the nuclear envelope.

### 10.2.2 Metaphase

The complete disintegration of the nuclear envelope marks the start of the second phase of mitosis, hence the chromosomes are spread through the cytoplasm of the cell. By this stage, condensation of chromosomes is completed and they can be observed clearly under the microscope. This then, is the stage at which morphology of chromosomes is most easily studied. At this stage, metaphase chromosome is made up of two sister chromatids, which are held together by the centromere (Figure 10.2 b). Small disc-shaped structures at the surface of the centromeres are called kinetochores. These structures serve as the sites of attachment of spindle fibres (formed by the spindle fibres) to the chromosomes that are moved into position at the centre of the cell. Hence, the metaphase is characterised by all the chromosomes coming to lie at the equator with one chromatid of each chromosome connected by its kinetochore to spindle fibres from one pole and its sister chromatid connected by its kinetochore to spindle fibres from the opposite pole (Figure 10.2 b). The plane of alignment of the chromosomes at metaphase is referred to as the **metaphase plate.** The key features of metaphase are:

- Spindle fibres attach to kinetochores of chromosomes.
- Chromosomes are moved to spindle equator and get aligned along metaphase plate through spindle fibres to both poles.

### 10.2.3 Anaphase

At the onset of anaphase, each chromosome arranged at the metaphase plate is split simultaneously and the two daughter chromatids, now referred to as daughter chromosomes of the future daughter nuclei, begin their migration towards the two opposite poles. As each chromosome moves away from the equatorial plate, the centromere of each chromosome remains directed towards the pole and hence at the leading edge, with the arms of the chromosome trailing behind (Figure 10.2 c). Thus, anaphase stage is characterised by

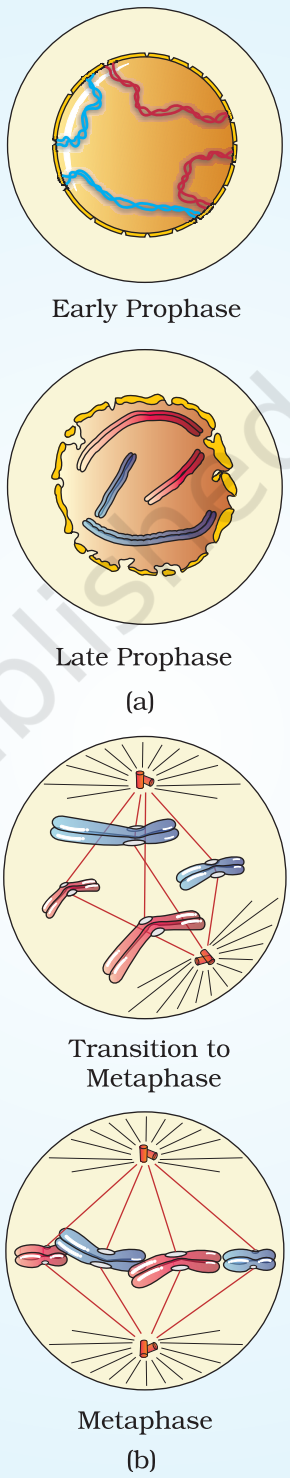
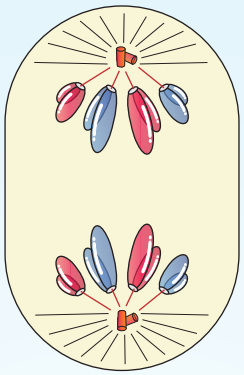
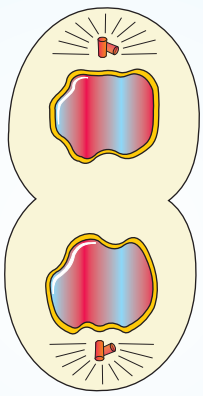



**Figure 10.2 a and b** : A diagrammatic view of stages in mitosis



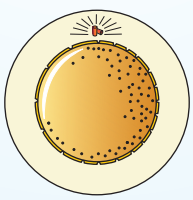
Anaphase

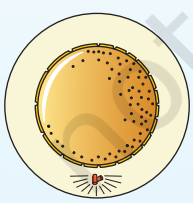
(c)



Telophase

(d)





Interphase

(e)

**Figure 10.2 c to e** : A diagrammatic view of stages in Mitosis

the following key events:

- Centromeres split and chromatids separate.
- Chromatids move to opposite poles.

### 10.2.4 Telophase

At the beginning of the final stage of karyokinesis, i.e., telophase, the chromosomes that have reached their respective poles decondense and lose their individuality. The individual chromosomes can no longer be seen and each set of chromatin material tends to collect at each of the two poles (Figure 10.2 d). This is the stage which shows the following key events:

- Chromosomes cluster at opposite spindle poles and their identity is lost as discrete elements.
- Nuclear envelope develops around the chromosome clusters at each pole forming two daughter nuclei.
- Nucleolus, golgi complex and ER reform.

### 10.2.5 Cytokinesis

Mitosis accomplishes not only the segregation of duplicated chromosomes into daughter nuclei (karyokinesis), but the cell itself is divided into two daughter cells by the separation of cytoplasm called cytokinesis at the end of which cell division gets completed (Figure 10.2 e). In an animal cell, this is achieved by the appearance of a furrow in the plasma membrane. The furrow gradually deepens and ultimately joins in the centre dividing the cell cytoplasm into two. Plant cells however, are enclosed by a relatively inextensible cell wall, thererfore they undergo cytokinesis by a different mechanism. In plant cells, wall formation starts in the centre of the cell and grows outward to meet the existing lateral walls. The formation of the new cell wall begins with the formation of a simple precursor, called the **cell-plate** that represents the middle lamella between the walls of two adjacent cells. At the time of cytoplasmic division, organelles like mitochondria and plastids get distributed between the two daughter cells. In some organisms karyokinesis is not followed by cytokinesis as a result of which multinucleate condition arises leading to the formation of syncytium (e.g., liquid endosperm in coconut).

## 10.3 Significance of Mitosis

Mitosis or the equational division is usually restricted to the diploid cells only. However, in some lower plants and in some social insects haploid cells also divide by mitosis. It is very essential to understand the significance of this division in the life of an organism. Are you aware of some examples where you have studied about haploid and diploid insects?

Mitosis usually results in the production of diploid daughter cells with identical genetic complement. The growth of multicellular organisms is due to mitosis. Cell growth results in disturbing the ratio between the nucleus and the cytoplasm. It therefore becomes essential for the cell to divide to restore the nucleo-cytoplasmic ratio. A very significant contribution of mitosis is cell repair. The cells of the upper layer of the epidermis, cells of the lining of the gut, and blood cells are being constantly replaced. Mitotic divisions in the meristematic tissues – the apical and the lateral cambium, result in a continuous growth of plants throughout their life.

## 10.4 MEIOSIS

The production of offspring by sexual reproduction includes the fusion of two gametes, each with a complete haploid set of chromosomes. Gametes are formed from specialised diploid cells. This specialised kind of cell division that reduces the chromosome number by half results in the production of haploid daughter cells. This kind of division is called **meiosis.** Meiosis ensures the production of haploid phase in the life cycle of sexually reproducing organisms whereas fertilisation restores the diploid phase. We come across meiosis during gametogenesis in plants and animals. This leads to the formation of haploid gametes. The key features of meiosis are as follows:

- Meiosis involves two sequential cycles of nuclear and cell division called **meiosis I** and **meiosis II** but only a single cycle of DNA replication.
- Meiosis I is initiated after the parental chromosomes have replicated to produce identical sister chromatids at the S phase.
- Meiosis involves pairing of homologous chromosomes and recombination between non-sister chromatids of homologous chromosomes.
- Four haploid cells are formed at the end of meiosis II.

Meiotic events can be grouped under the following phases:

| Meiosis I | Meiosis II |
|---|---|
| Prophase I | Prophase II |
| Metaphase I | Metaphase II |
| Anaphase I | Anaphase II |
| Telophase I | Telophase II |

### 10.4.1 Meiosis I

**Prophase I:** Prophase of the first meiotic division is typically longer and more complex when compared to prophase of mitosis. It has been further subdivided into the following five phases based on chromosomal behaviour, i.e., Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis.

During **leptotene** stage the chromosomes become gradually visible under the light microscope. The compaction of chromosomes continues throughout leptotene. This is followed by the second stage of prophase I called **zygotene**. During this stage chromosomes start pairing together and this process of association is called synapsis. Such paired chromosomes are called homologous chromosomes. Electron micrographs of this stage indicate that chromosome synapsis is accompanied by the formation of complex structure called **synaptonemal complex.** The complex formed by a pair of synapsed homologous chromosomes is called a **bivalent** or a tetrad. However, these are more clearly visible at the next stage. The first two stages of prophase I are relatively short-lived compared to the next stage that is **pachytene**. During this stage, the four chromatids of each bivalent chromosomes becomes distinct and clearly appears as tetrads. This stage is characterised by the appearance of recombination nodules, the sites at which crossing over occurs between non-sister chromatids of the homologous chromosomes. Crossing over is the exchange of genetic material between two homologous chromosomes. Crossing over is also an enzyme-mediated process and the enzyme involved is called recombinase. Crossing over leads to recombination of genetic material on the two chromosomes. Recombination between homologous chromosomes is completed by the end of pachytene, leaving the chromosomes linked at the sites of crossing over.

The beginning of **diplotene** is recognised by the dissolution of the synaptonemal complex and the tendency of the recombined homologous chromosomes of the bivalents to separate from each other except at the sites of crossovers. These X-shaped structures, are called **chiasmata.** In oocytes of some vertebrates, diplotene can last for months or years.

The final stage of meiotic prophase I is **diakinesis.** This is marked by terminalisation of chiasmata. During this phase the chromosomes are fully condensed and the meiotic spindle is assembled to prepare the homologous chromosomes for separation. By the end of diakinesis, the nucleolus disappears and the nuclear envelope also breaks down. Diakinesis represents transition to metaphase.

**Metaphase I:** The bivalent chromosomes align on the equatorial plate (Figure 10.3). The microtubules from the opposite poles of the spindle attach to the kinetochore of homologous chromosomes.

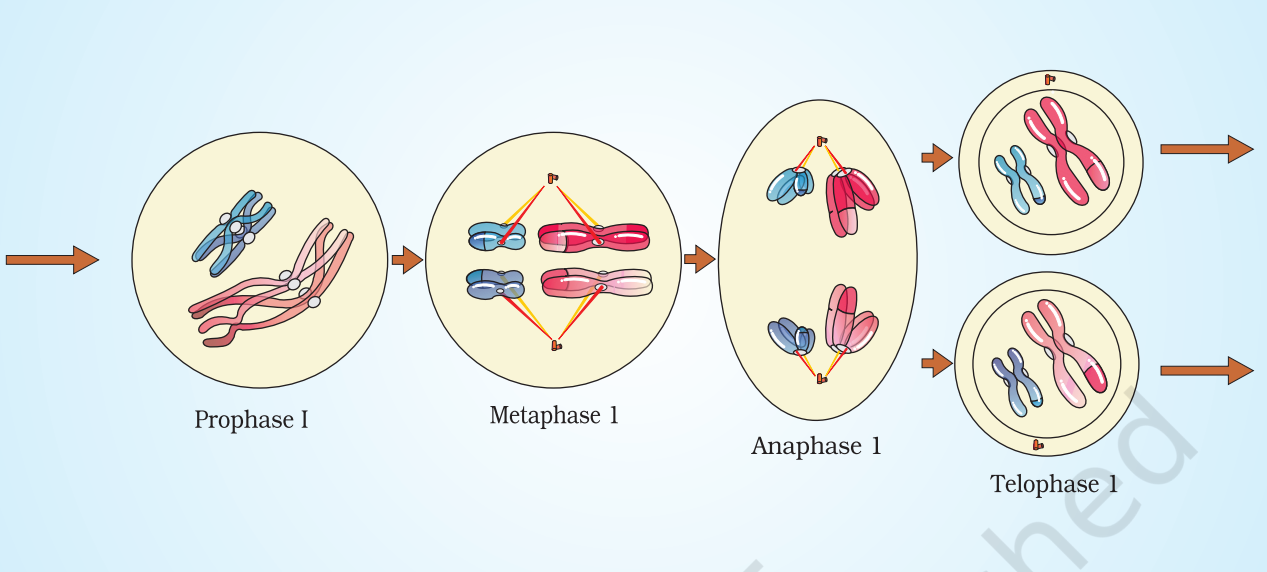



**Figure 10.3** Stages of Meiosis I

**Anaphase I:** The homologous chromosomes separate, while sister chromatids remain associated at their centromeres (Figure 10.3).

**Telophase I:** The nuclear membrane and nucleolus reappear, cytokinesis follows and this is called as dyad of cells (Figure 10.3). Although in many cases the chromosomes do undergo some dispersion, they do not reach the extremely extended state of the interphase nucleus. The stage between the two meiotic divisions is called interkinesis and is generally short lived. There is no replication of DNA during interkinesis. Interkinesis is followed by prophase II, a much simpler prophase than prophase I.

### 10.4.2 Meiosis II

**Prophase II:** Meiosis II is initiated immediately after cytokinesis, usually before the chromosomes have fully elongated. In contrast to meiosis I, meiosis II resembles a normal mitosis. The nuclear membrane disappears by the end of prophase II (Figure 10.4). The chromosomes again become compact.

**Metaphase II**: At this stage the chromosomes align at the equator and the microtubules from opposite poles of the spindle get attached to the kinetochores (Figure 10.4) of sister chromatids.

**Anaphase II:** It begins with the simultaneous splitting of the centromere of each chromosome (which was holding the sister chromatids together), allowing them to move toward opposite poles of the cell (Figure 10.4) by shortening of microtubules attached to kinetochores.

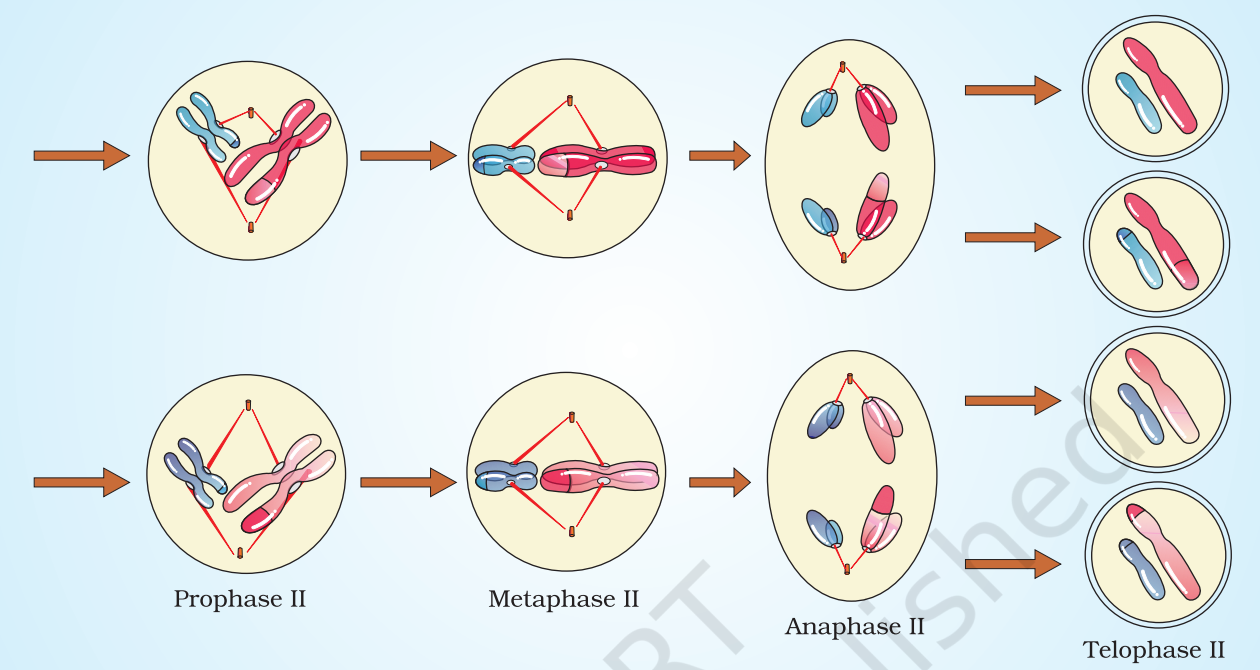



**Figure 10.4** Stages of Meiosis II

**Telophase II:** Meiosis ends with telophase II, in which the two groups of chromosomes once again get enclosed by a nuclear envelope; cytokinesis follows resulting in the formation of tetrad of cells i.e., four haploid daughter cells (Figure 10.4).

## 10.5 Significance of Meiosis

Meiosis is the mechanism by which conservation of specific chromosome number of each species is achieved across generations in sexually reproducing organisms, even though the process, per se, paradoxically, results in reduction of chromosome number by half. It also increases the genetic variability in the population of organisms from one generation to the next. Variations are very important for the process of evolution.

## Summary

According to the cell theory, cells arise from preexisting cells. The process by which this occurs is called cell division. Any sexually reproducing organism starts its life cycle from a single-celled zygote. Cell division does not stop with the formation of the mature organism but continues throughout its life cycle.

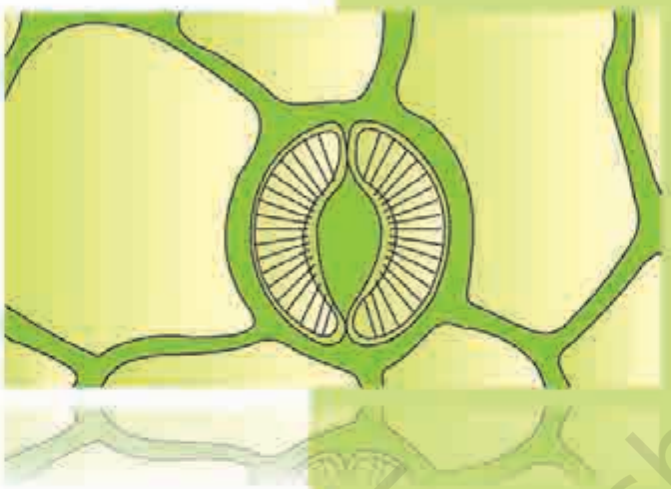
The stages through which a cell passes from one division to the next is called the cell cycle. Cell cycle is divided into two phases called (i) Interphase – a period of preparation for cell division, and (ii) Mitosis (M phase) – the actual period of cell division. Interphase is further subdivided into $G_1$, S and $G_2$. $G_1$ phase is the period when the cell grows and carries out normal metabolism. Most of the organelle duplication also occurs during this phase. S phase marks the phase of DNA replication and chromosome duplication. $G_2$ phase is the period of cytoplasmic growth. Mitosis is also divided into four stages namely prophase, metaphase, anaphase and telophase. Chromosome condensation occurs during prophase. Simultaneously, the centrioles move to the opposite poles. The nuclear envelope and the nucleolus disappear and the spindle fibres start appearing. Metaphase is marked by the alignment of chromosomes at the equatorial plate. During anaphase the centromeres divide and the chromatids start moving towards the two opposite poles. Once the chromatids reach the two poles, the chromosomal elongation starts, nucleolus and the nuclear membrane reappear. This stage is called the telophase. Nuclear division is then followed by the cytoplasmic division and is called cytokinesis. Mitosis thus, is the equational division in which the chromosome number of the parent is conserved in the daughter cell.

In contrast to mitosis, meiosis occurs in the diploid cells, which are destined to form gametes. It is called the reduction division since it reduces the chromosome number by half while making the gametes. In sexual reproduction when the two gametes fuse the chromosome number is restored to the value in the parent. Meiosis is divided into two phases – meiosis I and meiosis II. In the first meiotic division the homologous chromosomes pair to form bivalents, and undergo crossing over. Meiosis I has a long prophase, which is divided further into five phases. These are leptotene, zygotene, pachytene, diplotene and diakinesis. During metaphase I the bivalents arrange on the equatorial plate. This is followed by anaphase I in which homologous chromosomes move to the opposite poles with both their chromatids. Each pole receives half the chromosome number of the parent cell. In telophase I, the nuclear membrane and nucleolus reappear. Meiosis II is similar to mitosis. During anaphase II the sister chromatids separate. Thus at the end of meiosis four haploid cells are formed.

## EXERCISES

1. What is the average cell cycle span for a mammalian cell?
2. Distinguish cytokinesis from karyokinesis.
3. Describe the events taking place during interphase.
4. What is $G_0$ (quiescent phase) of cell cycle?

5. Why is mitosis called equational division?
6. Name the stage of cell cycle at which one of the following events occur:
   (i) Chromosomes are moved to spindle equator.
   (ii) Centromere splits and chromatids separate.
   (iii) Pairing between homologous chromosomes takes place.
   (iv) Crossing over between homologous chromosomes takes place.
7. Describe the following:
   (a) synapsis (b) bivalent (c) chiasmata
   Draw a diagram to illustrate your answer.
8. How does cytokinesis in plant cells differ from that in animal cells?
9. Find examples where the four daughter cells from meiosis are equal in size and where they are found unequal in size.
10. Distinguish anaphase of mitosis from anaphase I of meiosis.
11. List the main differences between mitosis and meiosis.
12. What is the significance of meiosis?
13. Discuss with your teacher about
    (i) haploid insects and lower plants where cell-division occurs, and
    (ii) some haploid cells in higher plants where cell-division does not occur.
14. Can there be mitosis without DNA replication in 'S' phase?
15. Can there be DNA replication without cell division?
16. Analyse the events during every stage of cell cycle and notice how the following two parameters change
    (i) number of chromosomes (N) per cell
    (ii) amount of DNA content (C) per cell



# UNIT 4

## PLANT PHYSIOLOGY



The description of structure and variation of living organisms over a period of time, ended up as two, apparently irreconcilable perspectives on biology. The two perspectives essentially rested on two levels of organisation of life forms and phenomena. One described at organismic and above level of organisation while the second described at cellular and molecular level of organisation. The first resulted in ecology and related disciplines. The second resulted in physiology and biochemistry. Description of physiological processes, in flowering plants as an example, is what is given in the chapters in this unit. The processes of mineral nutrition of plants, photosynthesis, transport, respiration and ultimately plant growth and development are described in molecular terms but in the context of cellular activities and even at organism level. Wherever appropriate, the relation of the physiological processes to environment is also discussed.

**Melvin Calvin**

MELVIN CALVIN born in Minnesota in April, 1911, received his Ph.D. in Chemistry from the University of Minnesota. He served as Professor of Chemistry at the University of California, Berkeley.

Just after world war II, when the world was under shock after the Hiroshima-Nagasaki bombings, and seeing the ill-effects of radio-activity, Calvin and co-workers put radio-activity to beneficial use. He along with J.A. Bassham studied reactions in green plants forming sugar and other substances from raw materials like carbon dioxide, water and minerals by labelling the carbon dioxide with $C^{14}$. Calvin proposed that plants change light energy to chemical energy by transferring an electron in an organised array of pigment molecules and other substances. The mapping of the pathway of carbon assimilation in photosynthesis earned him Nobel Prize in 1961.

The principles of photosynthesis as established by Calvin are, at present, being used in studies on renewable resource for energy and materials and basic studies in solar energy research.

# Chapter 11

# Transport in Plants



Have you ever wondered how water reaches the top of tall trees, or for that matter how and why substances move from one cell to the other, whether all substances move in a similar way, in the same direction and whether metabolic energy is required for moving substances. Plants need to move molecules over very long distances, much more than animals do; they also do not have a circulatory system in place. Water taken up by the roots has to reach all parts of the plant, up to the very tip of the growing stem. The photosynthates or food synthesised by the leaves have also to be moved to all parts including the root tips embedded deep inside the soil. Movement across short distances, say within the cell, across the membranes and from cell to cell within the tissue has also to take place. To understand some of the transport processes that take place in plants, one would have to recollect one's basic knowledge about the structure of the cell and the anatomy of the plant body. We also need to revisit our understanding of diffusion, besides gaining some knowledge about chemical potential and ions.

When we talk of the movement of substances we need first to define what kind of movement we are talking about, and also what substances we are looking at. In a flowering plant the substances that would need to be transported are water, mineral nutrients, organic nutrients and plant growth regulators. Over small distances substances move by diffusion and by cytoplasmic streaming supplemented by active transport. Transport over longer distances proceeds through the vascular system (the xylem and the phloem) and is called **translocation.**

An important aspect that needs to be considered is the direction of transport. In rooted plants, transport in xylem (of water and minerals) is essentially unidirectional, from roots to the stems. Organic and mineral nutrients however, undergo multidirectional transport. Organic

compounds synthesised in the photosynthetic leaves are exported to all other parts of the plant including storage organs. From the storage organs they are later re-exported. The mineral nutrients are taken up by the roots and transported upwards into the stem, leaves and the growing regions. When any plant part undergoes senescence, nutrients may be withdrawn from such regions and moved to the growing parts. Hormones or plant growth regulators and other chemical signals are also transported, though in very small amounts, sometimes in a strictly polarised or unidirectional manner from where they are synthesised to other parts. Hence, in a flowering plant there is a complex traffic of compounds (but probably very orderly) moving in different directions, each organ receiving some substances and giving out some others.

## 11.1 MEANS OF TRANSPORT

### 11.1.1 Diffusion

Movement by **diffusion** is passive, and may be from one part of the cell to the other, or from cell to cell, or over short distances, say, from the inter-cellular spaces of the leaf to the outside. No energy expenditure takes place. In diffusion, molecules move in a random fashion, the net result being substances moving from regions of higher concentration to regions of lower concentration. Diffusion is a slow process and is not dependent on a 'living system'. Diffusion is obvious in gases and liquids, but diffusion *in solids* rather than *of solids* is more likely. Diffusion is very important to plants since it is the only means for gaseous movement within the plant body.

Diffusion rates are affected by the gradient of concentration, the permeability of the membrane separating them, temperature and pressure.

### 11.1.2 Facilitated Diffusion

As pointed out earlier, a gradient must already be present for diffusion to occur. The diffusion rate depends on the size of the substances; obviously smaller substances diffuse faster. The diffusion of any substance across a membrane also depends on its solubility in lipids, the major constituent of the membrane. Substances soluble in lipids diffuse through the membrane faster. Substances that have a hydrophilic moiety, find it difficult to pass through the membrane; their movement has to be facilitated. Membrane proteins provide sites at which such molecules cross the membrane. They do not set up a concentration gradient: a concentration gradient must already be present for molecules to diffuse even if facilitated by the proteins. This process is called **facilitated diffusion**.

In facilitated diffusion special proteins help move substances across membranes without expenditure of ATP energy. Facilitated diffusion cannot cause net transport of molecules from a low to a high concentration – this would require input of energy. Transport rate reaches a maximum when all of the protein transporters are being used (saturation). Facilitated

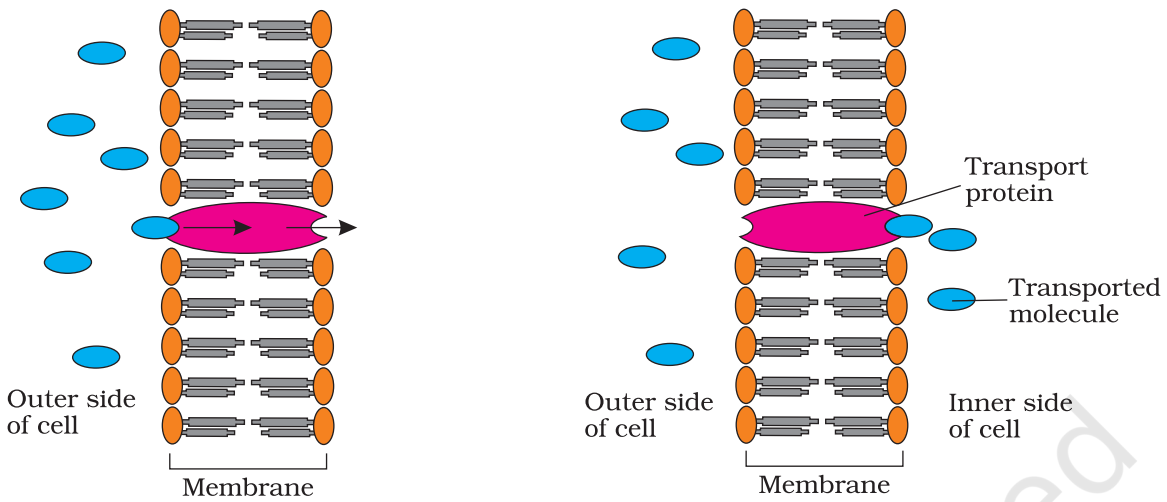



**Figure 11.1** Facilitated diffusion

diffusion is very specific: it allows cell to select substances for uptake. It is sensitive to inhibitors which react with protein side chains.

The proteins form channels in the membrane for molecules to pass through. Some channels are always open; others can be controlled. Some are large, allowing a variety of molecules to cross. The **porins** are proteins that form large pores in the outer membranes of the plastids, mitochondria and some bacteria allowing molecules up to the size of small proteins to pass through.

Figure 11.1 shows an extracellular molecule bound to the transport protein; the transport protein then rotates and releases the molecule inside the cell, e.g., water channels – made up of eight different types of **aquaporins**.

#### *11.1.2.1 Passive symports and antiports*

Some carrier or transport proteins allow diffusion only if two types of molecules move together. In a **symport**, both molecules cross the membrane in the same direction; in an **antiport**, they move in opposite directions (Figure 11.2). When a

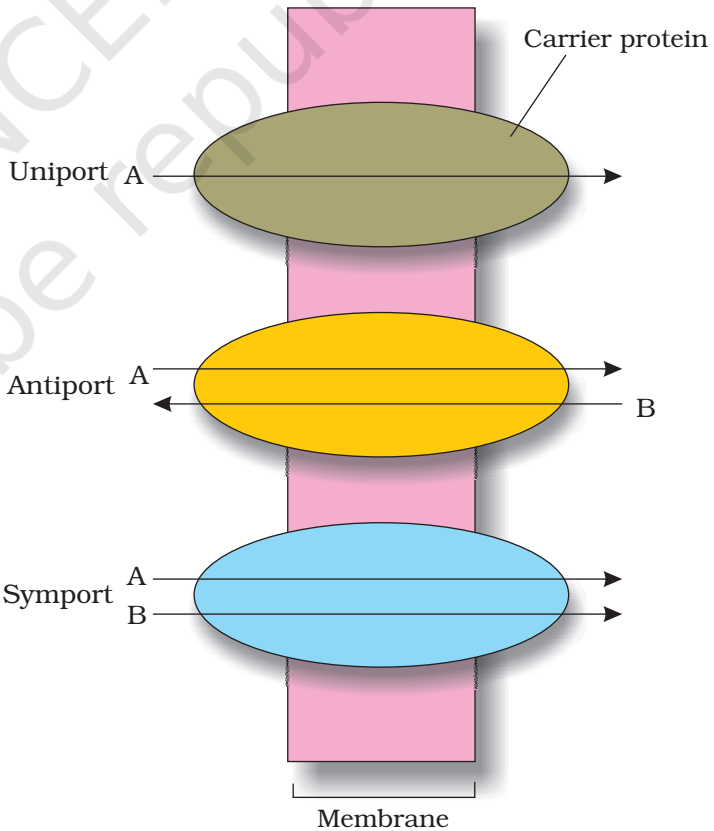



**Figure 11.2** Facilitated diffusion

molecule moves across a membrane independent of other molecules, the process is called **uniport**.

### 11.1.3 Active Transport

Active transport uses energy to transport and pump molecules against a concentration gradient. Active transport is carried out by specific membrane-proteins. Hence different proteins in the membrane play a major role in both active as well as passive transport. Pumps are proteins that use energy to carry substances across the cell membrane. These pumps can transport substances from a low concentration to a high concentration ('uphill' transport). Transport rate reaches a maximum when all the protein transporters are being used or are saturated. Like enzymes the carrier protein is very specific in what it carries across the membrane. These proteins are sensitive to inhibitors that react with protein side chains.

### 11.1.4 Comparison of Different Transport Processes

Table 11.1 gives a comparison of the different transport mechanisms. Proteins in the membrane are responsible for facilitated diffusion and active transport and hence show common characterstics of being highly selective; they are liable to saturate, respond to inhibitors and are under hormonal regulation. But diffusion whether facilitated or not – take place only along a gradient and do not use energy.

**TABLE 11.1 Comparison of Different Transport Mechanisms**

| Property | Simple Diffusion | Facilitated Transport | Active Transport |
|---|---|---|---|
| Requires special membrane proteins | No | Yes | Yes |
| Highly selective | No | Yes | Yes |
| Transport saturates | No | Yes | Yes |
| Uphill transport | No | No | Yes |
| Requires ATP energy | No | No | Yes |

## 11.2 PLANT-WATER RELATIONS

Water is essential for all physiological activities of the plant and plays a very important role in all living organisms. It provides the medium in which most substances are dissolved. The protoplasm of the cells is nothing but water in which different molecules are dissolved and (several particles) suspended. A watermelon has over 92 per cent water; most herbaceous plants have only about 10 to 15 per cent of its fresh weight as dry matter. Of course, distribution of water within a plant varies – woody parts have relatively very little water, while soft parts mostly contain

water. A seed may appear dry but it still has water – otherwise it would not be alive and respiring!

Terrestrial plants take up huge amount water daily but most of it is lost to the air through evaporation from the leaves, i.e., **transpiration**. A mature corn plant absorbs almost three litres of water in a day, while a mustard plant absorbs water equal to its own weight in about 5 hours. Because of this high demand for water, it is not surprising that water is often the limiting factor for plant growth and productivity in both agricultural and natural environments.

### 11.2.1 Water Potential

To comprehend plant-water relations, an understanding of certain standard terms is necessary. **Water potential ($\Psi_w$)** is a concept fundamental to understanding water movement. **Solute potential ($\Psi_s$)** and **pressure potential ($\Psi_p$)** are the two main components that determine water potential.

Water molecules possess kinetic energy. In liquid and gaseous form they are in random motion that is both rapid and constant. The greater the concentration of water in a system, the greater is its kinetic energy or 'water potential'. Hence, it is obvious that pure water will have the greatest water potential. If two systems containing water are in contact, random movement of water molecules will result in net movement of water molecules from the system with higher energy to the one with lower energy. Thus water will move from the system containing water at higher water potential to the one having low water potential. This process of movement of substances down a gradient of free energy is called diffusion. Water potential is denoted by the Greek symbol Psi or $\Psi$ and is expressed in pressure units such as pascals (Pa). By convention, the water potential of pure water at standard temperatures, which is not under any pressure, is taken to be zero.

If some solute is dissolved in pure water, the solution has fewer free water molecules and the concentration (free energy) of water decreases, reducing its water potential. Hence, all solutions have a lower water potential than pure water; the magnitude of this lowering due to dissolution of a solute is called **solute potential** or $\Psi_s$. $\Psi_s$ is always negative. The more the solute molecules, the lower (more negative) is the $\Psi_s$. For a solution at atmospheric pressure (water potential) $\Psi_w$ = (solute potential) $\Psi_s$.

If a pressure greater than atmospheric pressure is applied to pure water or a solution, its water potential increases. It is equivalent to pumping water from one place to another. Can you think of any system in our body where pressure is built up? Pressure can build up in a plant system when water enters a plant cell due to diffusion causing a pressure built up against the cell wall, it makes the cell **turgid** (see section 11.2.2);

this increases the **pressure potential**. Pressure potential is usually positive, though in plants negative potential or tension in the water column in the xylem plays a major role in water transport up a stem. Pressure potential is denoted as $\Psi_p$.

Water potential of a cell is affected by both solute and pressure potential. The relationship between them is as follows:

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

### 11.2.2 Osmosis

The plant cell is surrounded by a cell membrane and a cell wall. The cell wall is freely permeable to water and substances in solution hence is not a barrier to movement. In plants the cells usually contain a large central vacuole, whose contents, the vacuolar sap, contribute to the solute potential of the cell. In plant cells, the cell membrane and the membrane of the vacuole, the tonoplast together are important determinants of movement of molecules in or out of the cell.

**Osmosis** is the term used to refer specifically to the diffusion of water across a differentially- or selectively permeable membrane. Osmosis occurs spontaneously in response to a driving force. The net direction and rate of osmosis depends on both the **pressure gradient** and **concentration gradient**. Water will move from its region of higher chemical potential (or concentration) to its region of lower chemical potential until equilibrium is reached. At equilibrium the two chambers should have nearly the same water potential.

You may have made a potato osmometer in your earlier classes stage in school. If the tuber is placed in water, the water enters the cavity in the potato tuber containing a concentrated solution of sugar due to osmosis.

Study Figure 11.3 in which the two chambers, A and B, containing solutions are separated by a semi-permeable membrane.

*(a) Solution of which chamber has a lower water potential?*

*(b) Solution of which chamber has a lower solute potential?*

*(c) In which direction will osmosis occur?*

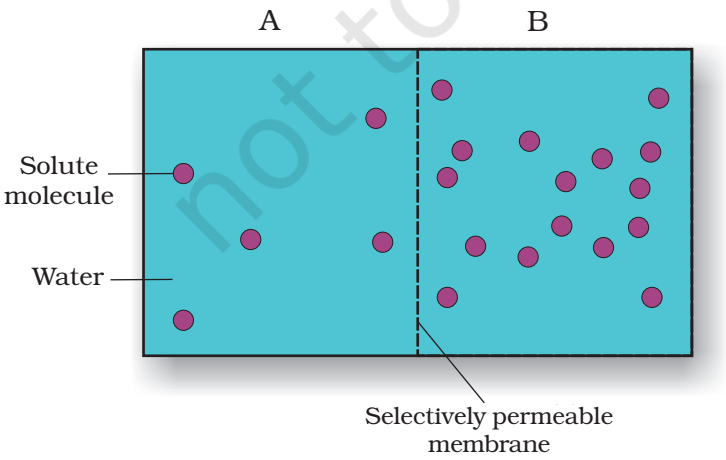



**Figure 11.3**

*(d) Which solution has a higher solute potential?*

*(e) At equilibrium which chamber will have lower water potential?*

*(f) If one chamber has a $\Psi$ of – 2000 kPa, and the other – 1000 kPa, which is the chamber that has the higher $\Psi$?*

(g) What will be the direction of the movement of water when two solutions with $\Psi_w$ = 0.2 MPa and $\Psi_w$ = 0.1 MPa are separated by a selectively permeable membrane?

Let us discuss another experiment where a solution of sucrose in water taken in a funnel is separated from pure water in a beaker by a selectively permeable membrane (Figure 11.4). *You can get this kind of a membrane in an egg. Remove the yolk and albumin through a small hole at one end of the egg, and place the shell in dilute solution of hydrochloric acid for a few hours. The egg shell dissolves leaving the membrane intact.* Water will move into the funnel, resulting in rise in the level of the solution in the funnel. This will continue till the equilibrium is reached. *In case sucrose does diffuse out through the membrane, will this equilibrium be ever reached?*

External pressure can be applied from the upper part of the funnel such that no water diffuses into the funnel through the membrane. This pressure required to prevent water from diffusing is in fact, the osmotic pressure and this is the function of the solute concentration; more the solute concentration, greater will be the pressure required to prevent water from diffusing in. Numerically osmotic pressure is equivalent to the osmotic potential, but the sign is opposite.Osmotic pressure is the positive pressure applied, while osmotic potential is negative.

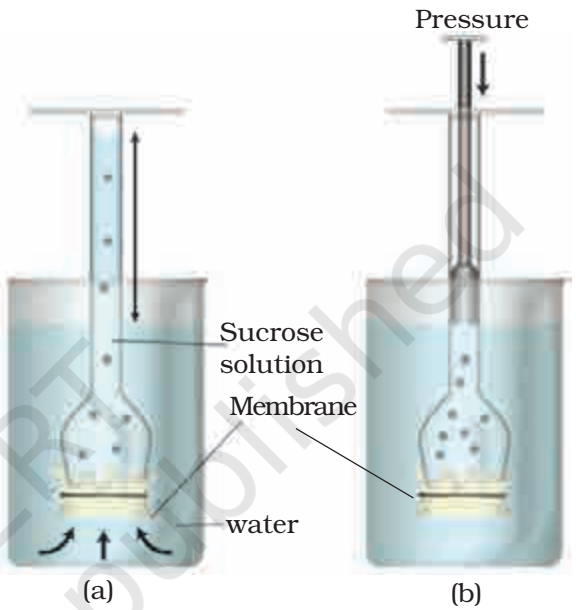



**Figure 11.4** A demonstration of osmosis. A thistle funnel is filled with sucrose solution and kept inverted in a beaker containing water. (a) Water will diffuse across the membrane (as shown by arrows) to raise the level of the solution in the funnel (b) Pressure can be applied as shown to stop the water movement into the funnel

### 11.2.3 Plasmolysis

The behaviour of the plant cells (or tissues) with regard to water movement depends on the surrounding solution. If the external solution balances the osmotic pressure of the cytoplasm, it is said to be **isotonic**. If the external solution is more dilute than the cytoplasm, it is **hypotonic** and if the external solution is more concentrated, it is **hypertonic**. Cells swell in hypotonic solutions and shrink in hypertonic ones.

**Plasmolysis** occurs when water moves out of the cell and the cell membrane of a plant cell shrinks away from its cell wall. This occurs when

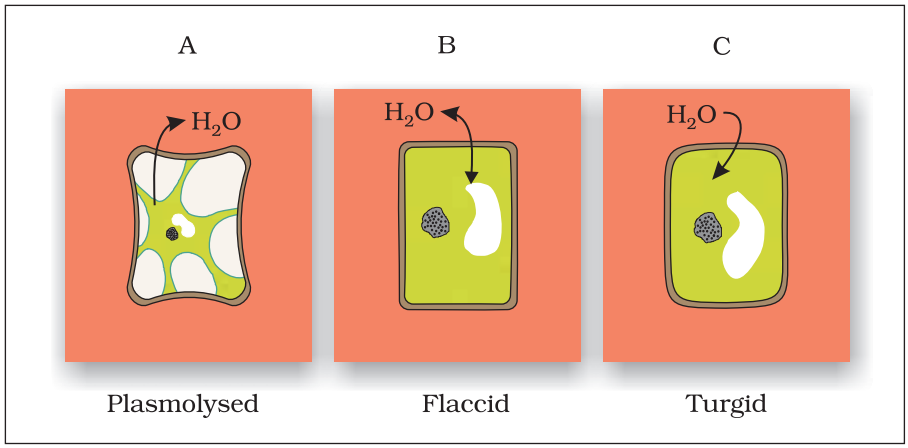



**Figure 11.5** Plant cell plasmolysis

the cell (or tissue) is placed in a solution that is hypertonic (has more solutes) to the protoplasm. Water moves out; it is first lost from the cytoplasm and then from the vacuole. The water when drawn out of the cell through diffusion into the extracellular (outside cell) fluid causes the protoplast to shrink away from the walls. The cell is said to be plasmolysed. The movement of water occurred across the membrane moving from an area of high water potential (i.e., the cell) to an area of lower water potential outside the cell (Figure 11.5).

*What occupies the space between the cell wall and the shrunken protoplast in the plasmolysed cell?*

When the cell (or tissue) is placed in an **isotonic** solution, there is no net flow of water towards the inside or outside. If the external solution balances the osmotic pressure of the cytoplasm it is said to be isotonic. When water flows into the cell and out of the cell and are in equilibrium, the cells are said to be **flaccid**.

The process of plasmolysis is usually reversible. When the cells are placed in a **hypotonic** solution (higher water potential or dilute solution as compared to the cytoplasm), water diffuses into the cell causing the cytoplasm to build up a pressure against the wall, that is called **turgor pressure**. The pressure exerted by the protoplasts due to entry of water against the rigid walls is called pressure potential $\Psi_{p.}$. Because of the rigidity of the cell wall, the cell does not rupture. This turgor pressure is ultimately responsible for enlargement and extension growth of cells.

*What would be the $\Psi_p$ of a flaccid cell? Which organisms other than plants possess cell wall ?*

### 11.2.4 Imbibition

**Imbibition** is a special type of diffusion when water is absorbed by solids – colloids – causing them to increase in volume. The classical

examples of imbibition are absorption of water by seeds and dry wood. The pressure that is produced by the swelling of wood had been used by prehistoric man to split rocks and boulders. If it were not for the pressure due to imbibition, seedlings would not have been able to emerge out of the soil into the open; they probably would not have been able to establish!

Imbibition is also diffusion since water movement is along a concentration gradient; the seeds and other such materials have almost no water hence they absorb water easily. Water potential gradient between the absorbent and the liquid imbibed is essential for imbibition. In addition, for any substance to imbibe any liquid, affinity between the adsorbant and the liquid is also a pre-requisite.

## 11.3 LONG DISTANCE TRANSPORT OF WATER

At some earlier stage you might have carried out an experiment where you had placed a twig bearing white flowers in coloured water and had watched it turn colour. On examining the cut end of the twig after a few hours you had noted the region through which the coloured water moved. That experiment very easily demonstrates that the path of water movement is through the vascular bundles, more specifically, the xylem. Now we have to go further and try and understand the mechanism of movement of water and other substances up a plant.

Long distance transport of substances within a plant cannot be by diffusion alone. Diffusion is a slow process. It can account for only short distance movement of molecules. For example, the movement of a molecule across a typical plant cell (about 50 µm) takes approximately 2.5 s. *At this rate, can you calculate how many years it would take for the movement of molecules over a distance of 1 m within a plant by diffusion alone?*

In large and complex organisms, often substances have to be moved to long distances. Sometimes the sites of production or absorption and sites of storage are too far from each other; diffusion or active transport would not suffice. Special long distance transport systems become necessary so as to move substances across long distances and at a much faster rate. Water and minerals, and food are generally moved by a **mass** or **bulk flow** system. Mass flow is the movement of substances in bulk or *en masse* from one point to another as a result of pressure differences between the two points. It is a characteristic of mass flow that substances, whether in solution or in suspension, are swept along at the same pace, as in a flowing river. This is unlike diffusion where different substances move independently depending on their concentration gradients. Bulk flow can be achieved either through a positive hydrostatic pressure gradient (e.g., a garden hose) or a negative hydrostatic pressure gradient (e.g., suction through a straw).

The bulk movement of substances through the conducting or vascular tissues of plants is called **translocation.**

Do you remember studying cross sections of roots, stems and leaves of higher plants and studying the vascular system? The higher plants have highly specialised vascular tissues – xylem and phloem. Xylem is associated with translocation of mainly water, mineral salts, some organic nitrogen and hormones, from roots to the aerial parts of the plants. The phloem translocates a variety of organic and inorganic solutes, mainly from the leaves to other parts of the plants.

### 11.3.1 How do Plants Absorb Water?

We know that the roots absorb most of the water that goes into plants; obviously that is why we apply water to the soil and not on the leaves. The responsibility of absorption of water and minerals is more specifically the function of the root hairs that are present in millions at the tips of the roots. Root hairs are thin-walled slender extensions of root epidermal cells that greatly increase the surface area for absorption. Water is absorbed along with mineral solutes, by the root hairs, purely by diffusion. Once water is absorbed by the root hairs, it can move deeper into root layers by two distinct pathways:

- apoplast pathway
- symplast pathway

The **apoplast** is the system of adjacent cell walls that is continuous throughout the plant, except at the **casparian** strips of the endodermis in the roots (Figure 11.6). The apoplastic movement of water occurs exclusively through the intercellular spaces and the walls of the cells. Movement through the apoplast does not involve crossing the cell

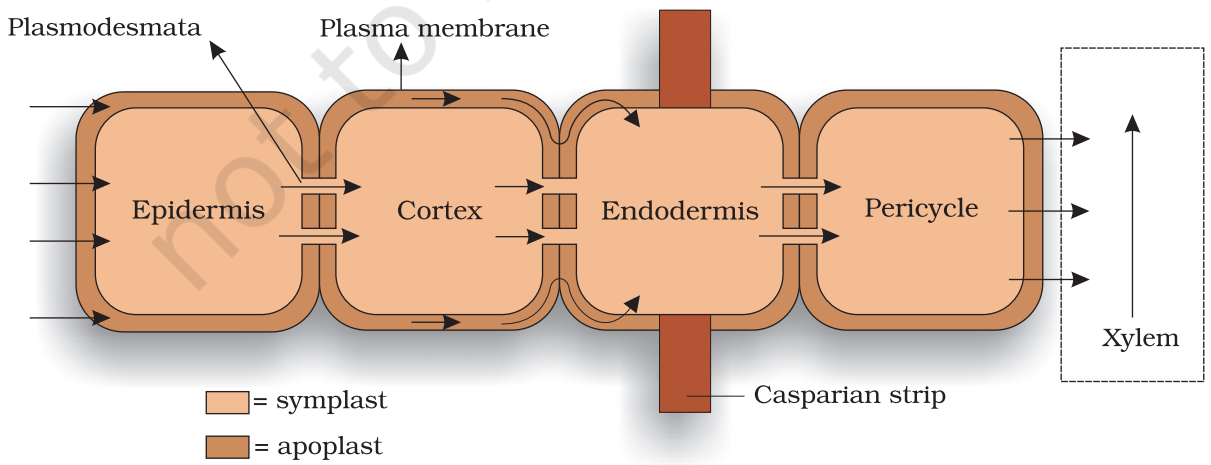



**Figure 11.6** Pathway of water movement in the root

membrane. This movement is dependent on the gradient. The apoplast does not provide any barrier to water movement and water movement is through mass flow. As water evaporates into the intercellular spaces or the atmosphere, tension develop in the continuous stream of water in the apoplast, hence mass flow of water occurs due to the adhesive and cohesive properties of water.

The **symplastic** system is the system of interconnected protoplasts. Neighbouring cells are connected through cytoplasmic strands that extend through **plasmodesmata.** During symplastic movement, the water travels through the cells – their cytoplasm; intercellular movement is through the plasmodesmata. Water has to enter the cells through the cell membrane, hence the movement is relatively slower. Movement is again down a potential gradient. Symplastic movement may be aided by cytoplasmic streaming. You may have observed cytoplasmic streaming in cells of the *Hydrilla* leaf; the movement of chloroplast due to streaming is easily visible.

Most of the water flow in the roots occurs via the apoplast since the cortical cells are loosely packed, and hence offer no resistance to water movement. However, the inner boundary of the cortex, the **endodermis**, is impervious to water because of a band of suberised matrix called the **casparian strip**. Water molecules are unable to penetrate the layer, so they are directed to wall regions that are not suberised, into the cells proper through the membranes. The water then moves through the symplast and again crosses a membrane to reach the cells of the xylem. The movement of water through the root layers is ultimately symplastic in the endodermis. This is the only way water and other solutes can enter the vascular cylinder.

Once inside the xylem, water is again free to move between cells as well as through them. In young roots, water enters directly into the xylem vessels and/or tracheids. These are non-living conduits and so are parts of the apoplast. The path of water and mineral ions into the root vascular system is summarised in Figure 11.7.

Some plants have additional structures associated with them that help in water (and mineral) absorption. A **mycorrhiza** is a symbiotic association of a fungus with a root system. The fungal

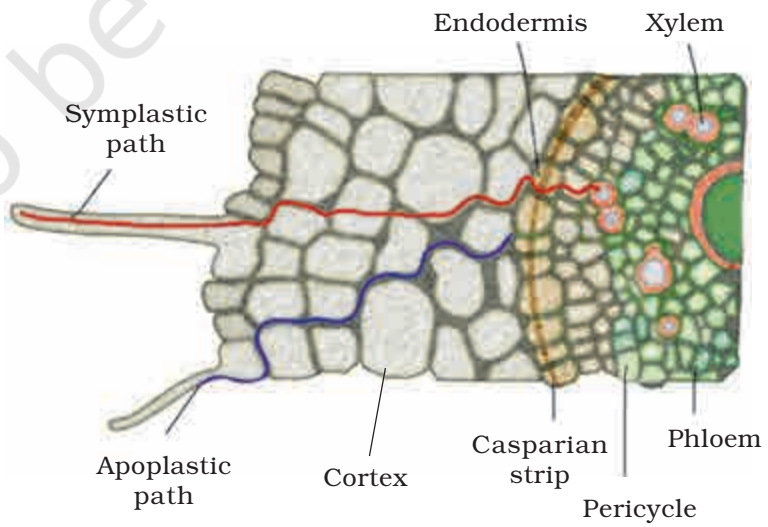



**Figure 11.7** Symplastic and apoplastic pathways of water and ion absorption and movement in roots

filaments form a network around the young root or they penetrate the root cells. The hyphae have a very large surface area that absorb mineral ions and water from the soil from a much larger volume of soil that perhaps a root cannot do. The fungus provides minerals and water to the roots, in turn the roots provide sugars and N-containing compounds to the mycorrhizae. Some plants have an obligate association with the mycorrhizae. For example, *Pinus* seeds cannot germinate and establish without the presence of mycorrhizae.

### 11.3.2 Water Movement up a Plant

We looked at how plants absorb water from the soil, and move it into the vascular tissues. We now have to try and understand how this water is transported to various parts of the plant. Is the water movement active, or is it still passive? Since the water has to be moved up a stem against gravity, what provides the energy for this?

#### *11.3.2.1 Root Pressure*

As various ions from the soil are actively transported into the vascular tissues of the roots, water follows (its potential gradient) and increases the **pressure** inside the xylem. This positive pressure is called **root pressure,** and can be responsible for pushing up water to small heights in the stem. How can we see that root pressure exists? Choose a small soft-stemmed plant and on a day, when there is plenty of atmospheric moisture, cut the stem horizontally near the base with a sharp blade, early in the morning. You will soon see drops of solution ooze out of the cut stem; this comes out due to the positive root pressure. If you fix a rubber tube to the cut stem as a sleeve you can actually collect and measure the rate of exudation, and also determine the composition of the exudates. Effects of root pressure is also observable at night and early morning when evaporation is low, and excess water collects in the form of droplets around special openings of veins near the tip of grass blades, and leaves of many herbaceous parts. Such water loss in its liquid phase is known as **guttation**.

Root pressure can, at best, only provide a modest push in the overall process of water transport. They obviously do not play a major role in water movement up tall trees. The greatest contribution of root pressure may be to re-establish the continuous chains of water molecules in the xylem which often break under the enormous tensions created by transpiration. Root pressure does not account for the majority of water transport; most plants meet their need by transpiratory pull.

#### *11.3.2.2 Transpiration pull*

Despite the absence of a heart or a circulatory system in plants, the upward flow of water through the xylem in plants can achieve fairly high

rates, 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 **stomata** (sing. : stoma). Besides the loss of water vapour in transpiration, exchange of oxygen and carbon dioxide in the leaf also occurs through these stomata. Normally stomata are open in the day time and close during the night. The immediate cause of the opening or closing of 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 stomata, water status of the plant, canopy structure etc.

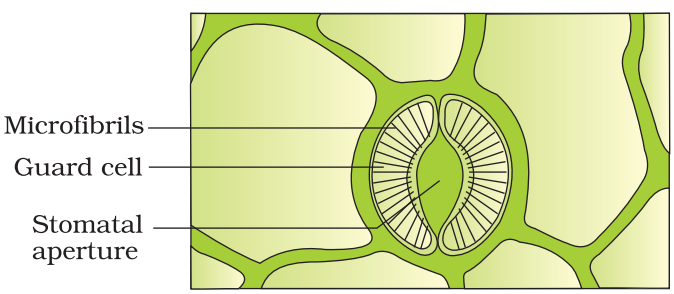



**Figure11.8** A stomatal aperture with guard cells

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.



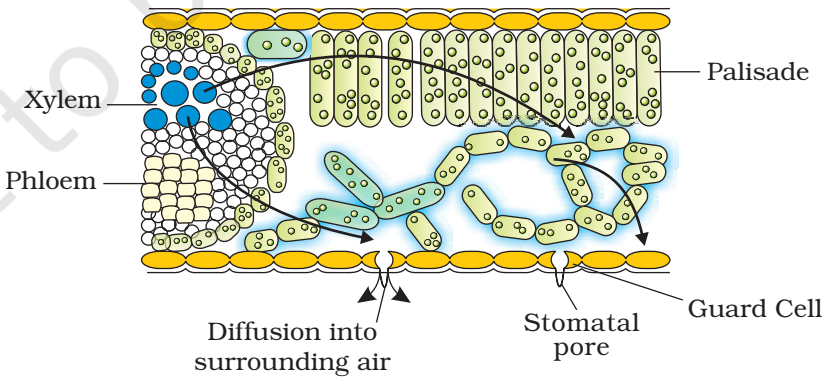



**Figure 11.9** Water movement in the leaf. Evaporation from the leaf sets up a pressure gradient between the outside air and the air spaces of the leaf. The gradient is transmitted into the photosynthetic cells and on the water-filled xylem in the leaf vein.

### 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 dioxide (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 water and minerals 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 cytoplasms 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 decidous 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 dissacharide). 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 sieve tube of phloem 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

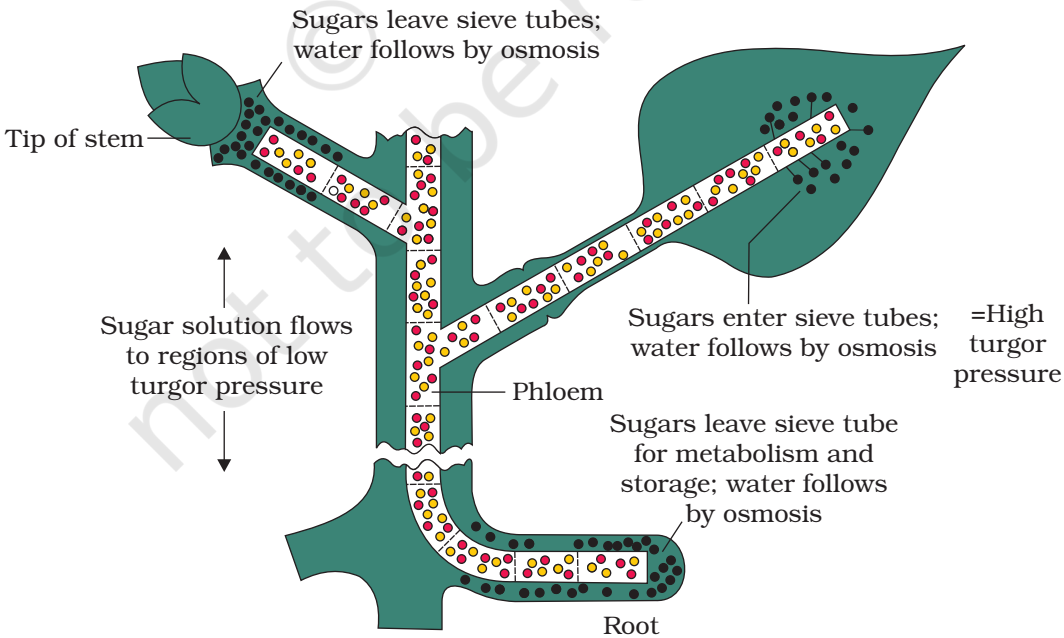



**Figure11.10** Diagrammatic presentation of mechanism of translocation

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 selectively 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 molecules which helps in the movement of water. It is determined by solute potential and pressure potential. The osmotic behaviour of 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 comprising of 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 into the root tissue 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



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 is this 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 / substituted / 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.

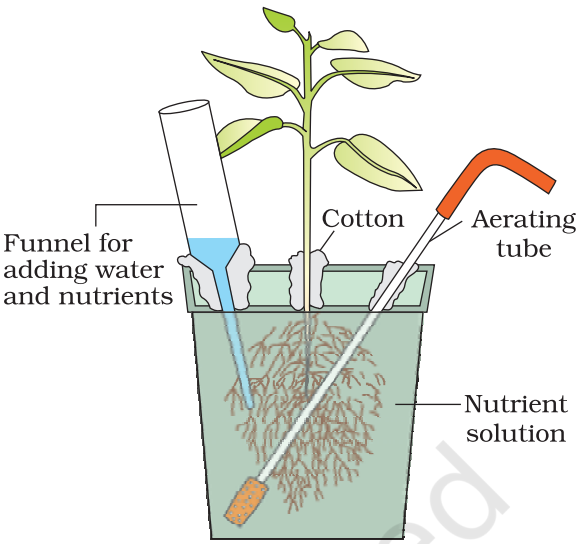



**Figure 12.1** Diagram of a typical set-up for nutrient solution culture

## 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:

(a) 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.

(b) 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.

(c) The element must be directly involved in the metabolism of the plant.

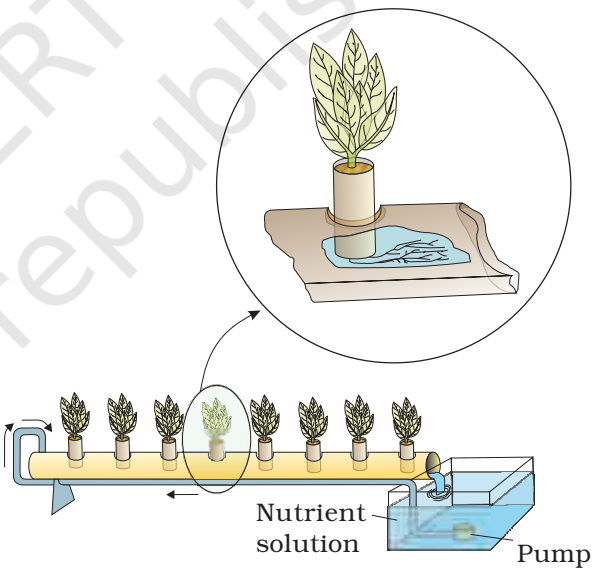



**Figure 12.2** Hydroponic plant production. Plants are grown in a tube or trough placed on a slight incline. A pump circulates a nutrient solution from a reservoir to the elevated end of the tube. The solution flows down the tube and returns to the reservoir due to gravity. Inset shows a plant whose 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 bisphosphate 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 $B_4O_7^{2-}$. 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 $(MoO_2^{2+})$. 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, element 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 inhibit 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 (N ≡ 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:

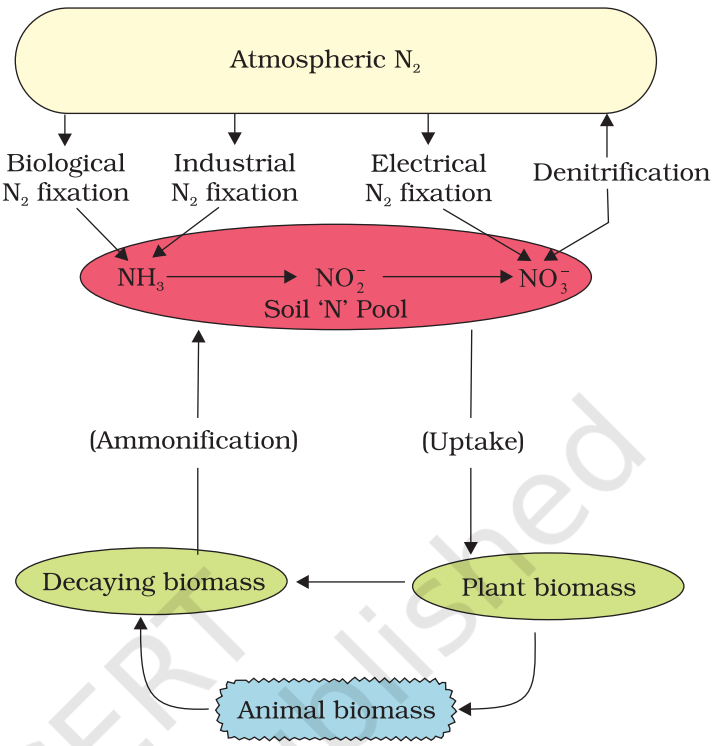



**Figure 12.3** The nitrogen cycle showing relationship between the three main nitrogen pools – atmospheric soil, and biomass

$$2NH_3 + 3O_2 \longrightarrow 2NO_2^- + 2H^+ + 2H_2O \quad \text{.... (i)}$$

$$2NO_2^- + O_2 \longrightarrow 2NO_3^- \quad \text{..... (ii)}$$

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

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.

$$N \equiv N \xrightarrow{\text{Nitrogenase}} NH_3$$

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.

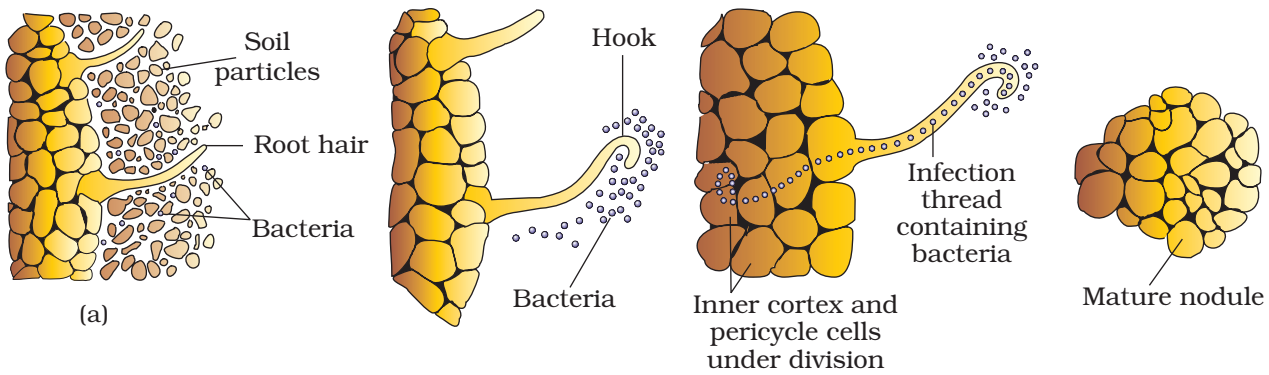



**Figure 12.4** Development of root nodules in soyabean : (a) *Rhizobium* bacteria contact a susceptible root hair, divide near it, (b) Successful infection of the root hair causes it to curl, (c) Infected thread carries the bacteria to the inner cortex. The bacteria get modified into rod-shaped bacteroids 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:

$$N_2 + 8e^- + 8H^+ + 16ATP \longrightarrow 2NH_3 + H_2 + 16ADP + 16P_i$$

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 nitrogenease requires a

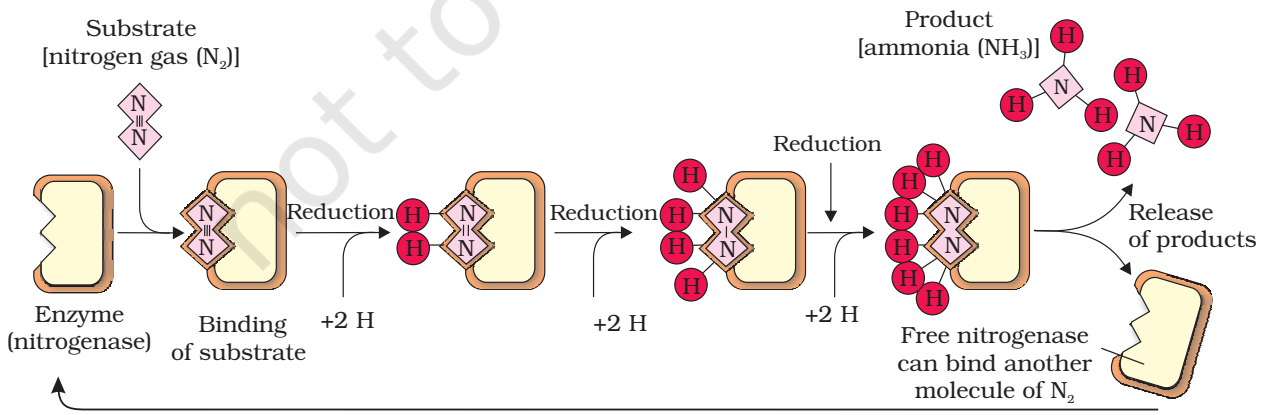



**Figure 12.5** Steps of conversion of atmospheric nitrogen to ammonia by nitrogenase enzyme complex found in nitrogen-fixing bacteria

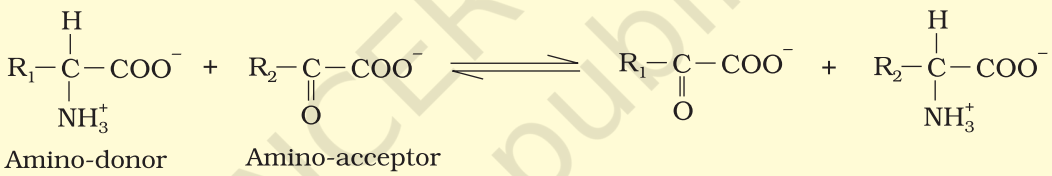
very high input of energy (8 ATP for each $NH_3$ produced). The energy required, thus, is obtained from the respiration of the host cells.

**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 :

$$\alpha - \text{ketoglutaric acid} + NH_4^+ + NADPH \xrightarrow[\text{Dehydrogenase}]{\text{Glutamate}} \text{glutamate} + H_2O + NADP$$

(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



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. The green plants make or rather synthesise the food they need through photosynthesis and are therefore called autotrophs. You have already learnt that the autotrophic nutrition is found only in plants and all other organisms that depend on the green plants for food are heterotrophs. 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 $CO_2$ 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 exposed to light. On testing these leaves for the presence of 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 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 the presence of starch later in the two plants 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?*

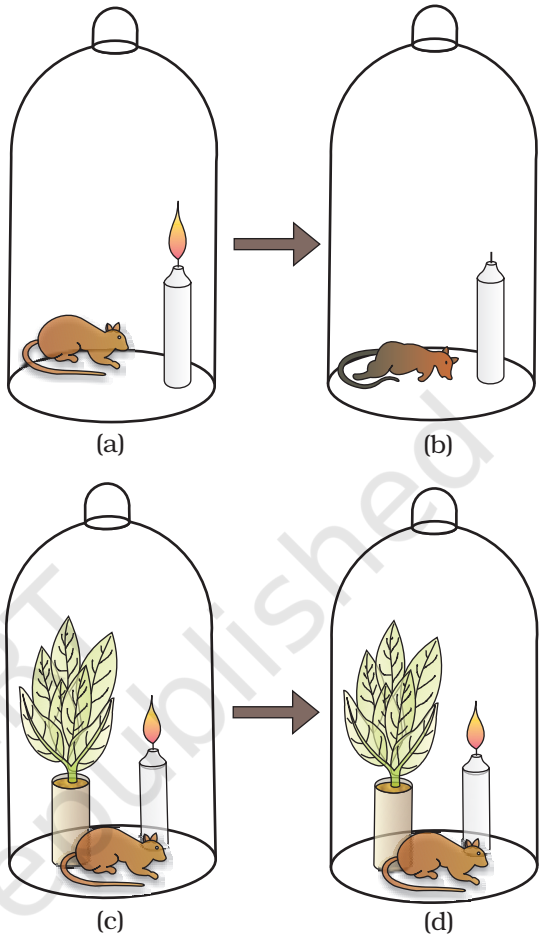



**Figure 13.1** Priestley's experiment

## 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.

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 $O_2$ 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 $CO_2$ and water. The empirical equation representing the total process of photosynthesis for oxygen evolving organisms was then understood as:

$$CO_2 + H_2O \xrightarrow{\text{Light}} [CH_2O] + O_2$$

where $[CH_2O]$ 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:

$$2H_2A + CO_2 \xrightarrow{\text{Light}} 2A + CH_2O + H_2O$$

In green plants $H_2O$ is the hydrogen donor and is oxidised to $O_2$. Some organisms do not release $O_2$ during photosynthesis. When $H_2S$, instead is the hydrogen donor for purple and green sulphur bacteria, the 'oxidation' product is sulphur or sulphate depending on the organism and not $O_2$. Hence, he inferred that the $O_2$ evolved by the green plant comes from $H_2O$, 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:

$$6CO_2 + 12H_2O \xrightarrow{\text{Light}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

where $C_6H_{12}O_6$ represents glucose. The $O_2$ 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 '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 membranous system consisting of grana, the stroma lamellae, and the matrix 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 synthesise sugar, which in turn forms starch. The former set of reactions, since they are directly light driven are called **light reactions** (photochemical 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** (carbon reactions). However, this should not be construed to mean that they occur in darkness or that they are not light-dependent.

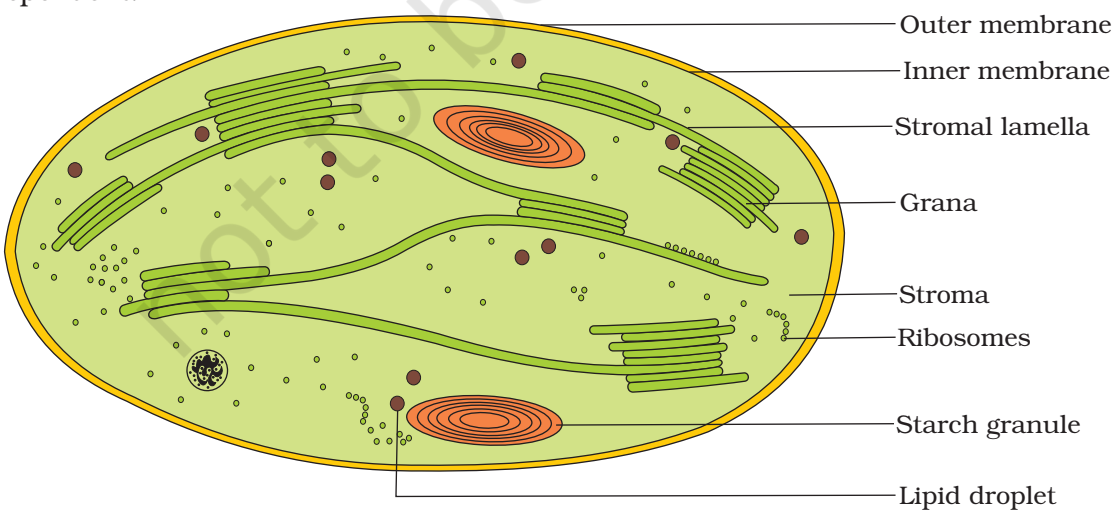



**Figure 13.2** Diagrammatic representation of an electron micrograph of a section of chloroplast

## 13.4 How many Types of Pigments are Involved in Photosynthesis?

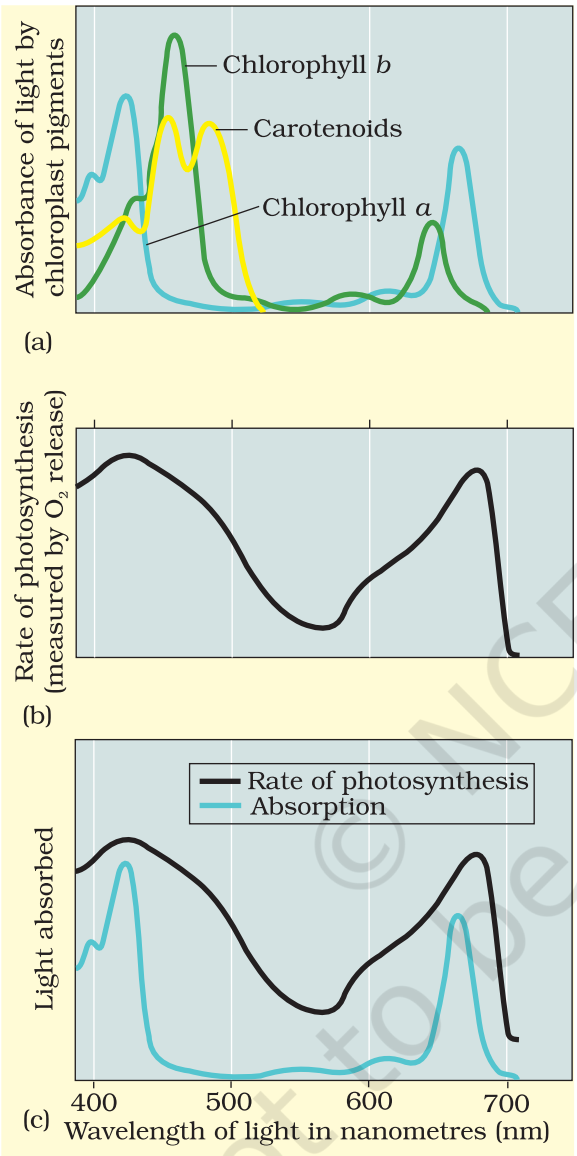



**Figure 13.3a** Graph showing the absorption spectrum of chlorophyll *a*, *b* and the carotenoids

**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 photosyntesis 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 protein 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**.

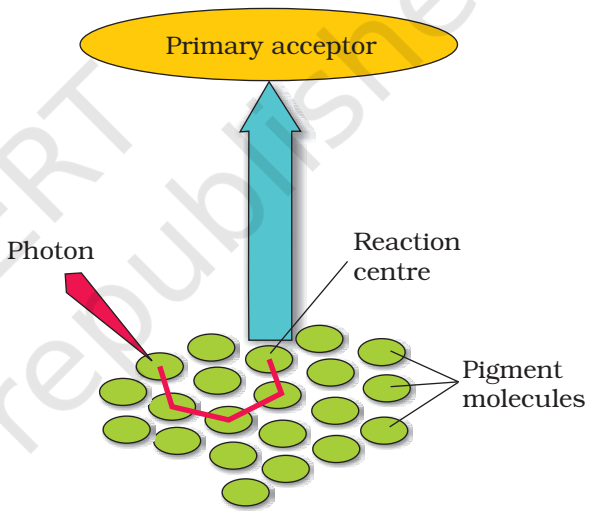



**Figure 13.4** The light harvesting complex

## 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**

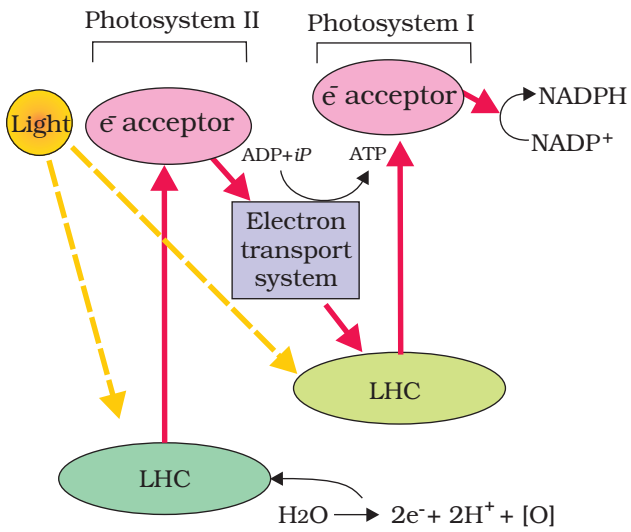



**Figure 13.5** Z scheme of light reaction

**system consisting of cytochromes** (Figure 13.5). This movement of electrons is downhill, in terms of an oxidation-reduction or redox potential scale. The electrons are not used up as they pass through the electron transport chain, but are passed on to the pigments of photosystem PS I. Simultaneously, electrons in the reaction centre of PS I are also excited when they receive red light of wavelength 700 nm and are transferred to another accepter molecule that has a greater redox potential. These electrons then are moved downhill again, this time to a molecule of energy-rich $NADP^+$. The addition of these electrons reduces $NADP^+$ to NADPH + $H^+$. This whole scheme of transfer of electrons, starting from the PS II, uphill to the acceptor, down the electron transport chain to PS I, excitation of electrons, transfer to another acceptor, and finally down hill to $NADP^+$ reducing it to NADPH + $H^+$ is called the **Z scheme**, due to its characterstic shape (Figure 13.5). This shape is formed when all the carriers are placed in a sequence on a redox potential scale.

### 13.6.1 Splitting of Water

You would then ask, *How does PS II supply electrons continuously?* The electrons that were moved from photosystem II must be replaced. This is achieved by electrons available due to splitting of water. The splitting of water is associated with the PS II; water is split into $2H^+$, [O] and electrons. This creates oxygen, one of the net products of photosynthesis. The electrons needed to replace those removed from photosystem I are provided by photosystem II.

$$2H_2O \longrightarrow 4H^+ + O_2 + 4e^-$$

We need to emphasise here that the water splitting complex is associated with the PS II, which itself is physically located on the inner side of the membrane of the thylakoid. *Then, where are the protons and $O_2$ formed likely to be released – in the lumen? or on the outer side of the membrane?*

### 13.6.2 Cyclic and Non-cyclic Photo-phosphorylation

Living organisms have the capability of extracting energy from oxidisable substances and store this in the form of bond energy. Special substances like ATP, carry this energy in their chemical bonds. The process through which

ATP is synthesised by cells (in mitochondria and chloroplasts) is named phosphorylation. Photo-phosphorylation is the synthesis of ATP from ADP and inorganic phosphate in the presence of light. When the two photosystems work in a series, first PS II and then the PS I, a process called non-cyclic photo-phosphorylation occurs. The two photosystems are connected through an electron transport chain, as seen earlier – in the Z scheme. Both ATP and NADPH + $H^+$ are synthesised by this kind of electron flow (Figure 13.5).

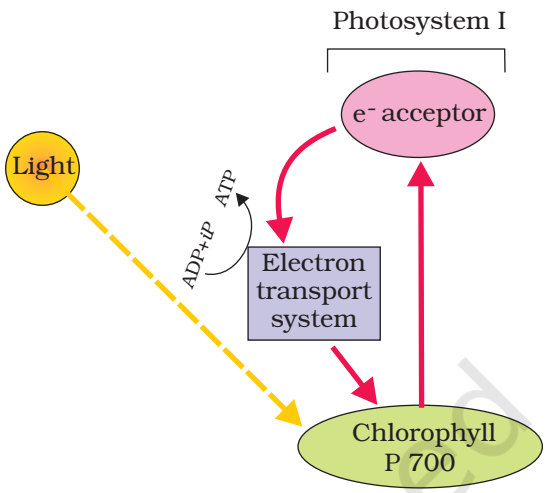



**Figure 13.6** Cyclic photophosphorylation

When only PS I is functional, the electron is circulated within the photosystem and the phosphorylation occurs due to cyclic flow of electrons (Figure 13.6). A possible location where this could be happening is in the stroma lamellae. While the membrane or lamellae of the grana have both PS I and PS II the stroma lamellae membranes lack PS II as well as NADP reductase enzyme. The excited electron does not pass on to $NADP^+$ but is cycled back to the PS I complex through the electron transport chain (Figure 13.6). The cyclic flow hence, results only in the synthesis of ATP, but not of NADPH + $H^+$. Cyclic photophosphorylation also occurs when only light of wavelengths beyond 680 nm are available for excitation.

### 13.6.3 Chemiosmotic Hypothesis

Let us now try and understand how actually ATP is synthesised in the chloroplast. The chemiosmotic hypothesis has been put forward to explain the mechanism. Like in respiration, in photosynthesis too, ATP synthesis is linked to development of a proton gradient across a membrane. This time these are the membranes of thylakoid. There is one difference though, here the proton accumulation is towards the inside of the membrane, i.e., in the lumen. In respiration, protons accumulate in the intermembrane space of the mitochondria when electrons move through the ETS (Chapter 14).

Let us understand what causes the proton gradient across the membrane. We need to consider again the processes that take place during the activation of electrons and their transport to determine the steps that cause a proton gradient to develop (Figure 13.7).

(a) Since splitting of the water molecule takes place on the inner side of the membrane, the protons or hydrogen ions that are produced by the splitting of water accumulate within the lumen of the thylakoids.

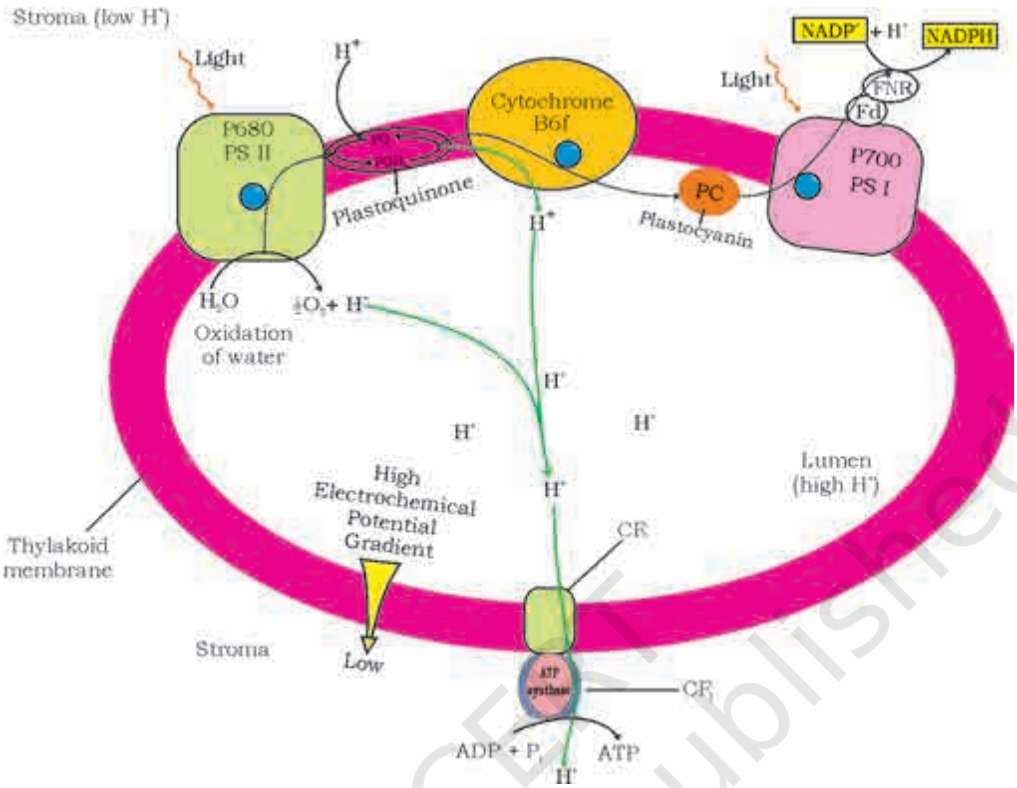



**Figure 13.7** ATP synthesis through chemiosmosis

(b) As electrons move through the photosystems, protons are transported across the membrane. This happens because the primary accepter of electron which is located towards the outer side of the membrane transfers its electron not to an electron carrier but to an H carrier. Hence, this molecule removes a proton from the stroma while transporting an electron. When this molecule passes on its electron to the electron carrier on the inner side of the membrane, the proton is released into the inner side or the lumen side of the membrane.

(c) The NADP reductase enzyme is located on the stroma side of the membrane. Along with electrons that come from the acceptor of electrons of PS I, protons are necessary for the reduction of $NADP^+$ to $NADPH + H^+$. These protons are also removed from the stroma.

Hence, within the chloroplast, protons in the stroma decrease in number, while in the lumen there is accumulation of protons. This creates a proton gradient across the thylakoid membrane as well as a measurable decrease in pH in the lumen.

Why are we so interested in the proton gradient? This gradient is important because it is the breakdown of this gradient that leads to the synthesis of ATP. The gradient is broken down due to the movement of protons across the membrane to the stroma through the transmembrane

channel of the $CF_0$ of the ATP synthase. The ATP synthase enzyme consists of two parts: one called the $CF_0$ is embedded in the thylakoid membrane and forms a transmembrane channel that carries out facilitated diffusion of protons across the membrane. The other portion is called $CF_1$ and protrudes on the outer surface of the thylakoid membrane on the side that faces the stroma. The break down of the gradient provides enough energy to cause a conformational change in the $CF_1$ particle of the ATP synthase, which makes the enzyme synthesise several molecules of energy-packed ATP.

Chemiosmosis requires a membrane, a proton pump, a proton gradient and ATP synthase. Energy is used to pump protons across a membrane, to create a gradient or a high concentration of protons within the thylakoid lumen. ATP synthase has a channel that allows diffusion of protons back across the membrane; this releases enough energy to activate ATP synthase enzyme that catalyses the formation of ATP.

Along with the NADPH produced by the movement of electrons, the ATP will be used immediately in the biosynthetic reaction taking place in the stroma, responsible for fixing $CO_2$, and synthesis of sugars.

## 13.7 WHERE ARE THE ATP AND NADPH USED?

We learnt that the products of light reaction are ATP, NADPH and $O_2$. Of these $O_2$ diffuses out of the chloroplast while ATP and NADPH are used to drive the processes leading to the synthesis of food, more accurately, sugars. This is the **biosynthetic phase** of photosynthesis. This process does not directly depend on the presence of light but is dependent on the products of the light reaction, i.e., ATP and NADPH, besides $CO_2$ and $H_2O$. You may wonder how this could be verified; it is simple: immediately after light becomes unavailable, the biosynthetic process continues for some time, and then stops. If then, light is made available, the synthesis starts again.

*Can we, hence, say that calling the biosynthetic phase as the* ***dark reaction*** *is a misnomer? Discuss this amongst yourselves.*

Let us now see how the ATP and NADPH are used in the biosynthetic phase. We saw earlier that $CO_2$ is combined with $H_2O$ to produce $(CH_2O)_n$ or sugars. It was of interest to scientists to find out how this reaction proceeded, or rather what was the first product formed when $CO_2$ is taken into a reaction or fixed. Just after world war II, among the several efforts to put radioisotopes to beneficial use, the work of Melvin Calvin is exemplary. The use of radioactive $^{14}C$ by him in algal photosynthesis studies led to the discovery that the first $CO_2$ fixation product was a 3-carbon organic acid. He also contributed to working out the complete biosynthetic pathway; hence it was called **Calvin cycle** after him. The first product identified was **3-phosphoglyceric** acid or in short **PGA**. *How many carbon atoms does it have?*

Scientists also tried to know whether all plants have PGA as the first product of $CO_2$ fixation, or whether any other product was formed in other plants. Experiments conducted over a wide range of plants led to the discovery of another group of plants, where the first stable product of $CO_2$ fixation was again an organic acid, but one which had 4 carbon atoms in it. This acid was identified to be **oxaloacetic acid** or OAA. Since then $CO_2$ assimilation during photosynthesis was said to be of two main types: those plants in which the first product of $CO_2$ fixation is a $C_3$ acid (PGA), i.e., the **$C_3$ pathway,** and those in which the first product was a $C_4$ acid (OAA), i.e., the **$C_4$ pathway**. These two groups of plants showed other associated characteristics that we will discuss later.

### 13.7.1 The Primary Acceptor of $CO_2$

Let us now ask ourselves a question that was asked by the scientists who were struggling to understand the 'dark reaction'. *How many carbon atoms would a molecule have which after accepting (fixing) $CO_2$, would have 3 carbons (of PGA)?*

The studies very unexpectedly showed that the acceptor molecule was a 5-carbon ketose sugar – ribulose bisphosphate (RuBP). *Did any of you think of this possibility?* Do not worry; the scientists also took a long time and conducted many experiments to reach this conclusion. They also believed that since the first product was a $C_3$ acid, the primary acceptor would be a 2-carbon compound; they spent many years trying to identify a 2-carbon compound before they discovered the 5-carbon RuBP.

### 13.7.2 The Calvin Cycle

Calvin and his co-workers then worked out the whole pathway and showed that the pathway operated in a cyclic manner; the RuBP was regenerated. Let us now see how the Calvin pathway operates and where the sugar is synthesised. Let us at the outset understand very clearly that the Calvin pathway occurs in **all photosynthetic plants**; it does not matter whether they have $C_3$ or $C_4$ (or any other) pathways (Figure 13.8).

For ease of understanding, the Calvin cycle can be described under three stages: carboxylation, reduction and regeneration.

1. **Carboxylation** – Carboxylation is the fixation of $CO_2$ into a stable organic intermediate. Carboxylation is the most crucial step of the Calvin cycle where $CO_2$ is utilised for the carboxylation of RuBP. This reaction is catalysed by the enzyme RuBP carboxylase which results in the formation of two molecules of 3-PGA. Since this enzyme also has an oxygenation activity it would be more correct to call it RuBP carboxylase-oxygenase or **RuBisCO**.

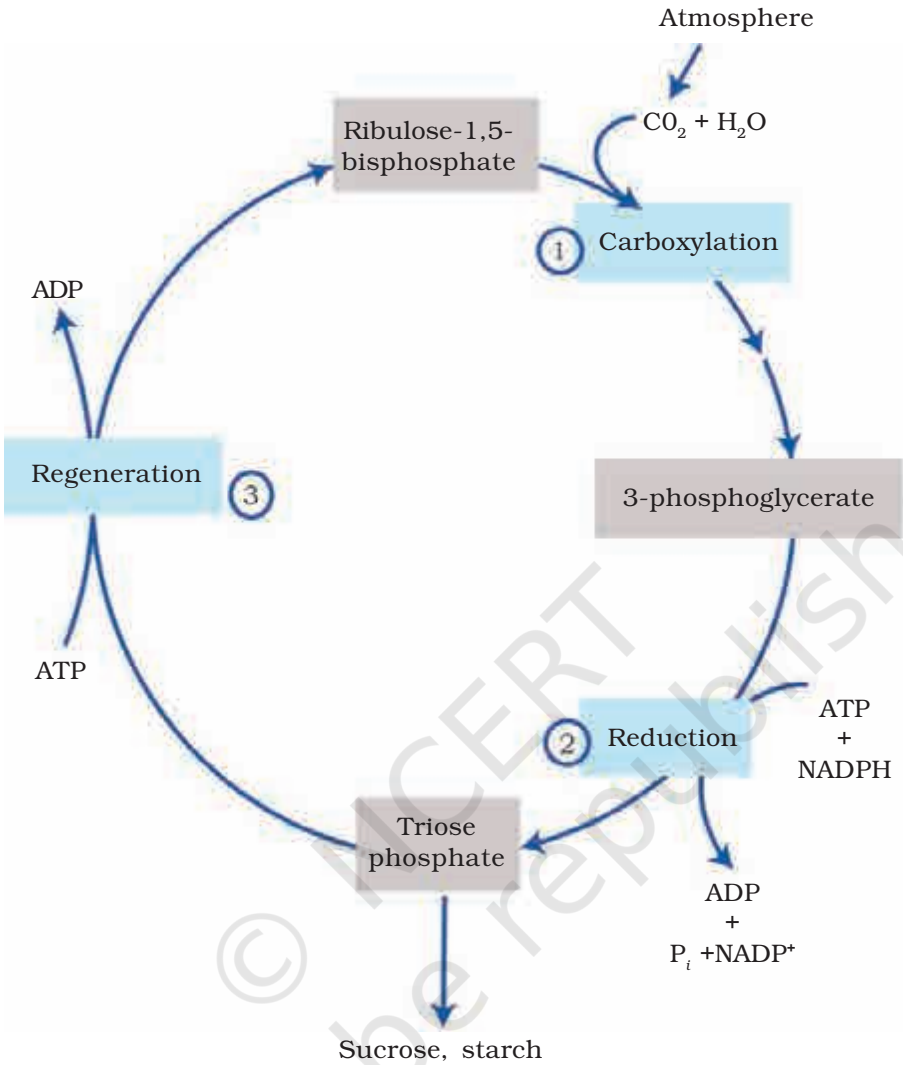



**Figure 13.8** The Calvin cycle proceeds in three stages : (1) carboxylation, during which $CO_2$ combines with ribulose-1,5-bisphosphate; (2) reduction, during which carbohydrate is formed at the expense of the photochemically made ATP and NADPH; and (3) regeneration during which the $CO_2$ acceptor ribulose-1,5-bisphosphate is formed again so that the cycle continues

2. **Reduction** – These are a series of reactions that lead to the formation of glucose. The steps involve utilisation of 2 molecules of ATP for phosphorylation and two of NADPH for reduction per $CO_2$ molecule fixed. The fixation of six molecules of $CO_2$ and 6 turns of the cycle are required for the formation of one molecule of glucose from the pathway.

3. **Regeneration** – Regeneration of the $CO_2$ acceptor molecule RuBP is crucial if the cycle is to continue uninterrupted. The regeneration steps require one ATP for phosphorylation to form RuBP.

Hence for every $CO_2$ molecule entering the Calvin cycle, 3 molecules of ATP and 2 of NADPH are required. It is probably to meet this difference in number of ATP and NADPH used in the dark reaction that the cyclic phosphorylation takes place.

To make one molecule of glucose 6 turns of the cycle are required. *Work out how many ATP and NADPH molecules will be required to make one molecule of glucose through the Calvin pathway.*

It might help you to understand all of this if we look at what goes in and what comes out of the Calvin cycle.

| In | Out |
|---|---|
| Six $CO_2$ | One glucose |
| 18 ATP | 18 ADP |
| 12 NADPH | 12 NADP |

## 13.8 The $C_4$ Pathway

Plants that are adapted to dry tropical regions have the $C_4$ pathway mentioned earlier. Though these plants have the $C_4$ oxaloacetic acid as the first $CO_2$ fixation product they use the $C_3$ pathway or the Calvin cycle as the main biosynthetic pathway. Then, in what way are they different from $C_3$ plants? This is a question that you may reasonably ask.

$C_4$ plants are special: They have a special type of leaf anatomy, they tolerate higher temperatures, they show a response to high light intensities, they lack a process called photorespiration and have greater productivity of biomass. Let us understand these one by one.

Study vertical sections of leaves, one of a $C_3$ plant and the other of a $C_4$ plant. *Do you notice the differences? Do both have the same types of mesophylls? Do they have similar cells around the vascular bundle sheath?*

The particularly large cells around the vascular bundles of the $C_4$ plants are called **bundle sheath cells**, and the leaves which have such anatomy are said to have **'Kranz' anatomy**. 'Kranz' means 'wreath' and is a reflection of the arrangement of cells. The bundle sheath cells may form **several layers** around the vascular bundles; they are characterised by having a large number of chloroplasts, thick walls impervious to gaseous exchange and no intercellular spaces. You may like to cut a section of the leaves of $C_4$ plants – maize or sorghum – to observe the Kranz anatomy and the distribution of mesophyll cells.

It would be interesting for you to collect leaves of diverse species of plants around you and cut vertical sections of the leaves. Observe under the microscope – look for the bundle sheath around the vascular bundles. The presence of the bundle sheath would help you identify the $C_4$ plants.

Now study the pathway shown in Figure 13.9. This pathway that has been named the Hatch and Slack Pathway, is again a cyclic process. Let us study the pathway by listing the steps.

The primary $CO_2$ acceptor is a 3-carbon molecule **phosphoenol pyruvate (PEP)** and is present in the mesophyll cells. The enzyme responsible for this fixation is **PEP carboxylase** or PEPcase. It is important to register that the mesophyll cells lack RuBisCO enzyme. The $C_4$ acid OAA is formed in the mesophyll cells.

It then forms other 4-carbon compounds like malic acid or aspartic acid in the mesophyll cells itself, which are transported to the bundle sheath cells. In the bundle sheath cells these $C_4$ acids are broken down to release $CO_2$ and a 3-carbon molecule.

The 3-carbon molecule is transported back to the mesophyll where it is converted to PEP again, thus, completing the cycle.

The $CO_2$ released in the bundle sheath cells enters the $C_3$ or the Calvin pathway, a pathway common to all plants. The bundle sheath cells are

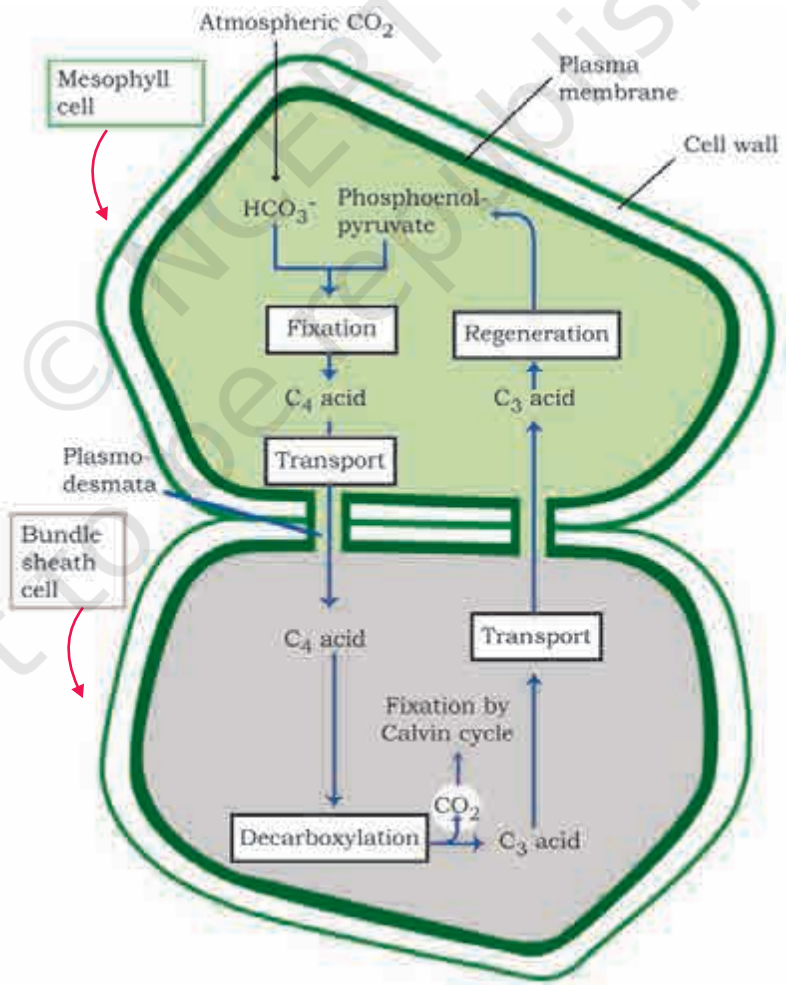



**Figure 13.9** Diagrammatic representation of the Hatch and Slack Pathway

rich in an enzyme Ribulose bisphosphate carboxylase-oxygenase **(RuBisCO)**, but lack PEPcase. Thus, the basic pathway that results in the formation of the sugars, the Calvin pathway, is common to the $C_3$ and $C_4$ plants.

Did you note that the Calvin pathway occurs in all the mesophyll cells of the $C_3$ plants? In the $C_4$ plants it does not take place in the mesophyll cells but does so only in the bundle sheath cells.

## 13.9 PHOTORESPIRATION

Let us try and understand one more process that creates an important difference between $C_3$ and $C_4$ plants – **Photorespiration**. To understand photorespiration we have to know a little bit more about the first step of the Calvin pathway – the first $CO_2$ fixation step. This is the reaction where RuBP combines with $CO_2$ to form 2 molecules of 3PGA, that is catalysed by RuBisCO.

$$RuBP + CO_2 \xrightarrow{RuBisCo} 2 \times 3PGA$$

RuBisCO that is the most abundant enzyme in the world (Do you wonder why?) is characterised by the fact that its active site can bind to both $CO_2$ and $O_2$ – hence the name. *Can you think how this could be possible?* RuBisCO has a much greater affinity for $CO_2$ when the $CO_2$: $O_2$ is nearly equal than for $O_2$. Imagine what would happen if this were not so! This binding is competitive. It is the relative concentration of $O_2$ and $CO_2$ that determines which of the two will bind to the enzyme.

In $C_3$ plants some $O_2$ does bind to RuBisCO, and hence $CO_2$ fixation is decreased. Here the RuBP instead of being converted to 2 molecules of PGA binds with $O_2$ to form one molecule of phosphoglycerate and phosphoglycolate (2 Carbon) in a pathway called photorespiration. In the photorespiratory pathway, there is neither synthesis of sugars, nor of ATP. Rather it results in the release of $CO_2$ with the utilisation of ATP. In the photorespiratory pathway there is no synthesis of ATP or NADPH. The biological function of photorespiration is not known yet.

In $C_4$ plants photorespiration does not occur. This is because they have a mechanism that increases the concentration of $CO_2$ at the enzyme site. This takes place when the $C_4$ acid from the mesophyll is broken down in the bundle sheath cells to release $CO_2$ – this results in increasing the intracellular concentration of $CO_2$. In turn, this ensures that the RuBisCO functions as a carboxylase minimising the oxygenase activity.

Now that you know that the $C_4$ plants lack photorespiration, you probably can understand why productivity and yields are better in these plants. In addition these plants show tolerance to higher temperatures.

*Based on the above discussion can you compare plants showing the $C_3$ and the $C_4$ pathway? Use the table format given and fill in the information.*

**TABLE 13.1 Fill in the Columns 2 and 3 in this table to highlight the differences between $C_3$ and $C_4$ Plants**

| Characteristics | $C_3$ Plants | $C_4$ Plants | Choose from |
|---|---|---|---|
| Cell type in which the Calvin cycle takes place | | | Mesophyll/Bundle sheath/both |
| Cell type in which the initial carboxylation reaction occurs | | | Mesophyll/Bundle sheath /both |
| How many cell types does the leaf have that fix $CO_2$. | | | Two: Bundle sheath and mesophyll<br>One: Mesophyll<br>Three: Bundle sheath, palisade, spongy mesophyll |
| Which is the primary $CO_2$ acceptor | | | RuBP/PEP/PGA |
| Number of carbons in the primary $CO_2$ acceptor | | | 5 / 4 / 3 |
| Which is the primary $CO_2$ fixation product | | | PGA/OAA/RuBP/PEP |
| No. of carbons in the primary $CO_2$ fixation product | | | 3 / 4 / 5 |
| Does the plant have RuBisCO? | | | Yes/No/Not always |
| Does the plant have PEP Case? | | | Yes/No/Not always |
| Which cells in the plant have Rubisco? | | | Mesophyll/Bundle sheath/none |
| $CO_2$ fixation rate under high light conditions | | | Low/ high/ medium |
| Whether photorespiration is present at low light intensities | | | High/negligible/sometimes |
| Whether photorespiration is present at high light intensities | | | High/negligible/sometimes |
| Whether photorespiration would be present at low $CO_2$ concentrations | | | High/negligible/sometimes |
| Whether photorespiration would be present at high $CO_2$ concentrations | | | High/negligible/sometimes |
| Temperature optimum | | | 30-40 C/20-25C/above 40 C |
| Examples | | | Cut vertical sections of leaves of different plants and observe under the microscope for Kranz anatomy and list them in the appropriate columns. |

## 13.10 FACTORS AFFECTING PHOTOSYNTHESIS

An understanding of the factors that affect photosynthesis is necessary. The rate of photosynthesis is very important in determining the yield of plants including crop plants. Photosynthesis is under the influence of several factors, both internal (plant) and external. The plant factors include the number, size, age and orientation of leaves, mesophyll cells and chloroplasts, internal $CO_2$ concentration and the amount of chlorophyll. The plant or internal factors are dependent on the genetic predisposition and the growth of the plant.

The external factors would include the availability of sunlight, temperature, $CO_2$ concentration and water. As a plant photosynthesises, all these factors will simultaneously affect its rate. Hence, though several factors interact and simultaneously affect photosynthesis or $CO_2$ fixation, usually one factor is the major cause or is the one that limits the rate. Hence, at any point the rate will be determined by the factor available at sub-optimal levels.

When several factors affect any [bio] chemical process, Blackman's (1905) **Law of Limiting Factors** comes into effect. This states the following:

*If a chemical process is affected by more than one factor, then its rate will be determined by the factor which is nearest to its minimal value: it is the factor which directly affects the process if its quantity is changed.*

For example, despite the presence of a green leaf and optimal light and $CO_2$ conditions, the plant may not photosynthesise if the temperature is very low. This leaf, if given the optimal temperature, will start photosynthesising.

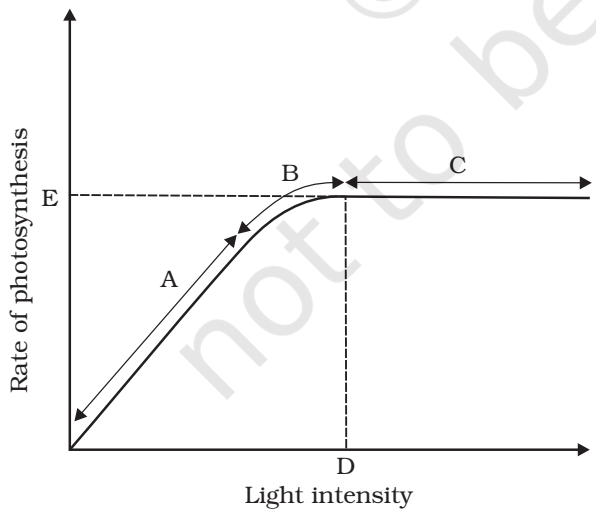



**Figure 13.10** Graph of light intensity on the rate of photosynthesis

### 13.10.1 Light

We need to distinguish between light quality, light intensity and the duration of exposure to light, while discussing light as a factor that affects photosynthesis. There is a linear relationship between incident light and $CO_2$ fixation rates at low light intensities. At higher light intensities, gradually the rate does not show further increase as other factors become limiting (Figure 13.10). What is interesting to note is that light saturation occurs at 10 per cent of the full sunlight. Hence, except for plants in shade or in dense forests, light is rarely a limiting factor in nature. Increase in

incident light beyond a point causes the breakdown of chlorophyll and a decrease in photosynthesis.

### 13.10.2 Carbon dioxide Concentration

Carbon dioxide is the major limiting factor for photosynthesis. The concentration of $CO_2$ is very low in the atmosphere (between 0.03 and 0.04 per cent). Increase in concentration upto 0.05 per cent can cause an increase in $CO_2$ fixation rates; beyond this the levels can become damaging over longer periods.

The $C_3$ and $C_4$ plants respond differently to $CO_2$ concentrations. At low light conditions neither group responds to high $CO_2$ conditions. At high light intensities, both $C_3$ and $C_4$ plants show increase in the rates of photosynthesis. What is important to note is that the $C_4$ plants show saturation at about 360 $\mu l L^{-1}$ while $C_3$ responds to increased $CO_2$ concentration and saturation is seen only beyond 450 $\mu l L^{-1}$. Thus, current availability of $CO_2$ levels is limiting to the $C_3$ plants.

The fact that $C_3$ plants respond to higher $CO_2$ concentration by showing increased rates of photosynthesis leading to higher productivity has been used for some greenhouse crops such as tomatoes and bell pepper. They are allowed to grow in carbon dioxide enriched atmosphere that leads to higher yields.

### 13.10.3 Temperature

The dark reactions being enzymatic are temperature controlled. Though the light reactions are also temperature sensitive they are affected to a much lesser extent. The $C_4$ plants respond to higher temperatures and show higher rate of photosynthesis while $C_3$ plants have a much lower temperature optimum.

The temperature optimum for photosynthesis of different plants also depends on the habitat that they are adapted to. Tropical plants have a higher temperature optimum than the plants adapted to temperate climates.

### 13.10.4 Water

Even though water is one of the reactants in the light reaction, the effect of water as a factor is more through its effect on the plant, rather than directly on photosynthesis. Water stress causes the stomata to close hence reducing the $CO_2$ availability. Besides, water stress also makes leaves wilt, thus, reducing the surface area of the leaves and their metabolic activity as well.

## SUMMARY

Green plants make their own food by photosynthesis. During this process carbon dioxide from the atmosphere is taken in by leaves through stomata and used for making carbohydrates, principally glucose and starch. Photosynthesis takes place only in the green parts of the plants, mainly the leaves. Within the leaves, the mesophyll cells have a large number of chloroplasts that are responsible for $CO_2$ fixation. Within the chloroplasts, the membranes are sites for the light reaction, while the chemosynthetic pathway occurs in the stroma. Photosynthesis has two stages: the light reaction and the carbon fixing reactions. In the light reaction the light energy is absorbed by the pigments present in the antenna, and funnelled to special chlorophyll *a* molecules called reaction centre chlorophylls. There are two photosystems, PS I and PS II. PS I has a 700 nm absorbing chlorophyll *a* P700 molecule at its reaction centre, while PS II has a P680 reaction centre that absorbs red light at 680 nm. After absorbing light, electrons are excited and transferred through PS II and PS I and finally to NAD forming NADH. During this process a proton gradient is created across the membrane of the thylakoid. The breakdown of the protons gradient due to movement through the $F_0$ part of the ATPase enzyme releases enough energy for synthesis of ATP. Splitting of water molecules is associated with PS II resulting in the release of $O_2$, protons and transfer of electrons to PS II.

In the carbon fixation cycle, $CO_2$ is added by the enzyme, RuBisCO, to a 5-carbon compound RuBP that is converted to 2 molecules of 3-carbon PGA. This is then converted to sugar by the Calvin cycle, and the RuBP is regenerated. During this process ATP and NADPH synthesised in the light reaction are utilised. RuBisCO also catalyses a wasteful oxygenation reaction in $C_3$ plants: photorespiration.

Some tropical plants show a special type of photosynthesis called $C_4$ pathway. In these plants the first product of $CO_2$ fixation that takes place in the mesophyll, is a 4-carbon compound. In the bundle sheath cells the Calvin pathway is carried out for the synthesis of carbohydrates.

## EXERCISES

1. By looking at a plant externally can you tell whether a plant is $C_3$ or $C_4$? Why and how?
2. By looking at which internal structure of a plant can you tell whether a plant is $C_3$ or $C_4$? Explain.
3. Even though a very few cells in a $C_4$ plant carry out the biosynthetic – Calvin pathway, yet they are highly productive. Can you discuss why?

4. RuBisCO is an enzyme that acts both as a carboxylase and oxygenase. Why do you think RuBisCO carries out more carboxylation in $C_4$ plants?
5. Suppose there were plants that had a high concentration of Chlorophyll *b*, but lacked chlorophyll *a*, would it carry out photosynthesis? Then why do plants have chlorophyll *b* and other accessory pigments?
6. Why is the colour of a leaf kept in the dark frequently yellow, or pale green? Which pigment do you think is more stable?
7. Look at leaves of the same plant on the shady side and compare it with the leaves on the sunny side. Or, compare the potted plants kept in the sunlight with those in the shade. Which of them has leaves that are darker green ? Why?
8. Figure 13.10 shows the effect of light on the rate of photosynthesis. Based on the graph, answer the following questions:

   (a) At which point/s (A, B or C) in the curve is light a limiting factor?
   (b) What could be the limiting factor/s in region A?
   (c) What do C and D represent on the curve?
9. Give comparison between the following:

   (a) $C_3$ and $C_4$ pathways
   (b) Cyclic and non-cyclic photophosphorylation
   (c) Anatomy of leaf in $C_3$ and $C_4$ plants

# Chapter 14
# Respiration in Plants



All of us breathe to live, but why is breathing so essential to life? What happens when we breathe? Also, do all living organisms, including plants and microbes, breathe? If so, how?

All living organisms need energy for carrying out daily life activities, be it absorption, transport, movement, reproduction or even breathing. Where does all this energy come from? We know we eat food for energy – but how is this energy taken from food? How is this energy utilised? Do all foods give the same amount of energy? Do plants 'eat'? Where do plants get their energy from? And micro-organisms – for their energy requirements, do they eat 'food'?

You may wonder at the several questions raised above – they may seem to be very disconnected. But in reality, the process of breathing is very much connected to the process of release of energy from food. Let us try and understand how this happens.

All the energy required for 'life' processes is obtained by oxidation of some macromolecules that we call 'food'. Only green plants and cyanobacteria can prepare their own food; by the process of photosynthesis they trap light energy and convert it into chemical energy that is stored in the bonds of carbohydrates like glucose, sucrose and starch. We must remember that in green plants too, not all cells, tissues and organs photosynthesise; only cells containing chloroplasts, that are most often located in the superficial layers, carry out photosynthesis. Hence, even in green plants all other organs, tissues and cells that are non-green, need food for oxidation. Hence, food has to be translocated to all non-green parts. Animals are heterotrophic, i.e., they obtain food from plants

directly (herbivores) or indirectly (carnivores). Saprophytes like fungi are dependent on dead and decaying matter. What is important to recognise is that ultimately all the food that is respired for life processes comes from photosynthesis. This chapter deals with **cellular respiration** or the mechanism of breakdown of food materials within the cell to release energy, and the trapping of this energy for synthesis of ATP.

Photosynthesis, of course, takes place within the chloroplasts (in the eukaryotes), whereas the breakdown of complex molecules to yield energy takes place in the cytoplasm and in the mitochondria (also only in eukaryotes). The breaking of the C-C bonds of complex compounds through oxidation within the cells, leading to release of considerable amount of energy is called **respiration**. The compounds that are oxidised during this process are known as **respiratory substrates.** Usually carbohydrates are oxidised to release energy, but proteins, fats and even organic acids can be used as respiratory substances in some plants, under certain conditions. During oxidation within a cell, all the energy contained in respiratory substrates is not released free into the cell, or in a single step. It is released in a series of slow step-wise reactions controlled by enzymes, and it is trapped as chemical energy in the form of ATP. Hence, it is important to understand that the energy released by oxidation in respiration is not (or rather cannot be) used directly but is used to synthesise ATP, which is broken down whenever (and wherever) energy needs to be utilised. Hence, ATP acts as the energy currency of the cell. This energy trapped in ATP is utilised in various energy-requiring processes of the organisms, and the carbon skeleton produced during respiration is used as precursors for biosynthesis of other molecules in the cell.

## 14.1 DO PLANTS BREATHE?

Well, the answer to this question is not quite so direct. Yes, plants require $O_2$ for respiration to occur and they also give out $CO_2$. Hence, plants have systems in place that ensure the availability of $O_2$. Plants, unlike animals, have no specialised organs for gaseous exchange but they have stomata and lenticels for this purpose. There are several reasons why plants can get along without respiratory organs. First, each plant part takes care of its own gas-exchange needs. There is very little transport of gases from one plant part to another. Second, plants do not present great demands for gas exchange. Roots, stems and leaves respire at rates far lower than animals do. Only during photosynthesis are large volumes of gases exchanged and, each leaf is well adapted to take care of its own needs during these periods. When cells photosynthesise, availability of $O_2$ is not a problem in these cells since $O_2$ is released within the cell. Third, the

distance that gases must diffuse even in large, bulky plants is not great. Each living cell in a plant is located quite close to the surface of the plant. 'This is true for leaves', you may ask, 'but what about thick, woody stems and roots?' In stems, the 'living' cells are organised in thin layers inside and beneath the bark. They also have openings called lenticels. The cells in the interior are dead and provide only mechanical support. Thus, most cells of a plant have at least a part of their surface in contact with air. This is also facilitated by the loose packing of parenchyma cells in leaves, stems and roots, which provide an interconnected network of air spaces.

The complete combustion of glucose, which produces $CO_2$ and $H_2O$ as end products, yields energy most of which is given out as heat.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{Energy}$$

If this energy is to be useful to the cell, it should be able to utilise it to synthesise other molecules that the cell requires. The strategy that the plant cell uses is to catabolise the glucose molecule in such a way that not all the liberated energy goes out as heat. The key is to oxidise glucose not in one step but in several small steps enabling some steps to be just large enough such that the energy released can be coupled to ATP synthesis. How this is done is, essentially, the story of respiration.

During the process of respiration, oxygen is utilised, and carbon dioxide, water and energy are released as products. The combustion reaction requires oxygen. But some cells live where oxygen may or may not be available. *Can you think of such situations (and organisms) where $O_2$ is not available?* There are sufficient reasons to believe that the first cells on this planet lived in an atmosphere that lacked oxygen. Even among present-day living organisms, we know of several that are adapted to anaerobic conditions. Some of these organisms are facultative anaerobes, while in others the requirement for anaerobic condition is obligate. In any case, all living organisms retain the enzymatic machinery to partially oxidise glucose without the help of oxygen. This breakdown of glucose to pyruvic acid is called **glycolysis.**

## 14.2 GLYCOLYSIS

The term glycolysis has originated from the Greek words, *glycos* for sugar, and *lysis* for splitting. The scheme of glycolysis was given by Gustav Embden, Otto Meyerhof, and J. Parnas, and is often referred to as the EMP pathway. In anaerobic organisms, it is the only process in respiration. Glycolysis occurs in the cytoplasm of the cell and is present in all living organisms. In this process, glucose undergoes partial oxidation to form two molecules of pyruvic acid. In plants, this glucose is derived from sucrose, which is the end product of photosynthesis, or from storage

carbohydrates. Sucrose is converted into glucose and fructose by the enzyme, invertase, and these two monosaccharides readily enter the glycolytic pathway. Glucose and fructose are phosphorylated to give rise to glucose-6-phosphate by the activity of the enzyme hexokinase. This phosphorylated form of glucose then isomerises to produce fructose-6-phosphate. Subsequent steps of metabolism of glucose and fructose are same. The various steps of glycolysis are depicted in Figure 14.1. In glycolysis, a chain of ten reactions, under the control of different enzymes, takes place to produce pyruvate from glucose. While studying the steps of glycolysis, please note the steps at which utilisation or synthesis of ATP or (in this case) NADH + $H^+$ take place.

ATP is utilised at two steps: first in the conversion of glucose into glucose 6-phosphate and second in the conversion of fructose 6-phosphate to fructose 1, 6-bisphosphate.

The fructose 1, 6-bisphosphate is split into dihydroxyacetone phosphate and 3-phosphoglyceraldehyde (PGAL). We find that there is one step where NADH + $H^+$ is formed from $NAD^+$; this is when 3-phosphoglyceraldehyde (PGAL) is converted to 1, 3-bisphosphoglycerate (BPGA). Two redox-equivalents are removed (in the form of two hydrogen atoms) from PGAL and transferred to a molecule of $NAD^+$. PGAL is oxidised and with inorganic phosphate to get converted into BPGA. The conversion of BPGA to 3-phosphoglyceric acid (PGA), is also an energy yielding process; this energy is trapped by the formation of ATP. Another ATP is synthesised during the conversion of PEP to pyruvic acid. ***Can you then calculate how many ATP molecules are directly synthesised in this pathway from one glucose molecule?***

Pyruvic acid is then the key product of glycolysis. What is the metabolic fate of pyruvate? This depends on the cellular need.

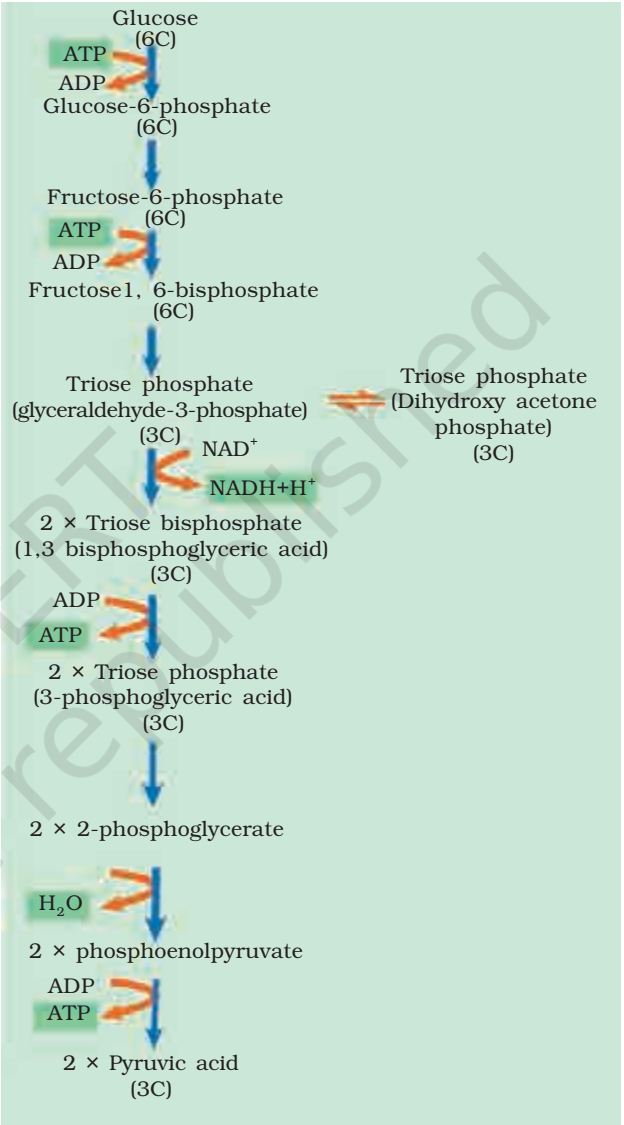



**Figure 14.1** Steps of glycolysis

There are three major ways in which different cells handle pyruvic acid produced by glycolysis. These are lactic acid fermentation, alcoholic fermentation and aerobic respiration. Fermentation takes place under anaerobic conditions in many prokaryotes and unicellular eukaryotes. For the complete oxidation of glucose to $CO_2$ and $H_2O$, however, organisms adopt Krebs' cycle which is also called as aerobic respiration. This requires $O_2$ supply.

## 14.3 FERMENTATION

In fermentation, say by yeast, the incomplete oxidation of glucose is achieved under anaerobic conditions by sets of reactions where pyruvic acid is converted to $CO_2$ and ethanol. The enzymes, pyruvic acid decarboxylase and alcohol dehydrogenase catalyse these reactions. Other organisms like some bacteria produce lactic acid from pyruvic acid. The steps involved are shown in Figure 14.2. In animal cells also, like muscles during exercise, when oxygen is inadequate for cellular respiration pyruvic acid is reduced to lactic acid by lactate dehydrogenase. The reducing agent is $NADH+H^+$ which is reoxidised to $NAD^+$ in both the processes.

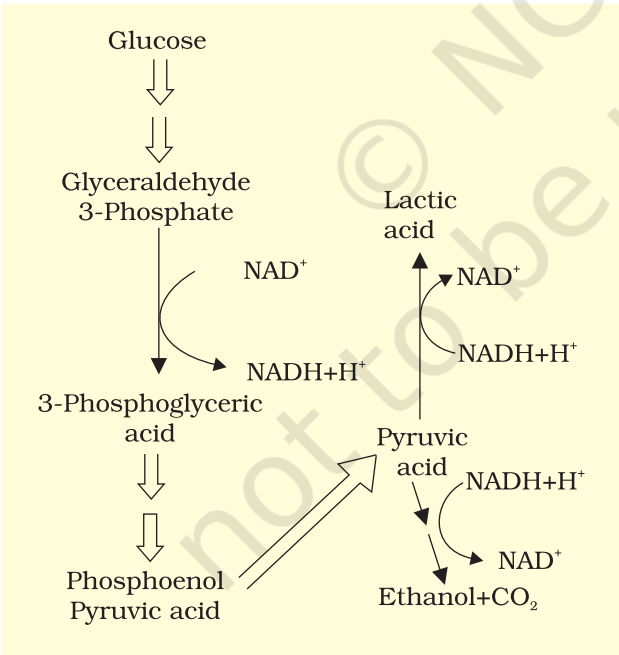



**Figure 14.2** Major pathways of anaerobic respiration

In both lactic acid and alcohol fermentation not much energy is released; less than seven per cent of the energy in glucose is released and not all of it is trapped as high energy bonds of ATP. Also, the processes are hazardous – either acid or alcohol is produced. What is the net ATPs that is synthesised (calculate how many ATP are synthesised and deduct the number of ATP utilised during glycolysis) when one molecule of glucose is fermented to alcohol or lactic acid? Yeasts poison themselves to death when the concentration of alcohol reaches about 13 per cent. ***What then would be the maximum concentration of alcohol in beverages that are naturally fermented?*** How do you think alcoholic beverages of alcohol content greater than this concentration are obtained?

What then is the process by which organisms can carry out complete oxidation of glucose and extract the energy stored to

synthesise a larger number of ATP molecules needed for cellular metabolism? In eukaryotes these steps take place within the mitochondria and this requires $O_2$. **Aerobic respiration** is the process that leads to a complete oxidation of organic substances in the presence of oxygen, and releases $CO_2$, water and a large amount of energy present in the substrate. This type of respiration is most common in higher organisms. We will look at these processes in the next section.

## 14.4 AEROBIC RESPIRATION

For aerobic respiration to take place within the mitochondria, the final product of glycolysis, pyruvate is transported from the cytoplasm into the mitochondria. The crucial events in aerobic respiration are:

- The complete oxidation of pyruvate by the stepwise removal of all the hydrogen atoms, leaving three molecules of $CO_2$.
- The passing on of the electrons removed as part of the hydrogen atoms to molecular $O_2$ with simultaneous synthesis of ATP.

What is interesting to note is that the first process takes place in the matrix of the mitochondria while the second process is located on the inner membrane of the mitochondria.

Pyruvate, which is formed by the glycolytic catabolism of carbohydrates in the cytosol, after it enters mitochondrial matrix undergoes oxidative decarboxylation by a complex set of reactions catalysed by pyruvic dehydrogenase. The reactions catalysed by pyruvic dehydrogenase require the participation of several coenzymes, including $NAD^+$ and Coenzyme A.

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

During this process, two molecules of NADH are produced from the metabolism of two molecules of pyruvic acid (produced from one glucose molecule during glycolysis).

The acetyl CoA then enters a cyclic pathway, tricarboxylic acid cycle, more commonly called as Krebs' cycle after the scientist Hans Krebs who first elucidated it.

### 14.4.1 Tricarboxylic Acid Cycle

The TCA cycle starts with the condensation of acetyl group with oxaloacetic acid (OAA) and water to yield citric acid (Figure 14.3). The reaction is catalysed by the enzyme citrate synthase and a molecule of CoA is released. Citrate is then isomerised to isocitrate. It is followed by two successive steps of decarboxylation, leading to the formation of α-ketoglutaric acid

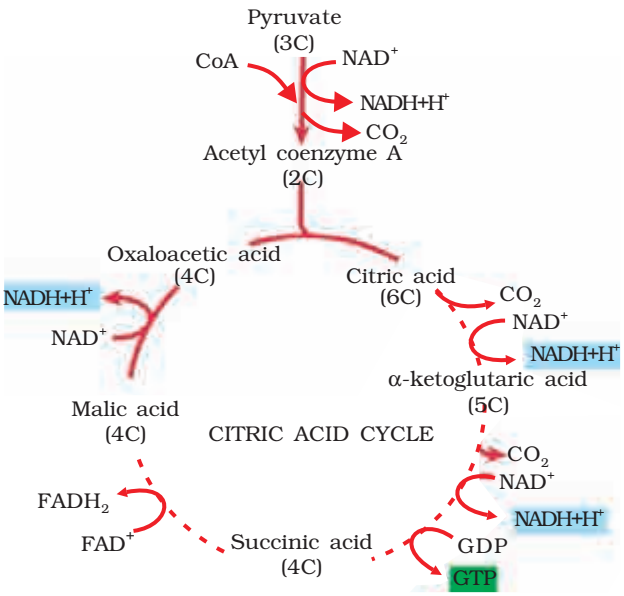



**Figure 14.3** The Citric acid cycle

and then succinyl-CoA. In the remaining steps of citric acid cycle, succinyl-CoA is oxidised to OAA allowing the cycle to continue. During the conversion of succinyl-CoA to succinic acid a molecule of GTP is synthesised. This is a substrate level phosphorylation. In a coupled reaction GTP is converted to GDP with the simultaneous synthesis of ATP from ADP. Also there are three points in the cycle where $NAD^+$ is reduced to $NADH + H^+$ and one point where $FAD^+$ is reduced to $FADH_2$. The continued oxidation of acetyl CoA via the TCA cycle requires the continued replenishment of oxaloacetic acid, the first member of the cycle. In addition it also requires regeneration of $NAD^+$ and $FAD^+$ from NADH and $FADH_2$ respectively. The summary equation for this phase of respiration may be written as follows:

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

We have till now seen that glucose has been broken down to release $CO_2$ and eight molecules of $NADH + H^+$; two of $FADH_2$ have been synthesised besides just two molecules of ATP in TCA cycle. You may be wondering why we have been discussing respiration at all – neither $O_2$ has come into the picture nor the promised large number of ATP has yet been synthesised. Also what is the role of the $NADH + H^+$ and $FADH_2$ that is synthesised? Let us now understand the role of $O_2$ in respiration and how ATP is synthesised.

### 14.4.2 Electron Transport System (ETS) and Oxidative Phosphorylation

The following steps in the respiratory process are to release and utilise the energy stored in $NADH+H^+$ and $FADH_2$. This is accomplished when they are oxidised through the electron transport system and the electrons are passed on to $O_2$ resulting in the formation of $H_2O$. The metabolic pathway through which the electron passes from one carrier to another, is called the ***electron transport system*** (ETS) (Figure 14.4) and it is present in the inner mitochondrial membrane. Electrons from NADH

produced in the mitochondrial matrix during citric acid cycle are oxidised by an NADH dehydrogenase (complex I), and electrons are then transferred to ubiquinone located within the inner membrane. Ubiquinone also receives reducing equivalents via $FADH_2$ (complex II) that is generated during oxidation of succinate in the citric acid cycle. The reduced ubiquinone (ubiquinol) is then oxidised with the transfer of electrons to cytochrome *c* via cytochrome $bc_1$ complex (complex III). Cytochrome *c* is a small protein attached to the outer surface of the inner membrane and acts as a mobile carrier for transfer of electrons between complex III and IV. Complex IV refers to cytochrome *c* oxidase complex containing cytochromes *a* and $a_3$, and two copper centres.

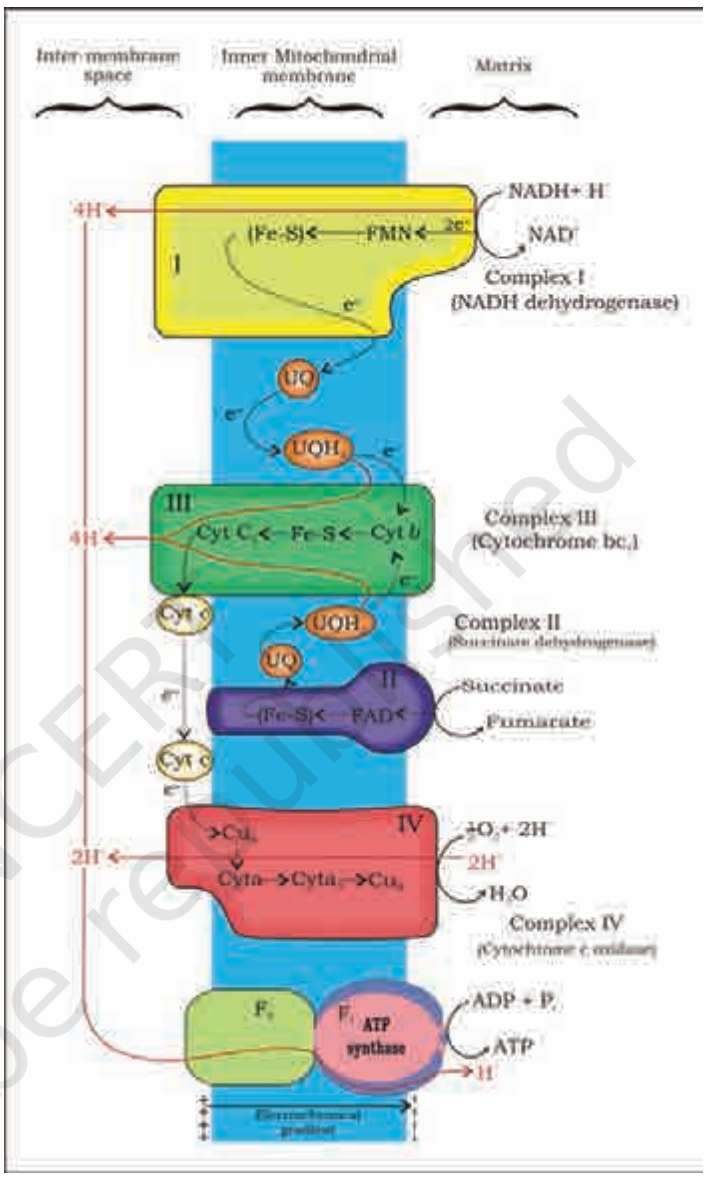



**Figure 14.4** Electron Transport System (ETS)

When the electrons pass from one carrier to another via complex I to IV in the electron transport chain, they are coupled to ATP synthase (complex V) for the production of ATP from ADP and inorganic phosphate. The number of ATP molecules synthesised depends on the nature of the electron donor. Oxidation of one molecule of NADH gives rise to 3 molecules of ATP, while that of one molecule of $FADH_2$ produces 2 molecules of ATP. Although the aerobic process of respiration takes place only in the presence of oxygen, the role of oxygen is limited to the terminal stage of the process. Yet, the presence of oxygen is vital, since it drives the whole process by removing hydrogen from the system. Oxygen acts as the final hydrogen acceptor. Unlike photophosphorylation where it is the light energy that is utilised for the production of proton gradient required for phosphorylation, in respiration it is the energy of oxidation-reduction utilised for the same process. It is for this reason that the process is called oxidative phosphorylation.

You have already studied about the mechanism of membrane-linked ATP synthesis as explained by chemiosmotic hypothesis in the earlier chapter. As mentioned earlier, the energy released during the electron

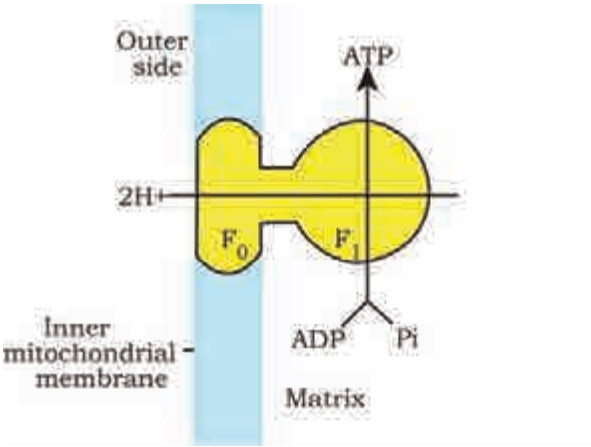



**Figure 14.5** Diagramatic presentation of ATP synthesis in mitochondria

transport system is utilised in synthesising ATP with the help of ATP synthase (complex V). This complex consists of two major components, $F_1$ and $F_0$ (Figure 14.5). The $F_1$ headpiece is a peripheral membrane protein complex and contains the site for synthesis of ATP from ADP and inorganic phosphate. $F_0$ is an integral membrane protein complex that forms the channel through which protons cross the inner membrane. The passage of protons through the channel is coupled to the catalytic site of the $F_1$ component for the production of ATP. For each ATP produced, $2H^+$ passes through $F_0$ from the intermembrane space to the matrix down the electrochemical proton gradient.

## 14.5 The Respiratory Balance Sheet

It is possible to make calculations of the net gain of ATP for every glucose molecule oxidised; but in reality this can remain only a theoretical exercise. These calculations can be made only on certain assumptions that:

- There is a sequential, orderly pathway functioning, with one substrate forming the next and with glycolysis, TCA cycle and ETS pathway following one after another.
- The NADH synthesised in glycolysis is transferred into the mitochondria and undergoes oxidative phosphorylation.
- None of the intermediates in the pathway are utilised to synthesise any other compound.
- Only glucose is being respired – no other alternative substrates are entering in the pathway at any of the intermediary stages.

But this kind of assumptions are not really valid in a living system; all pathways work simultaneously and do not take place one after another; substrates enter the pathways and are withdrawn from it as and when necessary; ATP is utilised as and when needed; enzymatic rates are controlled by multiple means. Yet, it is useful to do this exercise to appreciate the beauty and efficiency of the living system in extraction and storing energy. Hence, there can be a net gain of 38 ATP molecules during aerobic respiration of one molecule of glucose.

Now let us compare fermentation and aerobic respiration:

- Fermentation accounts for only a partial breakdown of glucose whereas in aerobic respiration it is completely degraded to $CO_2$ and $H_2O$.
- In fermentation there is a net gain of only two molecules of ATP for each molecule of glucose degraded to pyruvic acid whereas many more molecules of ATP are generated under aerobic conditions.
- NADH is oxidised to $NAD^+$ rather slowly in fermentation, however the reaction is very vigorous in case of aerobic respiration.

## 14.6 AMPHIBOLIC PATHWAY

Glucose is the favoured substrate for respiration. All carbohydrates are usually first converted into glucose before they are used for respiration. Other substrates can also be respired, as has been mentioned earlier, but then they do not enter the respiratory pathway at the first step. See Figure 14.6 to see the points of entry of different substrates in the respiratory pathway. Fats would need to be broken down into glycerol and fatty acids first. If fatty acids were to be respired they would first be degraded to acetyl CoA and enter the pathway. Glycerol would enter the pathway after being converted to PGAL. The proteins would be degraded by proteases and the individual amino acids (after deamination) depending on their structure would enter the pathway at some stage within the Krebs' cycle or even as pyruvate or acetyl CoA.

Since respiration involves breakdown of substrates, the respiratory process has traditionally been considered a catabolic process and the respiratory pathway as a catabolic pathway. But is this understanding correct? We have discussed above, at which points in the respiratory pathway different substrates would enter if they were to be respired and used to derive energy. What is important to recognise is that it is these very compounds that would be withdrawn from the respiratory pathway for the synthesis of the said substrates. Hence, fatty acids would be broken down to acetyl CoA before entering the respiratory pathway when it is used as a substrate. But when the organism needs to synthesise fatty acids, acetyl CoA would be withdrawn from the respiratory pathway for it. Hence, the respiratory pathway comes into the picture both during breakdown and synthesis of fatty acids. Similarly, during breakdown and synthesis of protein too, respiratory intermediates form the link. Breaking down processes within the living organism is catabolism, and synthesis is anabolism. Because the respiratory pathway is involved in both anabolism and catabolism, it would hence be better to consider the respiratory pathway as an **amphibolic pathway** rather than as a catabolic one.

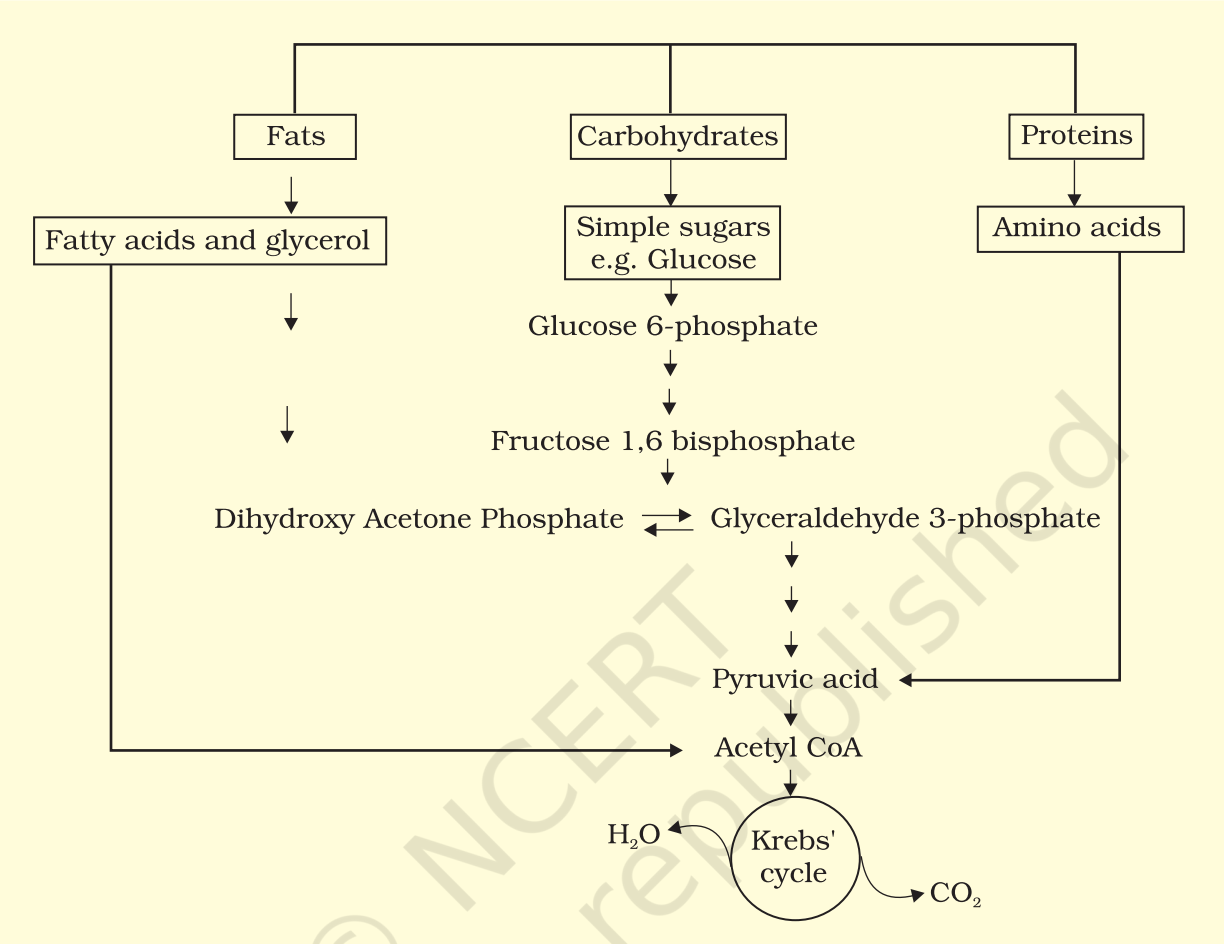



**Figure 14.6** Interrelationship among metabolic pathways showing respiration mediated breakdown of different organic molecules to $CO_2$ and $H_20$

## 14.7 Respiratory Quotient

Let us now look at another aspect of respiration. As you know, during aerobic respiration, $O_2$ is consumed and $CO_2$ is released. The ratio of the volume of $CO_2$ evolved to the volume of $O_2$ consumed in respiration is called the **respiratory quotient** (RQ) or respiratory ratio.

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

The respiratory quotient depends upon the type of respiratory substrate used during respiration.

When carbohydrates are used as substrate and are completely oxidised, the RQ will be 1, because equal amounts of $CO_2$ and $O_2$ are evolved and consumed, respectively, as shown in the equation below :

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{Energy}$$

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

When fats are used in respiration, the RQ is less than 1. Calculations for a fatty acid, tripalmitin, if used as a substrate is shown:

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

Tripalmitin

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

When proteins are respiratory substrates the ratio would be about 0.9.

What is important to recognise is that in living organisms respiratory substrates are often more than one; pure proteins or fats are never used as respiratory substrates.

## SUMMARY

Plants unlike animals have no special systems for breathing or gaseous exchange. Stomata and lenticels allow gaseous exchange by diffusion. Almost all living cells in a plant have their surfaces exposed to air.

The breaking of C-C bonds of complex organic molecules by oxidation cells leading to the release of a lot of energy is called cellular respiration. Glucose is the favoured substrate for respiration. Fats and proteins can also be broken down to yield energy. The initial stage of cellular respiration takes place in the cytoplasm. Each glucose molecule is broken through a series of enzyme catalysed reactions into two molecules of pyruvic acid. This process is called glycolysis. The fate of the pyruvate depends on the availability of oxygen and the organism. Under anaerobic conditions either lactic acid fermentation or alcohol fermentation occurs. Fermentation takes place under anaerobic conditions in many prokaryotes, unicellular eukaryotes and in germinating seeds. In eukaryotic organisms aerobic respiration occurs in the presence of oxygen. Pyruvic acid is transported into the mitochondria where it is converted into acetyl CoA with the release of $CO_2$. Acetyl CoA then enters the tricarboxylic acid pathway or Krebs' cycle operating in the matrix of the mitochondria. NADH + $H^+$ and $FADH_2$ are generated in the Krebs' cycle. The energy in these molecules as well as that in the NADH + $H^+$ synthesised during glycolysis are used to synthesise ATP. This is accomplished through a

system of electron carriers called electron transport system (ETS) located on the inner membrane of the mitochondria. The electrons, as they move through the system, release enough energy that are trapped to synthesise ATP. This is called oxidative phosphorylation. In this process $O_2$ is the ultimate acceptor of electrons and it gets reduced to water.

The respiratory pathway is an amphibolic pathway as it involves both anabolism and catabolism. The respiratory quotient depends upon the type of respiratory substance used during respiration.

## EXERCISES

1. Differentiate between
   (a) Respiration and Combustion
   (b) Glycolysis and Krebs' cycle
   (c) Aerobic respiration and Fermentation
2. What are respiratory substrates? Name the most common respiratory substrate.
3. Give the schematic representation of glycolysis?
4. What are the main steps in aerobic respiration? Where does it take place?
5. Give the schematic representation of an overall view of Krebs' cycle.
6. Explain ETS.
7. Distinguish between the following:
   (a) Aerobic respiration and Anaerobic respiration
   (b) Glycolysis and Fermentation
   (c) Glycolysis and Citric acid Cycle
8. What are the assumptions made during the calculation of net gain of ATP?
9. Discuss "The respiratory pathway is an amphibolic pathway."
10. Define RQ. What is its value for fats?
11. What is oxidative phosphorylation?
12. What is the significance of step-wise release of energy in respiration?

# Chapter 15
# Plant Growth and Development



You have already studied the organisation of a flowering plant in Chapter 5. Have you ever thought about where and how the structures like roots, stems, leaves, flowers, fruits and seeds arise and that too in an orderly sequence? You are, by now, aware of the terms seed, seedling, plantlet, mature plant. You have also seen that trees continue to increase in height or girth over a period of time. However, the leaves, flowers and fruits of the same tree not only have limited dimensions but also appear and fall periodically and some time repeatedly. Why does vegetative phase precede flowering in a plant? All plant organs are made up of a variety of tissues; is there any relationship between the structure of a cell, a tissue, an organ and the function they perform? Can the structure and the function of these be altered? All cells of a plant are descendents of the zygote. The question is, then, why and how do they have different structural and functional attributes? Development is the sum of two processes: growth and differentiation. To begin with, it is essential and sufficient to know that the development of a mature plant from a zygote (fertilised egg) follow a precise and highly ordered succession of events. During this process a complex body organisation is formed that produces roots, leaves, branches, flowers, fruits, and seeds, and eventually they die (Figure 15.1). The first step in the process of plant growth is seed germination. The seed germinates when favourable conditions for growth exist in the environment. In absence of such favourable conditions the seeds do not germinate and goes into a period of suspended growth or rest. Once favourable conditions return, the seeds resume metabolic activities and growth takes place.

In this chapter, you shall also study some of the factors which govern and control these developmental processes. These factors are both intrinsic (internal) and extrinsic (external) to the plant.

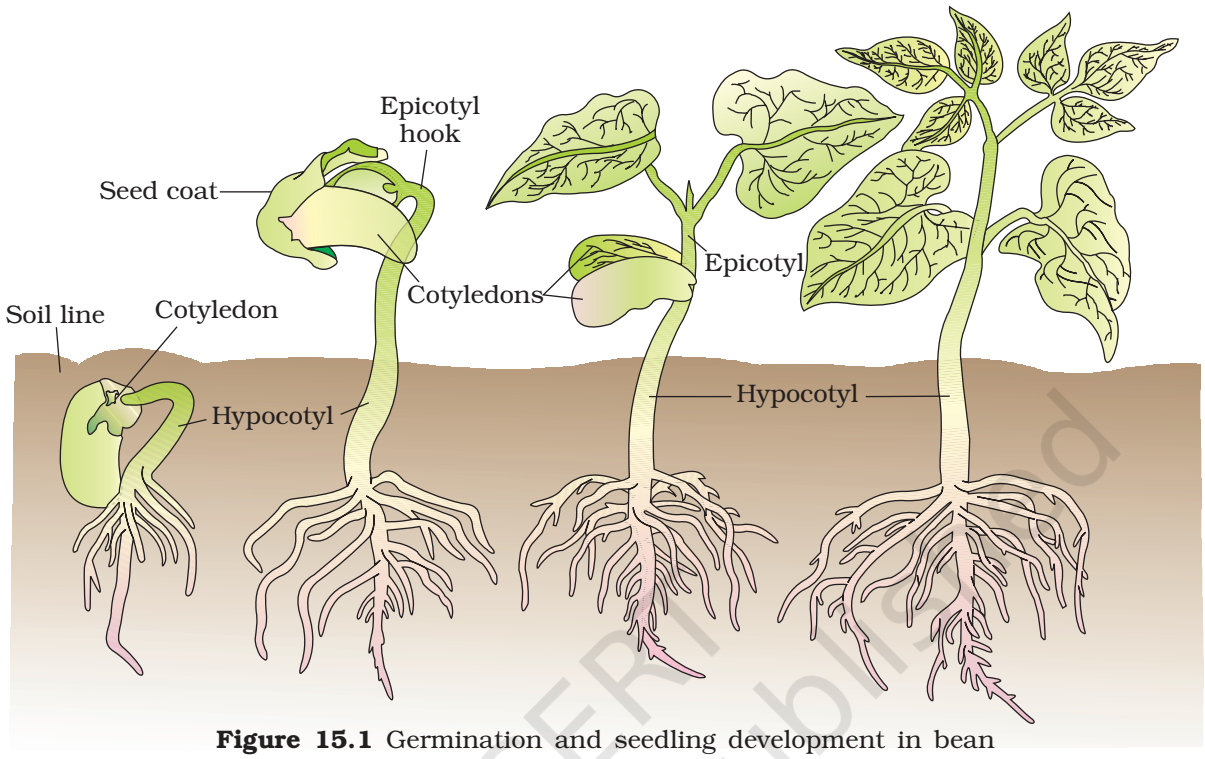



**Figure 15.1** Germination and seedling development in bean

## 15.1 GROWTH

Growth is regarded as one of the most fundamental and conspicuous characteristics of a living being. What is growth? Growth can be defined as an irreversible permanent increase in size of an organ or its parts or even of an individual cell. Generally, growth is accompanied by metabolic processes (both anabolic and catabolic), that occur at the expense of energy. Therefore, for example, expansion of a leaf is growth. How would you describe the swelling of piece of wood when placed in water?

### 15.1.1 Plant Growth Generally is Indeterminate

Plant growth is unique because plants retain the capacity for unlimited growth throughout their life. This ability of the plants is due to the presence of meristems at certain locations in their body. The cells of such meristems have the capacity to divide and self-perpetuate. The product, however, soon loses the capacity to divide and such cells make up the plant body. This form of growth wherein new cells are always being added to the plant body by the activity of the meristem is called the open form of growth. What would happen if the meristem ceases to divide? Does this ever happen?

In Chapter 6, you have studied about the root apical meristem and the shoot apical meristem. You know that they are responsible for the

primary growth of the plants and principally contribute to the elongation of the plants along their axis. You also know that in dicotyledonous plants and gymnosperms, the lateral meristems, vascular cambium and cork-cambium appear later in life. These are the meristems that cause the increase in the girth of the organs in which they are active. This is known as secondary growth of the plant (see Figure 15.2).

### 15.1.2 Growth is Measurable

Growth, at a cellular level, is principally a consequence of increase in the amount of protoplasm. Since increase in protoplasm is difficult to measure directly, one generally measures some quantity which is more or less proportional to it. Growth is, therefore, measured by a variety of parameters some of which are: increase in fresh weight, dry weight, length, area, volume and cell number. You may find it amazing to know that one single maize root apical mersitem can give rise to more than 17,500 new cells per hour, whereas cells in a watermelon may increase in size by upto 3,50,000 times. In the former, growth is expressed as increase in cell number; the latter expresses growth as increase in size of the cell. While the growth of a pollen tube is measured in terms of its length, an increase in surface area denotes the growth in a dorsiventral leaf.

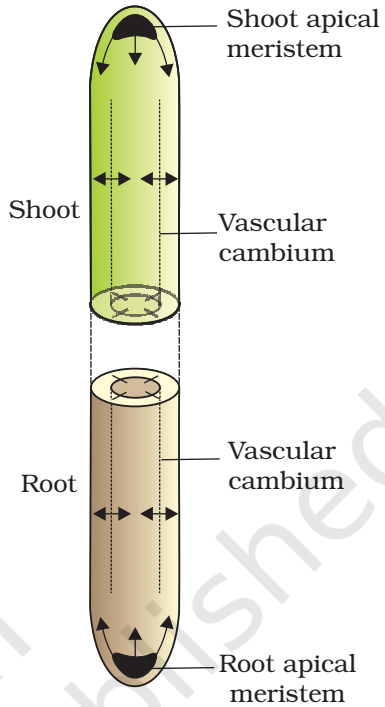



**Figure 15.2** Diagrammatic representation of locations of root apical meristem, shoot aplical meristem and vascular cambium. Arrows exhibit the direction of growth of cells and organ

### 15.1.3 Phases of Growth

The period of growth is generally divided into three phases, namely, meristematic, elongation and maturation (Figure 15.3). Let us understand this by looking at the root tips. The constantly dividing cells, both at the root apex and the shoot apex, represent the meristematic phase of growth. The cells in this region are rich in protoplasm, possess large conspicuous nuclei. Their cell walls are primary in nature, thin and cellulosic with abundant plasmodesmatal connections. The cells proximal (just next, away from the tip) to the

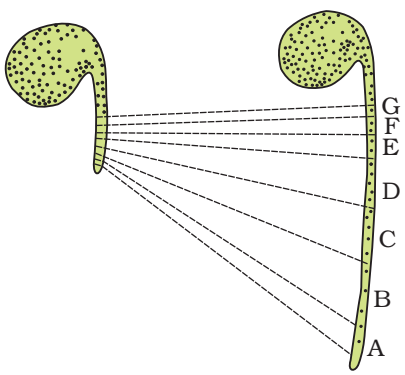



**Figure 15.3** Detection of zones of elongation by the parallel line technique. Zones A, B, C, D immediately behind the apex have elongated most.

meristematic zone represent the phase of elongation. Increased vacuolation, cell enlargement and new cell wall deposition are the characteristics of the cells in this phase. Further away from the apex, i.e., more proximal to the phase of elongation, lies the portion of axis which is undergoing the phase of maturation. The cells of this zone, attain their maximal size in terms of wall thickening and protoplasmic modifications. Most of the tissues and cell types you have studied in Chapter 6 represent this phase.

### 15.1.4 Growth Rates

The increased growth per unit time is termed as growth rate. Thus, rate of growth can be expressed mathematically. An organism, or a part of the organism can produce more cells in a variety of ways.

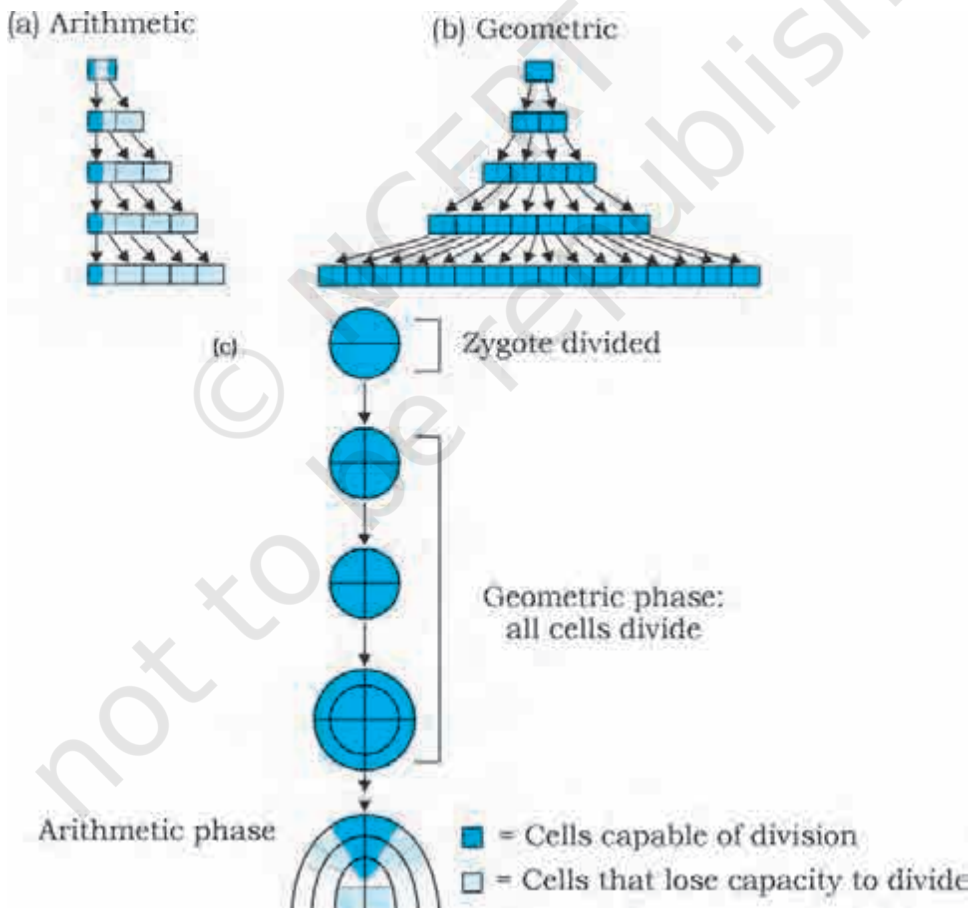



**Figure 15.4** Diagrammatic representation of : (a) Arithmetic (b) Geometric growth and (c) Stages during embryo development showing geometric and arithematic phases

The growth rate shows an increase that may be arithmetic or geometrical (Figure 15.4).

In arithmetic growth, following mitotic cell division, only one daughter cell continues to divide while the other differentiates and matures. The simplest expression of arithmetic growth is exemplified by a root elongating at a constant rate. Look at Figure 15.5. On plotting the length of the organ against time, a linear curve is obtained. Mathematically, it is expressed as

$L_t = L_0 + rt$

$L_t$ = length at time 't'

$L_0$ = length at time 'zero'

r = growth rate / elongation per unit time.

Let us now see what happens in geometrical growth. In most systems, the initial growth is slow (lag phase), and it increases rapidly thereafter – at an exponential rate (log or exponential phase). Here, both the progeny cells following mitotic cell division retain the ability to divide and continue to do so. However, with limited nutrient supply, the growth slows down leading to a stationary phase. If we plot the parameter of growth against time, we get a typical sigmoid or S-curve (Figure 15.6). A sigmoid curve is a characteristic of living organism growing in a natural environment. It is typical for all cells, tissues and organs of a plant. *Can you think of more similar examples? What kind of a curve can you expect in a tree showing seasonal activities?*

The exponential growth can be expressed as

$W_1 = W_0 e^{rt}$

$W_1$ = final size (weight, height, number etc.)

$W_0$ = initial size at the beginning of the period

r = growth rate

t = time of growth

e = base of natural logarithms

Here, r is the relative growth rate and is also the measure of the ability of the plant to produce new plant material, referred to as efficiency index. Hence, the final size of $W_1$ depends on the initial size, $W_0$.

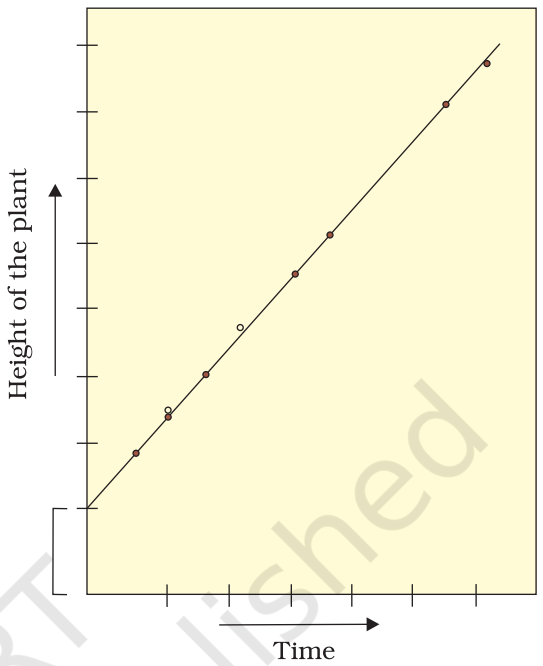



**Figure 15.5** Constant linear growth, a plot of length L against time t

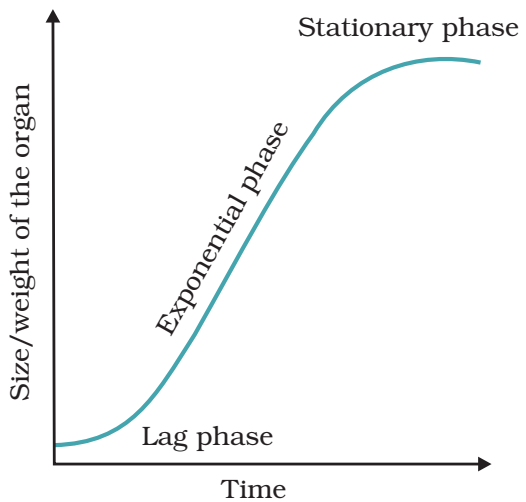



**Figure 15.6** An idealised sigmoid growth curve typical of cells in culture, and many higher plants and plant organs

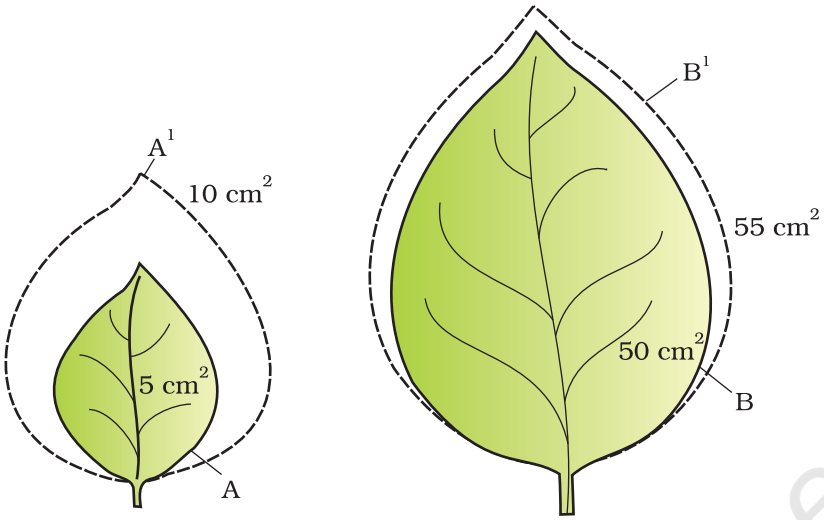



**Figure 15.7** Diagrammatic comparison of absolute and relative growth rates. Both leaves A and B have increased their area by 5 $cm^2$ in a given time to produce $A^1$, $B^1$ leaves.

Quantitative comparisons between the growth of living system can also be made in two ways : (i) measurement and the comparison of total growth per unit time is called the absolute growth rate. (ii) The growth of the given system per unit time expressed on a common basis, e.g., per unit initial parameter is called the relative growth rate. In Figure 15.7 two leaves, A and B, are drawn that are of different sizes but shows absolute increase in area in the given time to give leaves, $A^1$ and $B^1$. However, one of them shows much higher relative growth rate. Which one and why?

### 15.1.5 Conditions for Growth

Why do you not try to write down what you think are necessary conditions for growth? This list may have water, oxygen and nutrients as very essential elements for growth. The plant cells grow in size by cell enlargement which in turn requires water. Turgidity of cells helps in extension growth. Thus, plant growth and further development is intimately linked to the water status of the plant. Water also provides the medium for enzymatic activities needed for growth. Oxygen helps in releasing metabolic energy essential for growth activities. Nutrients (macro and micro essential elements) are required by plants for the synthesis of protoplasm and act as source of energy.

In addition, every plant organism has an optimum temperature range best suited for its growth. Any deviation from this range could be detrimental to its survival. Environmental signals such as light and gravity also affect certain phases/stages of growth.

## 15.2 DIFFERENTIATION, DEDIFFERENTIATION AND REDIFFERENTIATION

The cells derived from root apical and shoot-apical meristems and cambium differentiate and mature to perform specific functions. This act leading to maturation is termed as **differentiation**. During differentiation, cells undergo few to major structural changes both in their cell walls and protoplasm. For example, to form a tracheary element, the cells would lose their protoplasm. They also develop a very strong, elastic, lignocellulosic secondary cell walls, to carry water to long distances even under extreme tension. Try to correlate the various anatomical features you encounter in plants to the functions they perform.

Plants show another interesting phenomenon. The living differentiated cells, that by now have lost the capacity to divide can regain the capacity of division under certain conditions. This phenomenon is termed as **dedifferentiation**. For example, formation of meristems – interfascicular cambium and cork cambium from fully differentiated parenchyma cells. While doing so, such meristems/tissues are able to divide and produce cells that once again lose the capacity to divide but mature to perform specific functions, i.e., get **redifferentiated**. List some of the tissues in a woody dicotyledenous plant that are the products of redifferentiation. How would you describe a tumour? What would you call the parenchyma cells that are made to divide under controlled laboratory conditions during plant tissue culture?

Recall, in Section 15.1.1, we have mentioned that the growth in plants is open, i.e., it can be indeterminate or determinate. Now, we may say that even differentiation in plants is open, because cells/tissues arising out of the same meristem have different structures at maturity. The final structure at maturity of a cell/tissue is also determined by the location of the cell within. For example, cells positioned away from root apical meristems differentiate as root-cap cells, while those pushed to the periphery mature as epidermis. Can you add a few more examples of open differentiation correlating the position of a cell to its position in an organ?

## 15.3 DEVELOPMENT

Development is a term that includes all changes that an organism goes through during its life cycle from germination of the seed to senescence. Diagrammatic representation of the sequence of processes which constitute the development of a cell of a higher plant is given in Figure 15.8. It is also applicable to tissues/organs.

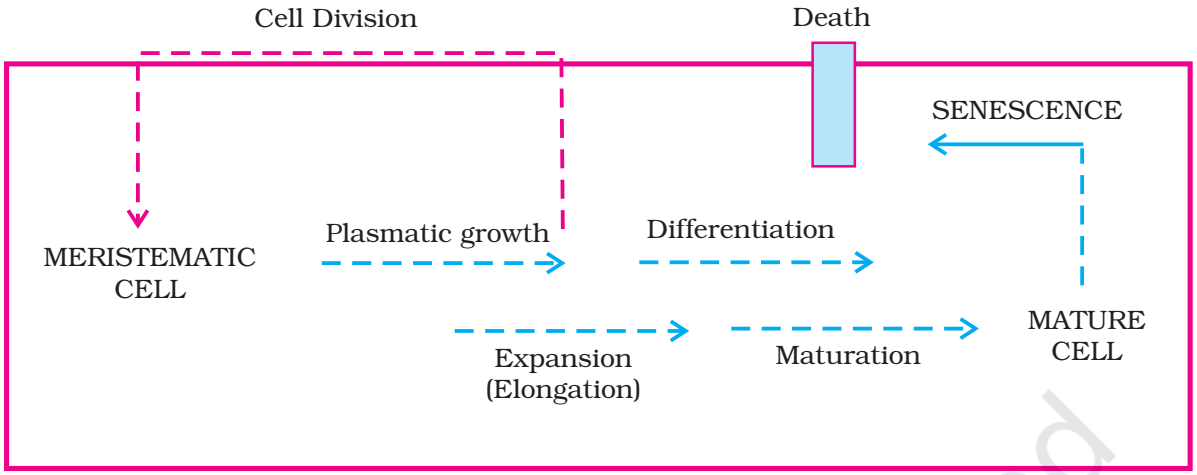



**Figure 15.8** Sequence of the developmental process in a plant cell

Plants follow different pathways in response to environment or phases of life to form different kinds of structures. This ability is called **plasticity,** e.g., heterophylly in cotton, coriander and larkspur. In such plants, the leaves of the juvenile plant are different in shape from those in mature plants. On the other hand, difference in shapes of leaves produced in air and those produced in water in buttercup also represent the heterophyllous development due to environment (Figure 15.9). This phenomenon of heterophylly is an example of plasticity.

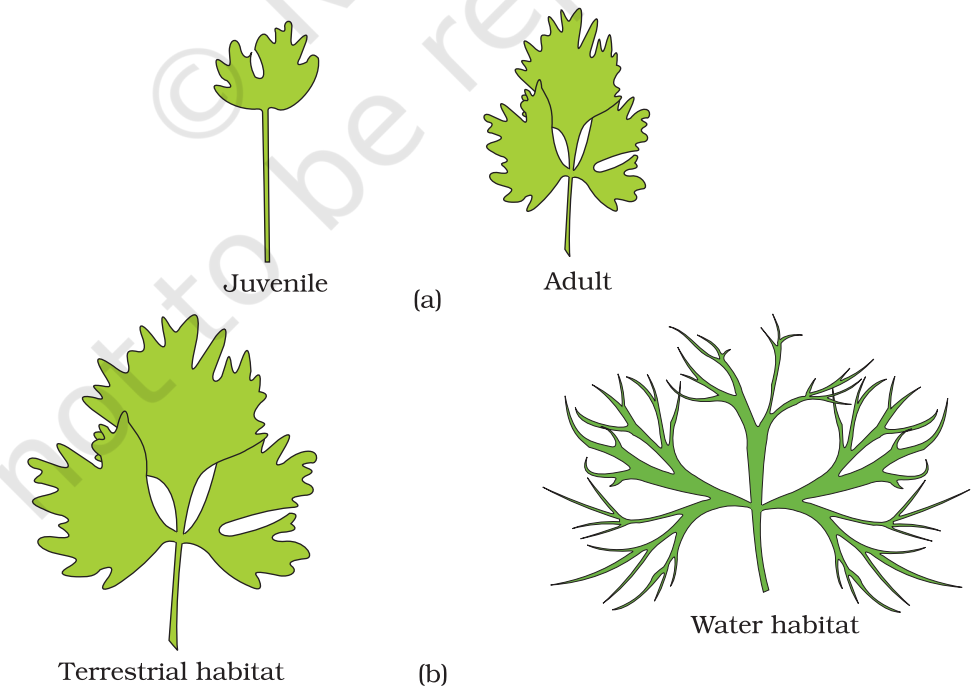



**Figure 15.9** Heterophylly in (a) larkspur and (b) buttercup

Thus, growth, differentiation and development are very closely related events in the life of a plant. Broadly, development is considered as the sum of growth and differentiation. Development in plants (i.e., both growth and differentiation) is under the control of intrinsic and extrinsic factors. The former includes both intracellular (genetic) or intercellular factors (chemicals such as plant growth regulators) while the latter includes light, temperature, water, oxygen, nutrition, etc.

## 15.4 PLANT GROWTH REGULATORS

### 15.4.1 Characteristics

The plant growth regulators (PGRs) are small, simple molecules of diverse chemical composition. They could be indole compounds (indole-3-acetic acid, IAA); adenine derivatives ($N^6$-furfurylamino purine, kinetin), derivatives of carotenoids (abscisic acid, ABA); terpenes (gibberellic acid, $GA_3$) or gases (ethylene, $C_2H_4$). Plant growth regulators are variously described as plant growth substances, plant hormones or phytohormones in literature.

The PGRs can be broadly divided into two groups based on their functions in a living plant body. One group of PGRs are involved in growth promoting activities, such as cell division, cell enlargement, pattern formation, tropic growth, flowering, fruiting and seed formation. These are also called plant growth promoters, e.g., auxins, gibberellins and cytokinins. The PGRs of the other group play an important role in plant responses to wounds and stresses of biotic and abiotic origin. They are also involved in various growth inhibiting activities such as dormancy and abscission. The PGR abscisic acid belongs to this group. The gaseous PGR, ethylene, could fit either of the groups, but it is largely an inhibitor of growth activities.

### 15.4.2 The Discovery of Plant Growth Regulators

Interestingly, the discovery of each of the five major groups of PGRs have been accidental. All this started with the observation of Charles Darwin and his son Francis Darwin when they observed that the coleoptiles of canary grass responded to unilateral illumination by growing towards the light source (phototropism). After a series of experiments, it was concluded that the tip of coleoptile was the site of transmittable influence that caused the bending of the entire coleoptile (Figure 15.10). Auxin was isolated by F.W. Went from tips of coleoptiles of oat seedlings.

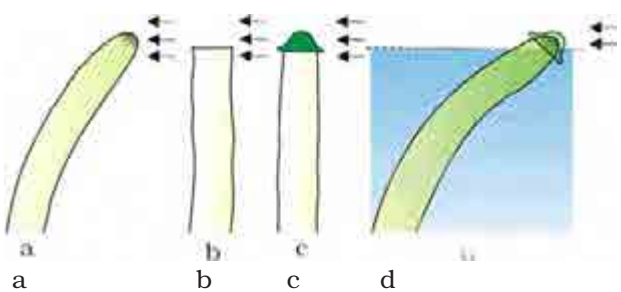



**Figure 15.10** Experiment used to demonstrate that tip of the coleoptile is the source of auxin. Arrows indicate direction of light

The 'bakanae' (foolish seedling) disease of rice seedlings, was caused by a fungal pathogen *Gibberella fujikuroi*. E. Kurosawa (1926) reported the appearance of symptoms of the disease in rice seedlings when they were treated with sterile filtrates of the fungus. The active substances were later identified as gibberellic acid.

F. Skoog and his co-workers observed that from the internodal segments of tobacco stems the callus (a mass of undifferentiated cells) proliferated only if, in addition to auxins the nutrients medium was supplemented with one of the following: extracts of vascular tissues, yeast extract, coconut milk or DNA. Skoog and Miller, later identified and crystallised the cytokinesis promoting active substance that they termed kinetin.

During mid-1960s, three independent researches reported the purification and chemical characterisation of three different kinds of inhibitors: inhibitor-B, abscission II and dormin. Later all the three were proved to be chemically identical. It was named abscisic acid (ABA).

Cousins confirmed the release of a volatile substance from ripened oranges that hastened the ripening of stored unripened bananas. Later this volatile substance was identified as ethylene, a gaseous PGR.

Let us study some of the physiological effects of these five categories of PGRs in the next section.

## 15.4.3 Physiological Effects of Plant Growth Regulators

### *15.4.3.1 Auxins*

Auxins (from Greek 'auxein' : to grow) was first isolated from human urine. The term 'auxin' is applied to the indole-3-acetic acid (IAA), and to other natural and synthetic compounds having certain growth regulating properties. They are generally produced by the growing apices of the stems and roots, from where they migrate to the regions of their action. Auxins like IAA and indole butyric acid (IBA) have been isolated from plants. NAA (naphthalene acetic acid) and 2, 4-D (2, 4-dichlorophenoxyacetic) are synthetic auxins. All these auxins have been used extensively in agricultural and horticultural practices.

They help to initiate rooting in stem cuttings, an application widely used for plant propagation. Auxins promote flowering e.g. in pineapples. They help to prevent fruit and leaf drop at early stages but promote the abscission of older mature leaves and fruits.

In most higher plants, the growing apical bud inhibits the growth of the lateral (axillary) buds, a phenomenon called **apical dominance**. Removal of shoot tips (decapitation) usually results in the growth of lateral buds (Figure 15.11). It is widely applied in tea plantations, hedge-making. Can you explain why?

Auxins also induce parthenocarpy, e.g., in tomatoes. They are widely used as herbicides. 2, 4-D, widely used to kill dicotyledonous weeds, does not affect mature monocotyledonous plants. It is used to prepare weed-free lawns by gardeners. Auxin also controls xylem differentiation and helps in cell division.

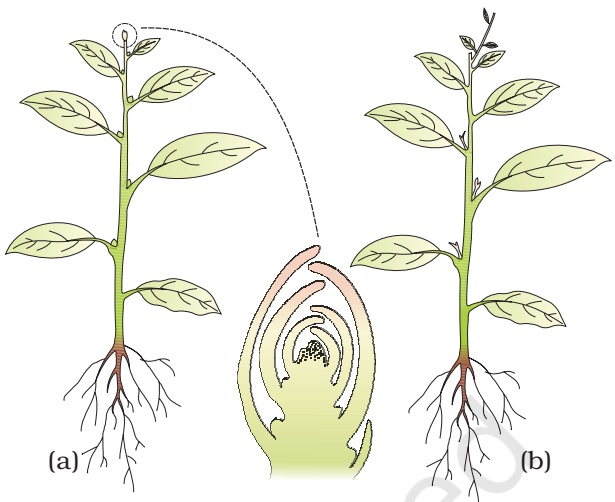



**Figure 15.11** Apical dominance in plants : (a) A plant with apical bud intact (b) A plant with apical bud removed Note the growth of lateral buds into branches after decapitation.

#### 15.4.3.2 Gibberellins

Gibberellins are another kind of promotory PGR. There are more than 100 gibberellins reported from widely different organisms such as fungi and higher plants. They are denoted as $GA_1$, $GA_2$, $GA_3$ and so on. However, Gibberellic acid ($GA_3$) was one of the first gibberellins to be discovered and remains the most intensively studied form. All GAs are acidic. They produce a wide range of physiological responses in the plants. Their ability to cause an increase in length of axis is used to increase the length of grapes stalks. Gibberellins, cause fruits like apple to elongate and improve its shape. They also delay senescence. Thus, the fruits can be left on the tree longer so as to extend the market period. $GA_3$ is used to speed up the malting process in brewing industry.

Sugarcane stores carbohydrate as sugar in their stems. Spraying sugarcane crop with gibberellins increases the length of the stem, thus increasing the yield by as much as 20 tonnes per acre.

Spraying juvenile conifers with GAs hastens the maturity period, thus leading to early seed production. Gibberellins also promotes bolting (internode elongation just prior to flowering) in beet, cabbages and many plants with rosette habit.

#### 15.4.3.3 Cytokinins

Cytokinins have specific effects on cytokinesis, and were discovered as kinetin (a modified form of adenine, a purine) from the autoclaved herring sperm DNA. Kinetin does not occur naturally in plants. Search for natural substances with cytokinin-like activities led to the isolation of zeatin from corn-kernels and coconut milk. Since the discovery of zeatin, several naturally occurring cytokinins, and some synthetic compounds with cell division promoting activity, have been identified. Natural cytokinins are synthesised in regions where rapid cell division occurs, for example, root apices, developing shoot buds, young fruits etc. It helps to produce new

leaves, chloroplasts in leaves, lateral shoot growth and adventitious shoot formation. Cytokinins help overcome the apical dominance. They promote nutrient mobilisation which helps in the delay of leaf senescence.

#### 15.4.3.4 Ethylene

Ethylene is a simple gaseous PGR. It is synthesised in large amounts by tissues undergoing senescence and ripening fruits. Influences of ethylene on plants include horizontal growth of seedlings, swelling of the axis and apical hook formation in dicot seedlings. Ethylene promotes senescence and abscission of plant organs especially of leaves and flowers. Ethylene is highly effective in fruit ripening. It enhances the respiration rate during ripening of the fruits. This rise in rate of respiration is called respiratory climactic.

Ethylene breaks seed and bud dormancy, initiates germination in peanut seeds, sprouting of potato tubers. Ethylene promotes rapid internode/petiole elongation in deep water rice plants. It helps leaves/upper parts of the shoot to remain above water. Ethylene also promotes root growth and root hair formation, thus helping the plants to increase their absorption surface.

Ethylene is used to initiate flowering and for synchronising fruit-set in pineapples. It also induces flowering in mango. Since ethylene regulates so many physiological processes, it is one of the most widely used PGR in agriculture. The most widely used compound as source of ethylene is ethephon. Ethephon in an aqueous solution is readily absorbed and transported within the plant and releases ethylene slowly. Ethephon hastens fruit ripening in tomatoes and apples and accelerates abscission in flowers and fruits (thinning of cotton, cherry, walnut). It promotes female flowers in cucumbers thereby increasing the yield.

#### 15.4.3.5 Abscisic acid

As mentioned earlier, abscisic acid **(ABA)** was discovered for its role in regulating abscission and dormancy. But like other PGRs, it also has other wide ranging effects on plant growth and development. It acts as a general plant growth inhibitor and an inhibitor of plant metabolism. ABA inhibits seed germination. ABA stimulates the closure of stomata in the epidermis and increases the tolerance of plants to various kinds of stresses. Therefore, it is also called the stress hormone. ABA plays an important role in seed development, maturation and dormancy. By inducing dormancy, ABA helps seeds to withstand desiccation and other factors unfavourable for growth. In most situations, ABA acts as an antagonist to GAs.

We may summarise that for any and every phase of growth, differentiation and development of plants, one or the other PGR has some role to play. Such roles could be complimentary or antagonistic. These could be individualistic or synergistic.

Similarly, there are a number of events in the life of a plant where more than one PGR interact to affect that event, e.g., dormancy in seeds/ buds, abscission, senescence, apical dominance, etc.

Remember, the role of PGR is of only one kind of intrinsic control. Along with genomic control and extrinsic factors, they play an important role in plant growth and development. Many of the extrinsic factors such as temperature and light, control plant growth and development via PGR. Some of such events could be: vernalisation, flowering, dormancy, seed germination, plant movements, etc.

We shall discuss briefly the role of light and temperature (both of them, the extrinsic factors) on initiation of flowering.

## 15.5 PHOTOPERIODISM

It has been observed that some plants require a periodic exposure to light to induce flowering. It is also seen that such plants are able to measure the duration of exposure to light. For example, some plants require the exposure to light for a period exceeding a well defined critical duration, while others must be exposed to light for a period less than this critical duration before the flowering is initiated in them. The former group of plants are called **long day plants** while the latter ones are termed **short day plants**. The critical duration is different for different plants. There are many plants, however, where there is no such correlation between exposure to light duration and induction of flowering response; such plants are called **day-neutral plants** (Figure 15.12). It is now also

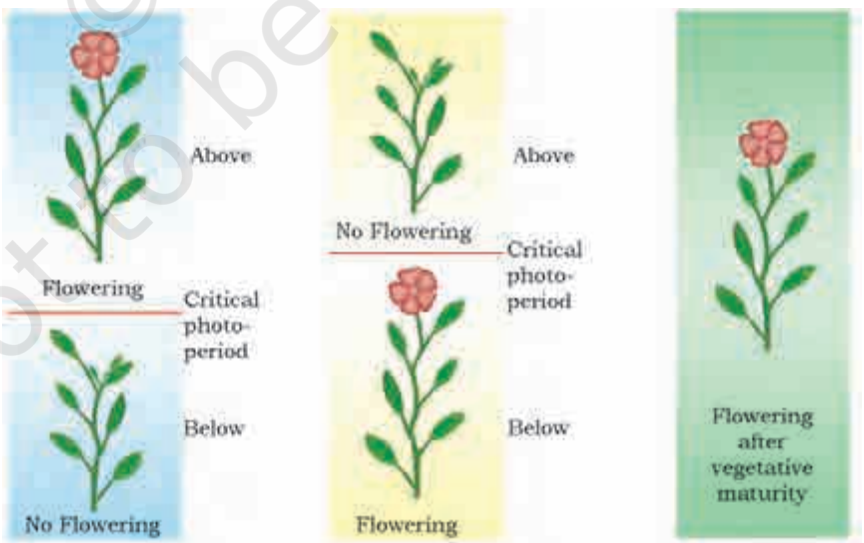



**Figure 15.12** Photoperiodism : Long day, short day and day neutral plants

known that not only the duration of light period but that the duration of dark period is also of equal importance. Hence, it can be said that flowering in certain plants depends not only on a combination of light and dark exposures but also their relative durations. This response of plants to periods of day/night is termed **photoperiodism**. It is also interesting to note that while shoot apices modify themselves into flowering apices prior to flowering, they (i.e., shoot apices of plants) by themselves cannot percieve photoperiods. The site of perception of light/dark duration are the leaves. It has been hypothesised that there is a hormonal substance(s) that is responsible for flowering. This hormonal substance migrates from leaves to shoot apices for inducing flowering only when the plants are exposed to the necessary inductive photoperiod.

## 15.6 VERNALISATION

There are plants for which flowering is either quantitatively or qualitatively dependent on exposure to low temperature. This phenomenon is termed **vernalisation**. It prevents precocious reproductive development late in the growing season, and enables the plant to have sufficient time to reach maturity. Vernalisation refers specially to the promotion of flowering by a period of low temperature. Some important food plants, wheat, barley, rye have two kinds of varieties: winter and spring varieties. The 'spring' variety are normally planted in the spring and come to flower and produce grain before the end of the growing season. Winter varieties, however, if planted in spring would normally fail to flower or produce mature grain within a span of a flowering season. Hence, they are planted in autumn. They germinate, and over winter come out as small seedlings, resume growth in the spring, and are harvested usually around mid-summer.

Another example of vernalisation is seen in biennial plants. Biennials are monocarpic plants that normally flower and die in the second season. Sugarbeet, cabbages, carrots are some of the common biennials. Subjecting the growing of a biennial plant to a cold treatment stimulates a subsequent photoperiodic flowering response.

## 15.7 SEED DORMANCY

There are certain seeds which fail to germinate even when external conditions are favourable. Such seeds are understood to be undergoing a period of dormancy which is controlled not by external environment but are under endogenous control or conditions within the seed itself. Impermeable and hard seed coat; presence of chemical inhibitors such as abscissic acids, phenolic acids, para-ascorbic acid; and immature

embryos are some of the reasons which causes seed dormancy. This dormancy however can be overcome through natural means and various other man-made measures. For example, the seed coat barrier in some seeds can be broken by mechanical abrasions using knives, sandpaper, etc. or vigorous shaking. In nature, these abrasions are caused by microbial action, and passage through digestive tract of animals. Effect of inhibitory substances can be removed by subjecting the seeds to chilling conditions or by application of certain chemicals like gibberellic acid and nitrates. Changing the environmental conditions, such as light and temperature are other methods to overcome seed dormancy.

## SUMMARY

Growth is one of the most conspicuous events in any living organism. It is an irreversible increase expressed in parameters such as size, area, length, height, volume, cell number etc. It conspicuously involves increased protoplasmic material. In plants, meristems are the sites of growth. Root and shoot apical meristems sometimes alongwith intercalary meristem, contribute to the elongation growth of plant axes. Growth is indeterminate in higher plants. Following cell division in root and shoot apical meristem cells, the growth could be arithmetic or geometrical. Growth may not be and generally is not sustained at a high rate throughout the life of cell/tissue/organ/organism. One can define three principle phases of growth – the lag, the log and the senescent phase. When a cell loses the capacity to divide, it leads to differentiation. Differentiation results in development of structures that is commensurate with the function the cells finally has to perform. General principles for differentiation for cell, tissues and organs are similar. A differentiated cell may dedifferentiate and then redifferentiate. Since differentiation in plants is open, the development could also be flexible, i.e., the development is the sum of growth and differentiation. Plant exhibit plasticity in development.

Plant growth and development are under the control of both intrinsic and extrinsic factors. Intercellular intrinsic factors are the chemical substances, called plant growth regulators (PGR). There are diverse groups of PGRs in plants, principally belonging to five groups: auxins, gibberellins, cytokinins, abscisic acid and ethylene. These PGRs are synthesised in various parts of the plant; they control different differentiation and developmental events. Any PGR has diverse physiological effects on plants. Diverse PGRs also manifest similar effects. PGRs may act synergistically or antagonistically. Plant growth and development is also affected by light, temperature, nutrition, oxygen status, gravity and such external factors.

Flowering in some plants is induced only when exposed to certain duration of photoperiod. Depending on the nature of photoperiod requirements, the plants are called short day plants, long day plants and day-neutral plants. Certain plants also need to be exposed to low temperature so as to hasten flowering later in life. This treatement is known as vernalisation.

## EXERCISES

1. Define growth, differentiation, development, dedifferentiation, redifferentiation, determinate growth, meristem and growth rate.
2. Why is not any one parameter good enough to demonstrate growth throughout the life of a flowering plant?
3. Describe briefly:
   (a) Arithmetic growth
   (b) Geometric growth
   (c) Sigmoid growth curve
   (d) Absolute and relative growth rates
4. List five main groups of natural plant growth regulators. Write a note on discovery, physiological functions and agricultural/horticultural applications of any one of them.
5. What do you understand by photoperiodism and vernalisation? Describe their significance.
6. Why is abscisic acid also known as stress hormone?
7. 'Both growth and differentiation in higher plants are *open*'. Comment.
8. 'Both a short day plant and a long day plant can produce can flower simultaneously in a given place'. Explain.
9. Which one of the plant growth regulators would you use if you are asked to:
   (a) induce rooting in a twig
   (b) quickly ripen a fruit
   (c) delay leaf senescence
   (d) induce growth in axillary buds
   (e) 'bolt' a rosette plant
   (f) induce immediate stomatal closure in leaves.
10. Would a defoliated plant respond to photoperiodic cycle? Why?
11. What would be expected to happen if:
    (a) $GA_3$ is applied to rice seedlings
    (b) dividing cells stop differentiating
    (c) a rotten fruit gets mixed with unripe fruits
    (d) you forget to add cytokinin to the culture medium.