# 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).

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.

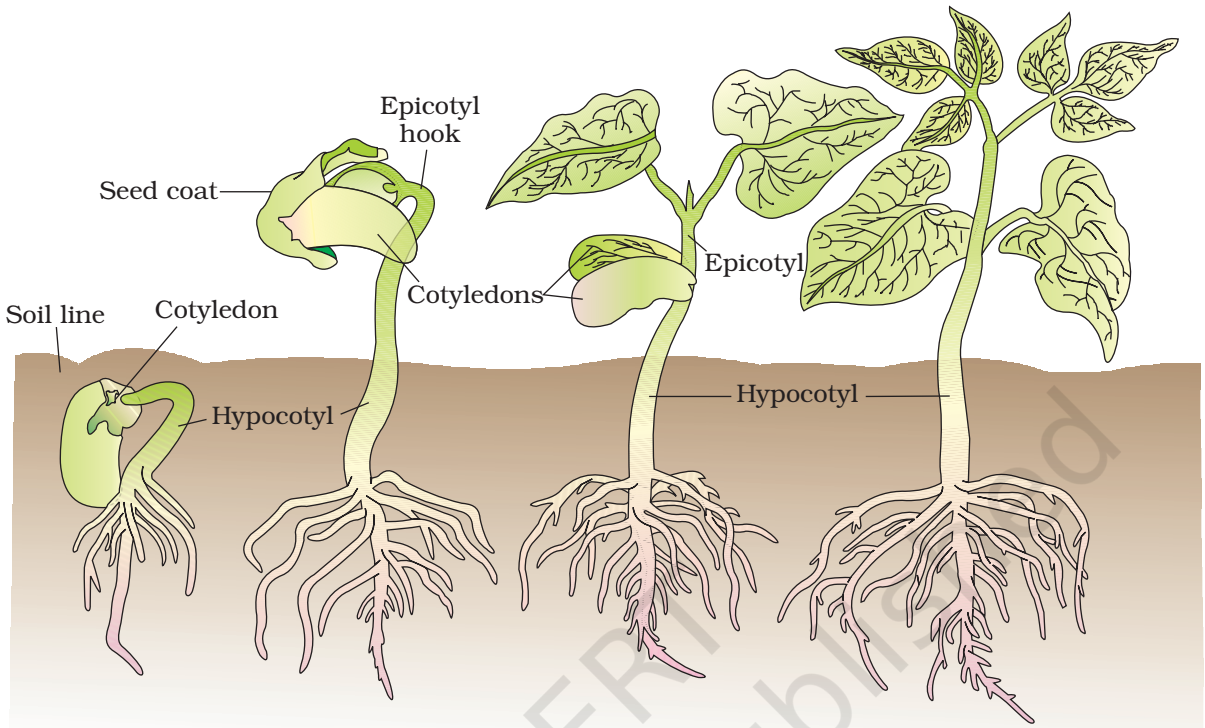


**Figure 15.1** Germination and seedling development in bean

## 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.

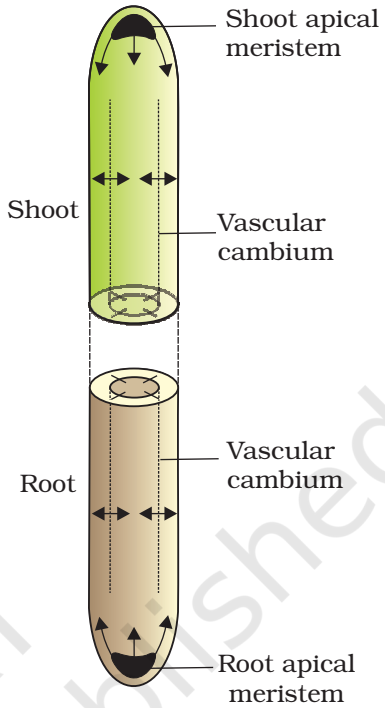


**Figure 15.2** Diagrammatic representation of locations of root apical meristem, shoot aplical meristem and vascular cambium. Arrows exhibit the direction of growth of cells and organ

### 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

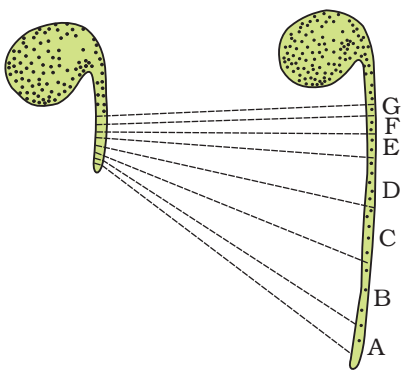


**Figure 15.3** Detection of zones of elongation by the parallel line technique. Zones A, B, C, D immediately behind the apex have elongated most.

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.

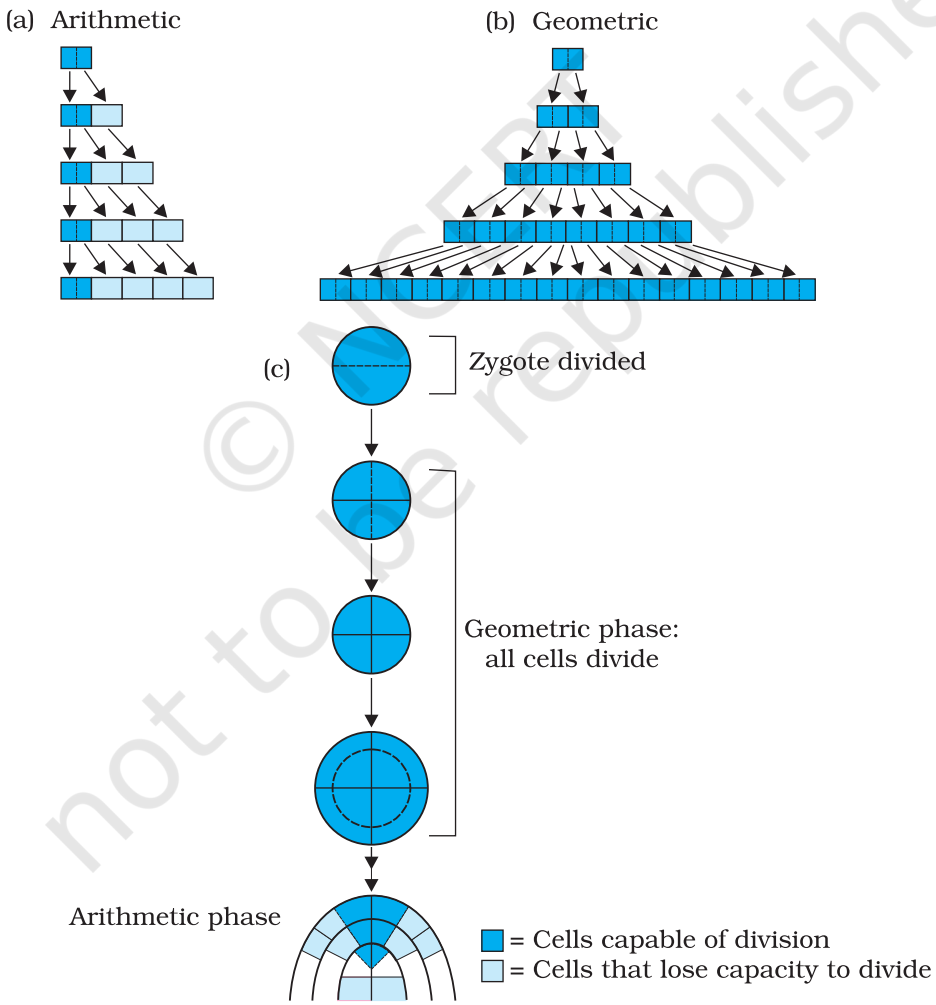


**Figure 15.4** Diagrammatic representation of : (a) Arithmetic (b) Geometric growth and (c) Stages during embryo development showing geometric and arithematic phases

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, $W_0$.

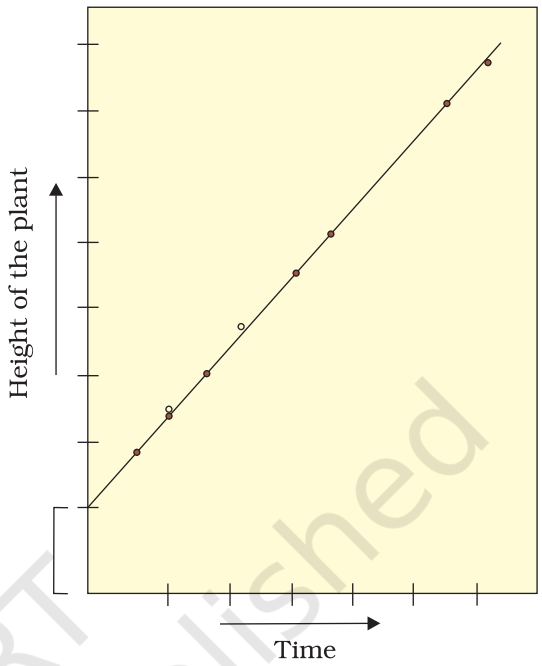


**Figure 15.5** Constant linear growth, a plot of length L against time t

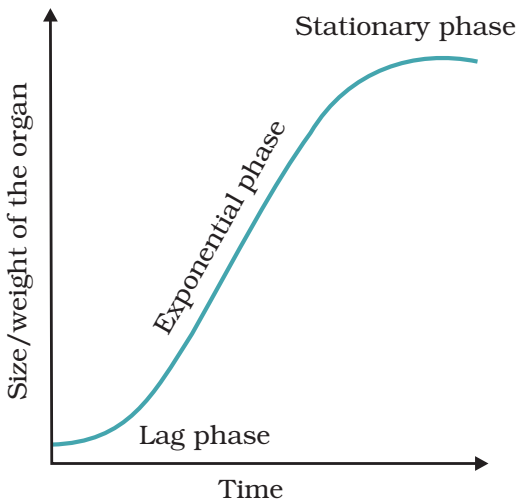


**Figure 15.6** An idealised sigmoid growth curve typical of cells in culture, and many higher plants and plant organs

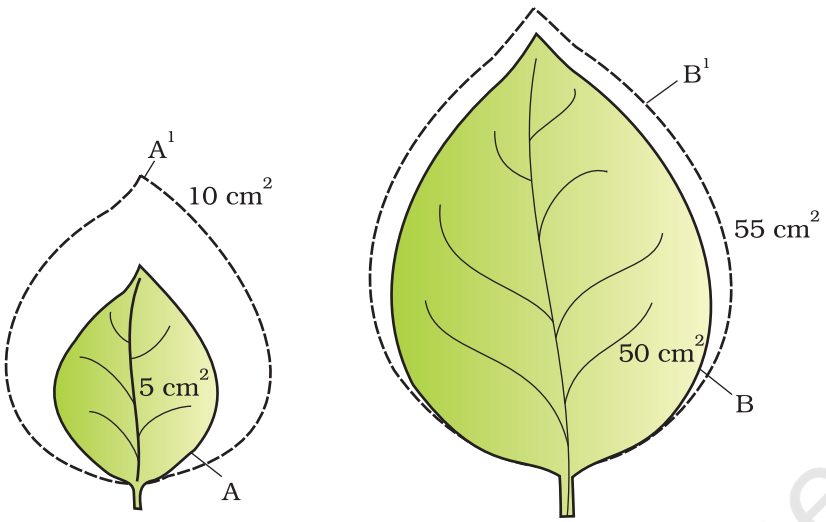


**Figure15.7** Diagrammatic comparison of absolute and relative growth rates. Both leaves A and B have increased their area by 5 $cm^2$ in a given time to produce $A^1$, $B^1$ leaves.

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.

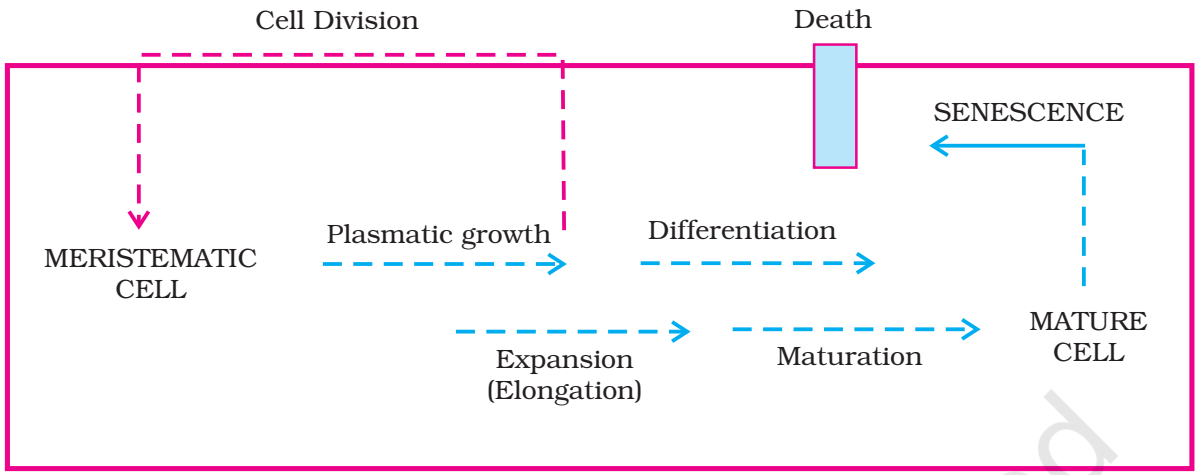


**Figure 15.8** Sequence of the developmental process in a plant cell

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.

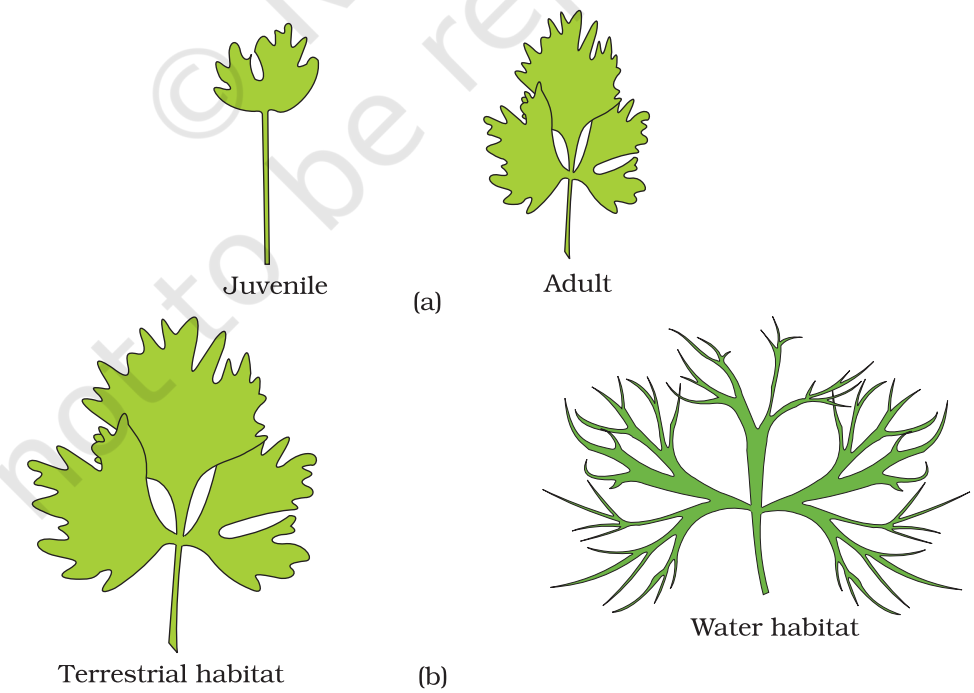


**Figure 15.9** Heterophylly in (a) larkspur and (b) buttercup

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.

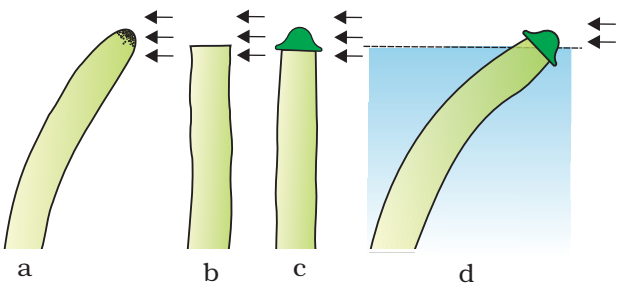


**Figure 15.10** Experiment used to demonstrate that tip of the coleoptile is the source of auxin. Arrows indicate direction of light

The 'bakane' (foolish seedling) disease of rice seedlings, was caused by a fungal pathogen *Gibberella fujikuroi*. E. Kurosawa reported the appearance of symptoms of the disease in uninfected 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.

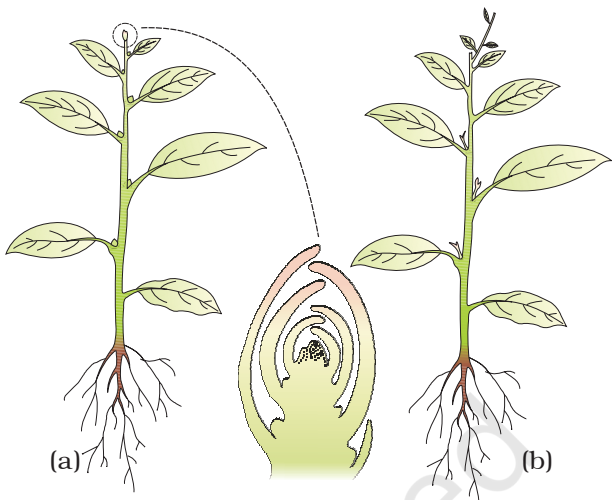


**Figure 15.11** Apical dominance in plants : (a) A plant with apical bud intact (b) A plant with apical bud removed Note the growth of lateral buds into branches after decapitation.

#### *15.4.3.2 Gibberellins*

Gibberellins are another kind of promotery 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

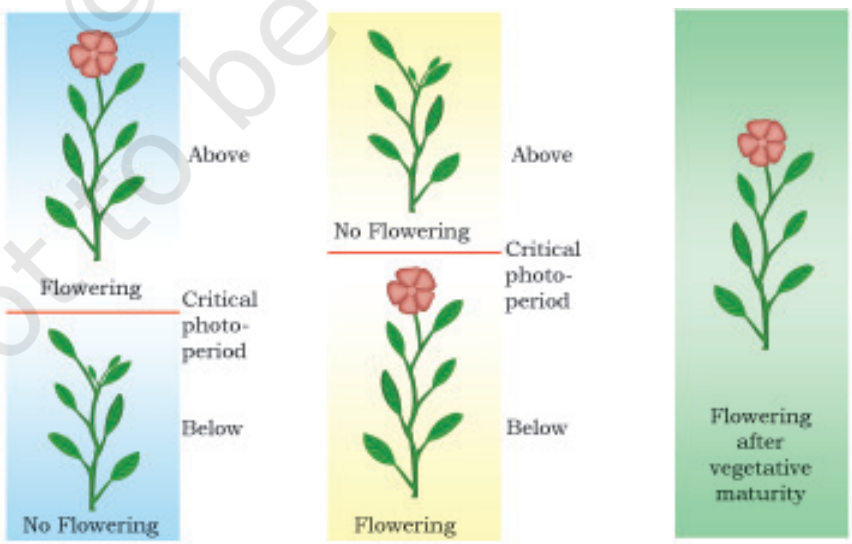


**Figure 15.12** Photoperiodism : Long day, short day and day neutral plants

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.



## 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.

# 10

# NITROGEN METABOLISM

All the living organisms are basically composed of carbon, hydrogen, oxygen, nitrogen and many other forms of chemical elements. These elements contribute to finally organize various biomolecules present in a cell. Nitrogen is next to carbon in importance in living organisms. In a living cell, nitrogen is an important constituent of amino acids, proteins, enzymes, vitamins, alkaloids and some growth hormones. Therefore, study of nitrogen metabolism is absolutely essential because the entire life process is dependent on these nitrogen-containing molecules. In this lesson, you will learn about various aspects of nitrogen metabolism including nitrogen fixation and nitrogen assimilation in plants.

## OBJECTIVES

After completing this lesson, you will be able to:

- *describe the modes of nitrogen fixation (both biological and abiological);*
- *explain the steps involved in nitrogen fixation by free living organisms;*
- *explain the mode of symbiotic nitrogen fixation in leguminous plants;*
- *describe the assimilation of nitrate and ammonia by plants;*
- *describe amino acid synthesis in plants.*

## 10.1 MOLECULAR NITROGEN

Nitrogen is primarily present in the atmosphere freely as dinitrogen or nitrogen gas. It is present in the combined form as Chile saltpetre or sodium nitrate and Chile in South America is the major source of this nitrate nitrogen.

Molecular Nitrogen or diatomic nitrogen ($N_2$) is highly stable as it is triple bonded (N≡N). Because of this stability, molecular nitrogen as such is not very reactive in the atmosphere under normal conditions. In the atmosphere molecular nitrogen is 78.03% by volume and it has a very low boiling point (-195.8$^{o}$C) which is even lower than oxygen. Proteins present in living organisms contain about 16% nitrogen.

## INTEXT QUESTIONS 10.1

1. What is the percent by volume of nitrogen gas in the atmosphere?

   .....

2. Name two biomolecules that contain nitrogen in plants.

   .....

3. Why nitrogen is a stable molecule?

   .....

4. What is the percentage of nitrogen in protein?

   .....

5. What is the boiling point of nitrogen?

   .....

## 10.2 NITROGEN FIXATION (BIOLOGICAL AND ABIOLOGICAL)

The conversion of molecular nitrogen into compounds of nitrogen especially ammonia is called **nitrogen fixation**. Nitrogen fixation, is a reductive process i.e., nitrogen fixation will stop if there is no reducing condition or if oxygen is present. This nitrogen fixation may take place by two different methods – abiological and biological.

### 10.2.1 Abiological nitrogen fixation

In abiological nitrogen fixation the nitrogen is reduced to ammonia without involving any living cell. Abiological fixation can be of two types : industrial and natural. For example, in the Haber's process, synthetic ammonia is produced by passing a mixture of nitrogen and hydrogen through a bed of catalyst (iron oxides) at a very high temperature and pressure.

$$N_2 + 3H_2 \xrightarrow[1000 \text{ atmosphere}]{500^\circ C} 2NH_3$$

This is industrial fixation and nitrogen reduced to ammonia.

In natural process nitrogen can be fixed especially during electrical discharges in the atmosphere. It may occur during lightning storms and nitrogen in the atmosphere can combine with oxygen to form oxides of nitrogen

$$N_2 + O_2 \longrightarrow 2NO \xrightarrow{O_2} 2NO_2$$

These oxides of nitrogen may be hydrated and trickle down to earth as combined nitrite and nitrate.

### 10.2.2 Biological nitrogen fixation

Chemically, this process is same as abiological. Biological nitrogen fixation is reduction of molecular nitrogen to ammonia by a living cell in the presence of an enzyme nitrogenase.

## INTEXT QUESTIONS 10.2

1. Define nitrogen fixation.

   .....

2. Which industrial process is utilized for converting nitrogen to ammonia?

   .....

3. Distinguish between biological and a biological nitrogen fixation.

   .....

4. Name the enzyme that helps in nitrogen fixation in lining cells.

   .....

5. Which gas prevents nitrogen fixation?

   .....

## 10.3 NITROGEN FIXATION BY FREE LIVING ORGANISMS AND SYMBIOTIC NITROGEN FIXATION

Nitrogen fixation is a distinctive property possessed by a select group of organisms, because of the presence of the enzyme nitrogenase in them.

The process of nitrogen fixation is primarily confined to microbial cells like bacteria and cyanobacteria. These microorganisms may be independent and free living (Table 10.1).

**Table 10.1 :** Some free living microbes which fix nitrogen

| Organisms | Status |
|---|---|
| *Clostridium* | Anaerobic bacteria (Non photosynthetic) |
| *Klebsiella* | Facultative bacteria (Non photosynthetic) |
| *Azotobacter* | Aerobic bacteria (Non photosynthetic) |
| *Rhodospirillum* | Purple, non-sulphur bacteria (Photosynthetic) |
| *Anabaena* | Cyanobacteria (Photosynthetic) |

Some microbes may become associated with other oragnisms and fix nitrogen. The host organism may be a lower plant or higher plant. The host organism and the

nitrogen fixing microbes establish a special relationship called **symbiosis** and this results in symbiotic nitrogen fixation (Table 10.2).

**Table 10.2 :** Some symbiotic nitrogen fixing organisms

| System | Symbionts |
|---|---|
| Lichens | Cyanobacteria and Fungus. |
| Bryophyte | Cyanobacteria and *Anthoceros.* |
| Pteridophyte | Cyanobacteria and *Azolla.* |
| Gymnosperm | Cyanobacteria and *Cycas.* |
| Angiosperms | Legumes and *Rhizobium.* |
| Angiosperms | Non leguminous and actinomycete (Such as *Alnus*, *Myrica*, *Purshia*). |
| Angiosperm | Brazilian grass (*Digitaria*), Corn and *Azospirillum.* |

### 10.3.1 Mechanism of Biological Fixation of Nitrogen

Nitrogen fixation requires

(i) the molecular nitrogen –

(ii) a strong reducing power to reduce nitrogen like FAD (Flavin adenine dinucleotide)

(iii) a source of energy (ATP) to transfer hydrogen atoms to dinitrogen and

(iv) enzyme nitrogenase

(v) compound for trapping the ammonia formed since it is toxic to cells.

The reducing agent and ATP are provided by photosynthesis and respiration.

The overall **biochemical process** involves stepwise reduction of nitrogen to ammonia. The enzyme nitrogenase is a Mo-Fe containing protein and binds with molecule of nitrogen ($N_2$) at its binding site. This molecule of nitrogen is then acted upon by hydrogen (from the reduced coenzymes) and reduced in a stepwise manner. It first produces dimide ($N_2H_2$) then hydrazime ($N_2H_4$) and finally ammonia ($2NH_3$).

$NH_3$ is not liberated by the nitrogen fixers. It is toxic to the cells and therefore these fixers combine $NH_3$ with organic acids in the cell and form amino acids.

The general equation for nitrogen fixation may be described as follows:

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

Molecular nitrogen is a very stable molecule. Therefore, sufficeient amount of cell energy in the form of ATP is required for stepwise reduction of nitrogen to ammonia.

In legumes, nitrogen fixation occurs in specialized bodies called **nodules**. The nodules develop due to interaction between the bacteria *Rhizobium* and the legume roots (see diagram 6.4c). The biochemical steps for nitrogen fixation are same. However, legume nodules possess special protein called LEGHEMOGLOBIN. The synthesis of leghemoglobin is the result of symbiosis because neither bacteria alone nor legume plant alone possess the protein. Recently it has been shown that a number of host genes are involved to achieve this. In addition to leghemoglobin, a group of proteins called **nodulins** are also synthesized which help in establishing symbiosis and maintaining nodule functioning.

Leghemoglobin is produced as a result of interaction between the bacterium and legume roots. Apparently, Rhizobium gene codes for Heme part and legume root cell gene codes for Globin moiety. Both the coded products together consitute the final protein leghemoglobin.

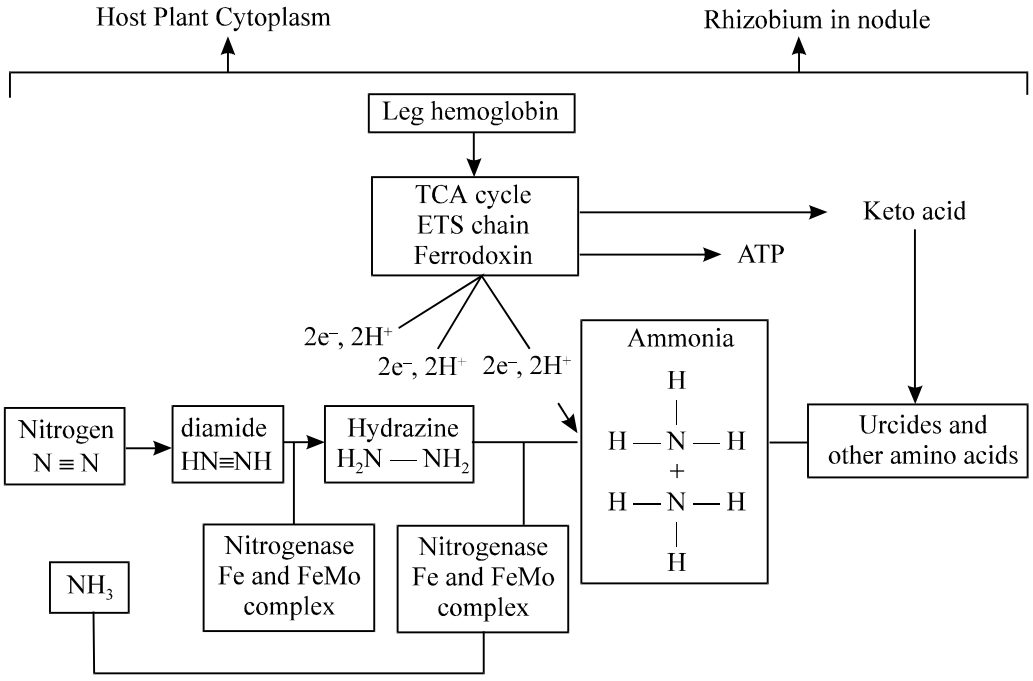


**Fig. 10.1** Simplified flowsheet of biochemical steps for nitrogen fixation

Leghemoglobin is considered to lower down the partial pressure of oxygen and helps in nitrogen fixation. However, this function is specific for legumes only because free living microbes do not possess nitrogen fixing leghemoglobin. Moreover, it has also not been found in cyanobacterial symbiosis with other plants.

## INTEXT QUESTIONS 10.3

1. Match the following:

| | A | B |
|---|---|---|
| (i) | *Azotobacter* | (a) anaerobic nitrogen fixer. |
| (ii) | *Clostridium* | (b) aerobic nitrogen fixer |
| (iii) | *Lichens* | (c) nitrogen fixing cyanobacterium |
| (iv) | *Anabaena* | (d) symbiotic nitrogen fixer. |

2. Which Gymnospermous plant fixes nitrogen?

   .....

3. Is there any other gas evovled during nitrogen fixation? If yes, name the gas evolved.

   .....

4. How many ATP molecules are required to reduce a single molecule of nitrogen?

   .....

5. What is the major source of electrons for reduction of nitrogen?

   .....

6. Match the following:

| | A | B |
|---|---|---|
| (i) | Leghemoglobin | (a) cyanobacterium |
| (ii) | *Anabaena* | (b) Legumes |
| (iii) | Reductive process | (c) nitrogen fixation |

7. Name the proteins that help in establishing symbiosis and maintain nodule functioning.

   .....

## 10.4 NITRATE AND AMMONIA ASSIMILATION BY PLANTS

As pointed in the previous section, nitrogen fixation is confined to selected microbes and plants. But all plants do require nitrogen because it has a role to play in the general metabolism. Therefore, plants which do not fix nitrogen, use other combined nitrogen sources such as nitrate and ammonia for carrying on metabolic activity.

Nitrate is absorbed by most plants and reduced to ammonia with the help of two different enzymes. The first step conversion of nitrate to nitrite is catalyzed by an enzyme called nitrate reductase. This enzyme has several other important constituents including FAD, cytochrome, NADPH or NADH and molybdenum.

$$NO_3^- + NADH + H^+ \xrightarrow{\text{Nitrate reductase}} NO_2^- + NAD^+ + H_2O$$

The overall process of nitrate reduction take place in the cytosol and is an energy dependent reaction.

The enzyme nitrate reductase has been studied in many plants and it is observed that the enzyme is continuously and synthesized and degraded. The enzyme nitrate reductase is inducible. This means that increase in nitrate concentration in the cytosol induces more of nitrate redutase to be synthesized. However, when excess $NH_4^+$ is produced then it has a negative effect on the synthesis of nitrate reductase. In plants, it has also been observed that light also increase nitrate reductase when nitrate is available.

In the second step the nitrite so formed is further reduced to ammonia and this is catalyzed by the enzyme nitrite reductase. Nitrite present in the cytosol is transported into chloroplast or plastids where it is reduced to ammonia.

$$NO_2^- + 3NADPH + 3H^+ \xrightarrow{\text{Nitrite reductase}} NH_3 + 3NADP^+ + H_2O + OH^-$$

The enzyme nitrite reductase is able to accept electrons from sources such as NADH, NADPH or $FADH_2$. Besides, reduced ferredoxin has also been shown to provide electrons to nitrite reductase for reducing nitrite to ammonia. Ammonia so formed has to be utilized quickly by plants because accumulation of ammonia has a toxic effect. Some plants including algae leach out excess ammonia which can further be oxidized to nitrite and nitrate by microorganisms in the soil or water.

## INTEXT QUESTIONS 10.4

1. Which is the most reduced form of inorganic nitrogen?

   .....

2. Match the following:

| | A | | B |
|---|---|---|---|
| (*i*) | Nitrate reductase | (a) | nitrogen fixation |
| (*ii*) | Nitrite reductase | b) | nitrate reduction |
| (*iii*) | Nitrogenase | c) | nitrite reduction |

3. In which pat of the cell, reduction of nitrate to nitrite occurs?

   .....

4 Which is the most oxidized form of inorganic nitrogen?

   .....

5 In which plant organelle reduction of nitrite to ammonia is catlyzed by the enzyme?

   .....

## 10.5 AMINO ACID SYSTHESIS BY PLANTS

As you have noticed that ammonia formation is achieved by plants either by (i.) nitrogen fixation or (ii) by reduction of nitrate to nitrite. Ammonium ($NH_4^+$) is the

most reduced form of inorganic combined nitrogen. This ammonium now becomes the major source for the production of amino acids, which are the building blocks of enzymes and proteins. Amino acids have two important chemical groups. (i) amino group (NH) and (ii) carboxy1 group (-COOH).

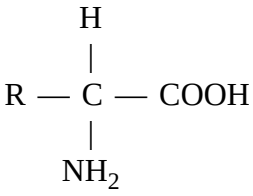

**Fig. 10.2** A typical amino acid with functional groups. R represents alkyl group.

Ammonium so produced is the major source of amino group. However, the carboxyl group has to be provided by other organic molecule synthesized by the plants. There are two major reactions for amino acid biosynthesis in plants:

### 10.5.1 Reductive amination reaction:

In this reaction, ammonia combines with a keto acid. The most important keto acid is the alpha ketoglutaric acid produced during the operation of Krebs cycle (see lesson 12 Plant Respiration). The keto acid then undergoes enzymatic reductive amination to produce an amino acid.

$$\underset{\text{(keto acid)}}{\alpha\text{-ketoglutaric acid}} + NH_3 \xrightarrow{\text{glutamate dehydrogenase}} \underset{\text{(amino acid)}}{\text{Glutamic acid}}$$

Similarly another amino acid called aspartic acid is produced by reductive amination of oxaloacetic acid.

> It has been noted that reductive amination respresents the major 'port of entry' for ammonia into the metabolic stream in plants. This initiates synthesis of glutamic acid followed by other amino acids.

### 10.5.2 Transamination reaction

This is another very important reaction for amino acid biosynthesis. The reaction involves transfer of amino group, from already synthesized amino acid, to the keto acid.

$$\underset{\text{(Keto acid)}}{\alpha\text{-Ketoglutaric acid}} + \underset{\text{(Amino acid)}}{\text{Aspartic acid}} \xrightarrow{\text{Transaminase}} \underset{\text{(Amino acid)}}{\text{Glutamic acid}} + \underset{\text{(Keto acid)}}{\text{Oxaloacetic acid}}$$

In the above reaction, aspartic acid has transferred its amino group ($NH_2$) to the α-ketoglutaric acid to synthesize glutamic acid and release keto acid. The reaction is catalyzed by enzymes called **transaminases**. A large number of amino acids are synthesized by this transamination reaction. Amino acids are organic molecule containing nitorgen. The incorporation of amino group, from ammonium, into keto acids represents the major step for synthesis of nitrogenous organic biomolecules.

## INTEXT QUESTIONS 10.5

1. Match the following:

| | A | B |
|---|---|---|
| (i) | Amino acid | (a) keto acid |
| (ii) | Glutamic acid | (b) amino group and carboxyl group |
| (iii) | α-ketoglutaric acid | (c) amino acid |

2. Name two biochemical reactions for biosynthesis of amino acids in plants.

   .....

3. Which group of enzymes catalyzes transamination reaction?

   .....

4. What is the source of amino group for amino acid synthesis in reductive amination reaction?

   .....

5. Which keto acid is the source for synthesis of glutamic acid?

   .....

## WHAT YOU HAVE LEARNT

- Nitrogen is an important constituent of several biomolecules such as amino acids, proteins and enzymes.
- Molecules such as vitamins, alkaloids, nucleic acids, pigments and some growth hormones also contain nitrogen.
- Molecular nitrogen is triple bonded and stable.
- Nitrogen fixation is the reduction of nitrogen to ammonia.
- Abiological nitrogen fixation is an industrial process (Haber's process)
- Biological nitrogen fixation takes place in a living cell.
- The enzyme that catalyzes nitrogen fixation is Nitrogenase.
- Nitrogen fixation may take place in free living organisms or in symbiotic systems.
- There are many symbiotic nitrogen fixation systems such as Lichens, Pteridophytes, Bryophytes, Gymnosperms and Legumes.
- Cyanobacteria is the symbiotic component in Lichens, Bryophytes, Pteridophytes and Gymnosperms.
- In Legumes, the symbiont is a species of bacterium *Rhizobium*.
- Source of electrons and energy for nitrogen fixation is generally pyruvic acid.

- Hydrogen gas evolution may also accompany nitrogen fixation process.
- Nitrate is the most oxidized form and ammonium is the most reduced form of nitrogen.
- Nitrate is reduced to nitrite by an enzyme nitrate reductase.
- Amino acids have two functional groups including amino group and carboxyl group.
- Amino acids may be produced by reductive amination of keto acids.
- Amino acids may be produced by transamination reaction.
- Reductive amination reactions are catalyzed by dehydrogenase..
- Transamination reactions are catalyzed by transaminases.

## TERMINAL EXERCISES

1. Define nitrogen fixation.
2. Which form of combined nitrogen may be formed during lightening storms?
3. Name three biomolecules other than enzymes and proteins, which contain nitrogen.
4. Name one aerobic and one anaerobic bacterium, which fixes nitrogen.
5. Which amino acid is synthesized due to reductive amination of α-ketoglutaric acid?
6. Differentiate between biological and abiological nitrogen fixation.
7. What is required for biological nitrogen fixation?
8. How does human hemoglobin differ from leghemoglobin?
9. What is the function of leghemoglobin?
10. What are the functional differences between nitrate reductase and nitrite reductase?
11. What is the difference between nitrogen fixation and nitrogen assimilation? Describe in brief the process of abiological nitrogen fixation.
12. Describe in brief various steps involved in biological nitrogen fixation.
13. Enumerate various free living and symbiotic nitrogen fixing system with suitable examples.
14. What are the major differences between free living and leguminous nitrogen fixing organisms?
15. Describe in brief nitrate and nitrite reduction in plants..
17. Describe in brief the reductive amination reactions for synthesis of amino acids in plants.
18. Describe the transamination reaction for synthesis of amino acids in plants. How does this differ from reductive amination?

## ANSWER TO INTEXT QUESTIONS

**10.1** 1. 78.03 percent 2. proteins and enzymes.
3. Because it is triple bonded. 4. 16 percent.
5. –195.8°C.

**10.2** 1. Conversion of molecular nitrogen to ammonia.
2. Haber's process.
3. Biological nitrogen fixation takes place in a living cell and abiological fixation without a living cell.
4. Nitrogenase. 5. Oxygen.

**10.3** 1. (i) b (ii) a (iii) d (iv) c
2. Cycas. 3. Yes, Hydrogen gas.
4. 16 ATP 5. Reduced coenzymes such as Ferrodoxin
6. (i) b (ii) a (iii) c 7. Nodulins.

**10.4** 1. NH
2. (i) b (ii) c (iii) a
3. Cytosol. 4. Nitrate. 5. Chloroplast.

**10.5** 1. (i) b (ii) c (iii) a
2. Reductive amination and transamination.
3. Transaminases. 4. Ammonia.
5. Alpha ketoglutaric acid.

CHAPTER 8

# CELL — STRUCTURE AND FUNCTIONS

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You have already learnt that things around us are either living or non-living. Further, you may recall that all living organisms carry out certain basic functions. Can you list these functions?

Different sets of organs perform the various functions you have listed. In this chapter, you shall learn about the basic structural unit of an organ, which is the **cell.** Cells may be compared to bricks. Bricks are assembled to make a building. Similarly, cells are assembled to make the body of every organism.

## 8.1 Discovery of the Cell

Robert Hooke in 1665 observed slices of cork under a simple magnifying device. Cork is a part of the bark of a tree. He took thin slices of cork and observed them under a microscope. He noticed partitioned boxes or compartments in the cork slice (Fig. 8.1).

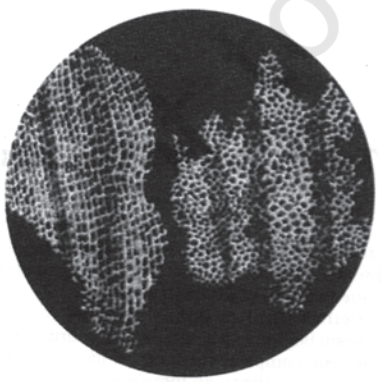


***Fig. 8.1:*** *Cork cells as observed by Robert Hooke*

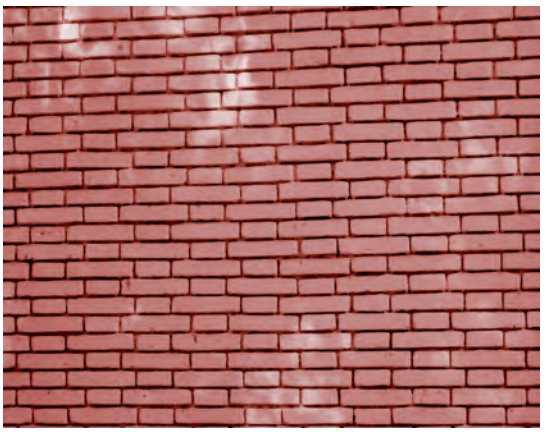
These boxes appeared like a honeycomb. He also noticed that one box was separated from the other by a wall or partition. Hooke coined the term 'cell' for each box. What Hooke observed as boxes or cells in the cork were actually dead cells.

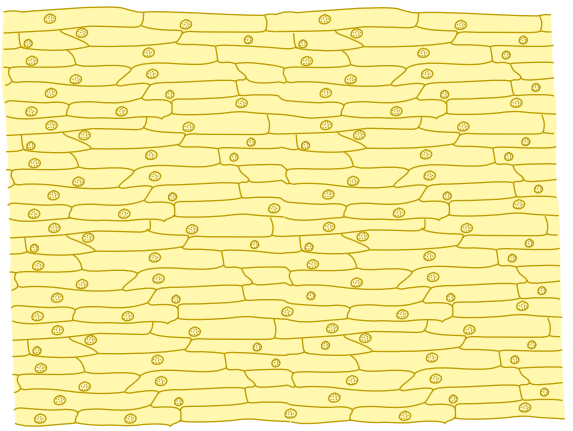
Cells of living organisms could be observed only after the discovery of improved microscopes. Very little was known about the cell for the next 150 years after Robert Hooke's observations. Today, we know a lot about cell structure and its functions because of improved microscopes having high magnification.

## 8.2 The Cell

Both, bricks in a building and cells in the living organisms, are **basic structural units** [Fig. 8.2(a), (b)]**.** The buildings, though built of similar bricks, have different designs, shapes and sizes. Similarly, in the living world, organisms differ from one another but all are made up of cells. Cells in the living organisms are complex living structures unlike non-living bricks.

A hen's egg can be seen easily. Is it a cell or a group of cells?





***(a)*** *Brick wall* ***(b)*** *Onion peel*

***Fig. 8.2 :*** *Brick wall and onion peel*

The egg of a hen represents a single cell and is big enough to be seen by the unaided eye.

## 8.3 Organisms show Variety in Cell Number, Shape and Size

How do scientists observe and study the living cells? They use microscopes which magnify objects. Stains (dyes) are used to colour parts of the cell to study the detailed structure.

There are millions of living organisms. They are of different shapes and sizes. Their organs also vary in shape, size and number of cells. Let us study about some of them.

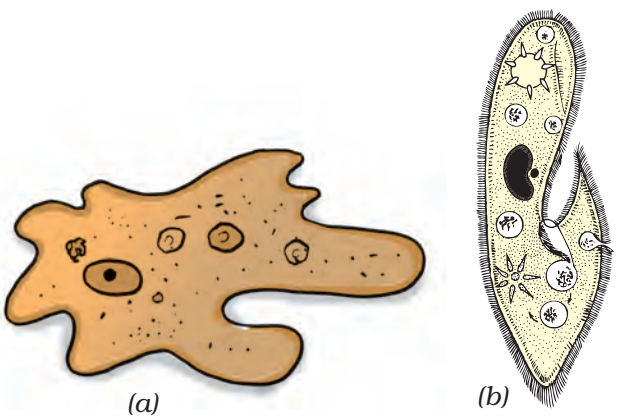
### Number of Cells

Can you guess the number of cells in a tall tree or in a huge animal like the elephant? The number runs into billions and trillions. Human body has trillions of cells which vary in shapes and sizes. Different groups of cells perform a variety of functions.

> A billion is a thousand million. A trillion is a thousand billion.

Organisms made of more than one cell are called **multicellular** (*multi* : many; *cellular* : cell) organisms. The number of cells being less in smaller organisms does not, in any way, affect the functioning of the organisms. You will be surprised to know that an organism with billions of cells begins life as a **single cell** which is the fertilised egg. The fertilised egg cell multiplies and the number of cells increase as development proceeds.

Look at Fig 8.3 (a) and (b). Both organisms are made up of a single cell. The single-celled organisms are called **unicellular** (*uni* : one; *cellular* : cell)



*(a)* *(b)*

***Fig. 8.3 : (a)*** *Amoeba* ***(b)*** *Paramecium*

organisms. A single-celled organism performs all the necessary functions that multicellular organisms perform.

A single-celled organism, like *amoeba,* captures and digests food, respires, excretes, grows and reproduces. Similar functions in multicellular organisms are carried out by groups of specialised cells forming different tissues. Tissues, in turn, form organs.

## Activity 8.1

The teacher may show a permanent slide of *amoeba* and *paramecium* under a microscope. Alternatively, the teacher can collect pond water and show these organisms by preparing the slides.

### Shape of Cells

Refer to Fig. 8.3 (a). How do you define the shape of *amoeba* in the figure? You may say that the shape appears irregular. Infact, *amoeba* has no definite shape, unlike other organisms. It keeps on changing its shape. Observe the projections of varying lengths protruding out of its body. These are called **pseudopodia** (*pseudo* : false; *podia* : feet), as you learnt in Class VII. These projections appear and disappear as *amoeba* moves or feeds.

What advantage does *amoeba* derive by changing shape?

The change in shape is due to formation of pseudopodia which facilitates movement and help in capturing food.

A white blood cell (WBC) in human blood is another example of a single cell which can change its shape. But while WBC is a cell, *amoeba* is a full fledged organism capable of independent existence.

What shape would you expect in organisms with millions of cells? Fig. 8.4 (a, b, c) shows different cells such as blood, muscle and nerve of human beings. The different shapes are related to their specific functions.

Generally, cells are round, spherical or elongated [Fig. 8.4(a)]. Some cells are long and pointed at both ends. They exhibit a spindle shape [Fig. 8.4(b)]. Cells sometimes are quite long. Some are branched like the nerve cell or a neuron [Fig. 8.4(c)]. The nerve cell receives and transfers messages, thereby

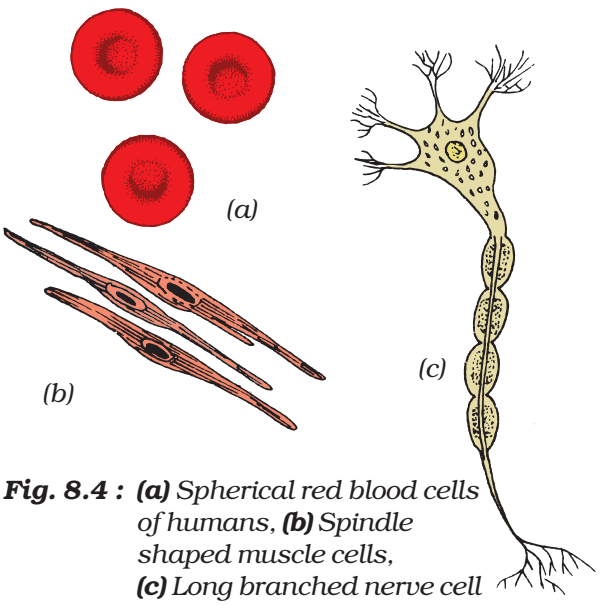



***Fig. 8.4 :*** ***(a)*** *Spherical red blood cells of humans,* ***(b)*** *Spindle shaped muscle cells,* ***(c)*** *Long branched nerve cell*

helping to control and coordinate the working of different parts of the body.

Can you guess, which part of the cell gives it shape? Components of the cell are enclosed in a membrane. This membrane provides shape to the cells of plants and animals. Cell wall is an additional covering over the cell membrane in plant cells. It gives shape and rigidity to these cells (Fig. 8.7). Bacterial cell also has a cell wall.

### Size of Cells

The size of cells in living organisms may be as small as a millionth of a metre (micrometre or micron) or may be as large as a few centimetres. However, most of the cells are microscopic in size and are not visible to the unaided eye. They need to be enlarged or magnified by a microscope. The smallest cell is 0.1 to 0.5 micrometre in bacteria. The largest cell measuring 170 mm ×130 mm, is the egg of an ostrich.

### Activity 8.2

Boil a hen's egg. Remove the shell. What do you observe? A white material surrounds the yellow part. White material is albumin which solidifies on boiling. The yellow part is yolk. It is part of the single cell. You can observe this single cell without any magnifying device.

Are the cells in an elephant larger than the cells in a rat?

The size of the cells has no relation with the size of the body of the animal or plant. It is not necessary that the cells in the elephant be much bigger than those in a rat. The size of the cell is related to its function. For example, nerve cells, both in the elephant and rat, are long and branched. They perform the same function, that of transferring messages.

## 8.4 Cell Structure and Function

You have learnt that each living organism has many organs. You have studied in Class VII about the digestive organs which together constitute the digestive system. Each organ in the system performs different functions such as digestion, assimilation and absorption. Similarly, different organs of a plant perform specific/specialised functions. For example, roots help in the absorption of water and minerals. Leaves, as you have learnt in Class VII, are responsible for synthesis of food.

Each organ is further made up of smaller parts called **tissues**. A tissue is a group of similar cells performing a specific function.

Paheli realised that an organ is made up of tissues which in turn, are made up of cells. The cell in a living organism is the basic structural unit.

## 8.5 Parts of the Cell

### Cell Membrane

The basic components of a cell are cell membrane, cytoplasm and nucleus (Fig. 8.7). The cytoplasm and nucleus are enclosed within the cell membrane, also called the plasma membrane. The membrane separates cells from one another and also the cell from the surrounding medium. The plasma membrane is porous and allows the

movement of substances or materials both inward and outward.

## Activity 8.3

In order to observe the basic components of the cell, take an onion bulb. Remove the dry pink coverings (peels). You can easily separate these from the fleshy white layers of the bulb with the help of forceps or even with your hand. You can also break the onion bulb and separate out thin layers. Place a small piece of the thin onion peel in a drop of water on a glass slide. The thin layer can be cut into smaller pieces with the help of a blade or forceps. Add a drop of methylene blue solution to the layer and place a coverslip on it. While placing the coverslip ensure that there are no air bubbles under the coverslip. Observe the slide under the microscope. Draw and label. You may compare it with Fig. 8.5.

The boundary of the onion cell is the **cell membrane** covered by another thick covering called the cell wall. The central dense round body in the centre is called the **nucleus**. The jelly-like substance between the nucleus and the cell membrane is called **cytoplasm.**

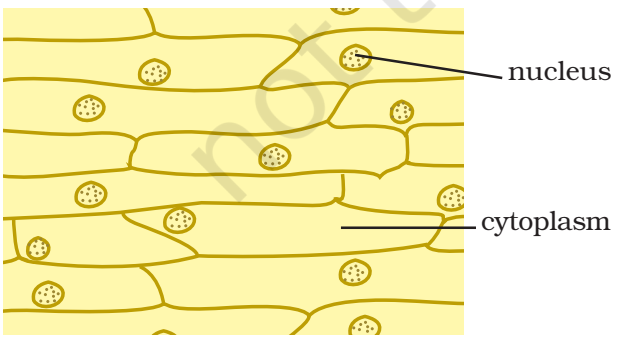



***Fig. 8.5 :*** *Cells observed in an onion peel*

I want to know why plant cells need cell walls?

You have learnt earlier that the cell membrane gives shape to the cell. In addition to the cell membrane, there is an outer thick layer in cells of plants called **cell wall**. This additional layer surrounding the cell membrane is required by plants for protection. Plant cells need protection against variations in temperature, high wind speed, atmospheric moisture etc. They are exposed to these variations because they cannot move. Cells can be observed in the leaf peel of *Tradescantia, Elodea* or *Rhoeo*. You can prepare a slide as in the case of onion.

Paheli asks Boojho if he can also observe animal cells.

## Activity 8.4

Take a clean tooth pick, or a matchstick with the tip broken. Scrape inside of your cheek without hurting it. Place it in a drop of water on a glass slide. Add a drop of iodine and place a coverslip over it. Alternatively, add 1-2 drops of methylene blue solution. Observe it under the microscope. You may notice several cells in the scraped material (Fig. 8.6). You can identify the cell membrane, the cytoplasm and nucleus. A cell wall is absent in animal cells.

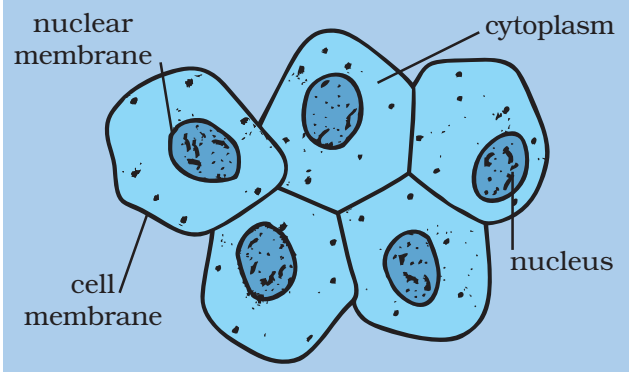



***Fig. 8.6 :*** *Human cheek cells*

## Cytoplasm

It is the jelly-like substance present between the cell membrane and the nucleus. Various other components, or **organelles**, of cells are present in the cytoplasm. These are mitochondria, golgi bodies, ribosomes, etc. You will learn about them in later classes.

## Nucleus

It is an important component of the living cell. It is generally spherical and located in the centre of the cell. It can be stained and seen easily with the help of a microscope. Nucleus is separated from the cytoplasm by a membrane called the **nuclear membrane**. This membrane is also porous and allows the movement of materials between the cytoplasm and the inside of the nucleus.

With a microscope of higher magnification, we can see a smaller spherical body in the nucleus. It is called the **nucleolus**. In addition, nucleus contains thread-like structures called **chromosomes**. These carry **genes** and help in inheritance or transfer of characters from the parents to the offspring. The chromosomes can be seen only when the cell divides.

### Gene

Gene is a unit of inheritance in living organisms. It controls the transfer of a hereditary characteristic from parents to offspring. This means that your parents pass some of their characteristics on to you. If your father has brown eyes, you may also have brown eyes. If your mother has curly hair, you might also end up having curly hair. However, the different combination of genes from parents result in different characteristics.

Nucleus, in addition to its role in inheritance, acts as control centre of the activities of the cell. The entire content of a living cell is known as protoplasm. It includes the cytoplasm and the nucleus. Protoplasm is called the living substance of the cell.

Paheli wants to know if the structure of the nucleus is the same in cells of plants, animals and bacteria.

The nucleus of the bacterial cell is not well-organised like the cells of multicellular organisms. There is no nuclear membrane. The cells having nuclear material without nuclear membrane are termed **prokaryotic cells.** The organisms with these kinds of cells are called **prokaryotes** (*pro* : primitive; *karyon* : nucleus). Examples are bacteria and blue green algae. The cells, like onion cells and cheek cells having well-organised nucleus with a nuclear membrane are designated as **eukaryotic cells**. All organisms other than bacteria and blue green algae are called **eukaryotes**. (*eu* : true; *karyon*: nucleus).

While observing the onion cells under the microscope, did you notice any blank-looking structures in the cytoplasm? It is called **vacuole**. It could be single and big as in an onion cell. Cheek cells have smaller vacuoles. Large vacuoles are common in plant cells. Vacuoles in animal cells are much smaller.

You might have noticed several small coloured bodies in the cytoplasm of the cells of *Tradescantia* leaf. They are scattered in the cytoplasm of the leaf cells. These are called **plastids**. They are of different colours. Some of them contain green pigment called chlorophyll. Green coloured plastids are called **chloroplasts**. They provide green colour to the leaves. You may recall that chlorophyll in the chloroplasts of leaves, is essential for photosynthesis.

## 8.6 Comparison of Plant and Animal Cells

If you recall Activities 8.3 and 8.4, you should be able to compare plant and animal cells. Observe the plant and animal cell carefully in Fig. 8.7 (a), (b).

Let us tabulate the similarities and disinguishing features of plant and animal cells. Only a few features are mentioned. You may add more in Table 8.1.

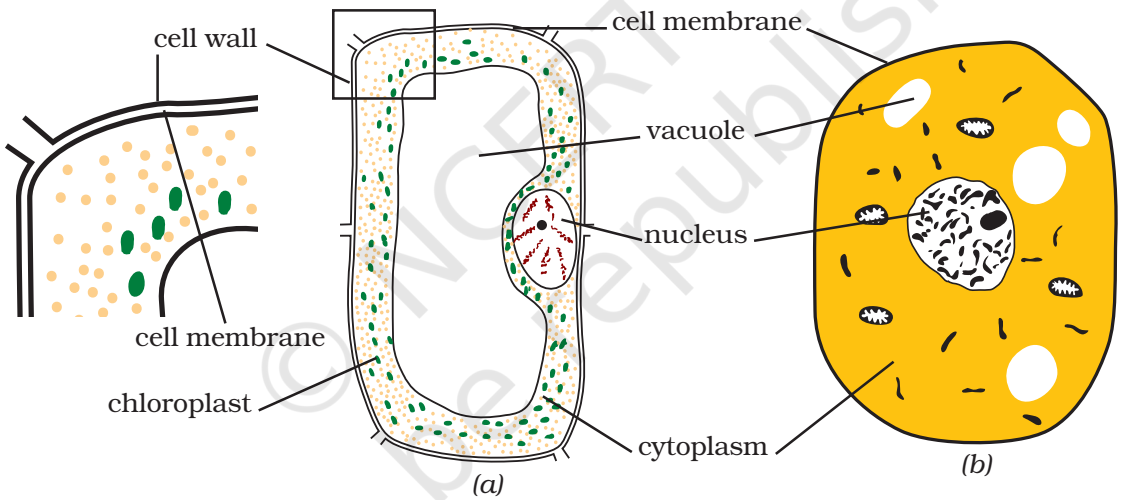



***Fig. 8.7 : (a)*** *Plant cell* ***(b)*** *Animal cell*

**Table 8.1 : Comparison of Plant Cell and Animal Cell**

| Sl. No. | Part | Plant Cell | Animal Cell |
|---|---|---|---|
| 1. | Cell membrane | Present | Present |
| 2. | Cell wall | Present | Absent |
| 3. | Nucleus | | |
| 4. | Nuclear membrane | | |
| 5. | Cytoplasm | | |
| 6. | Plastids | | |
| 7. | Vacuole | | |

## KEYWORDS

- CELL
- CELL MEMBRANE
- CELL WALL
- CHLOROPLAST
- CHROMOSOME
- CYTOPLASM
- EUKARYOTES
- GENE
- MULTICELLULAR
- NUCLEAR MEMBRANE
- NUCLEOLUS
- NUCLEUS
- ORGAN
- ORGANELLES
- PLASMA MEMBRANE
- PLASTID
- PROKARYOTES
- PSEUDOPODIA
- TISSUE
- UNICELLULAR
- VACUOLE
- WHITE BLOOD CELL (WBC)

## WHAT YOU HAVE LEARNT

- All organisms are made of smaller parts called organs.
- Organs are made of still smaller parts. The smallest living part of an organism is a 'cell'.
- Cells were first observed in cork by Robert Hooke in 1665.
- Cells exhibit a variety of shapes and sizes.
- Number of cells also varies from organism to organism.
- Some cells are big enough to be seen with the unaided eye. Hen's egg is an example.
- Some organisms are single-celled, while others contain large number of cells.
- The single cell of unicellular organisms performs all the basic functions performed by a variety of cells in multicellular organisms.
- The cell has three main parts: (i) the cell membrane, (ii) cytoplasm which contains smaller components called organelles, and (iii) the nucleus.
- Nucleus is separated from cytoplasm by a nuclear membrane.
- Cells without well-organised nucleus, i.e. lacking nuclear membrane, are called prokaryotic cells.
- Plant cells differ from animal cells in having an additional layer around the cell membrane termed cell wall.
- Coloured bodies called plastids are found in the plant cells only. Green plastids containing chlorophyll are called chloroplasts.
- Plant cell has a big central vacuole unlike a number of small vacuoles in animal cells.

## Exercises

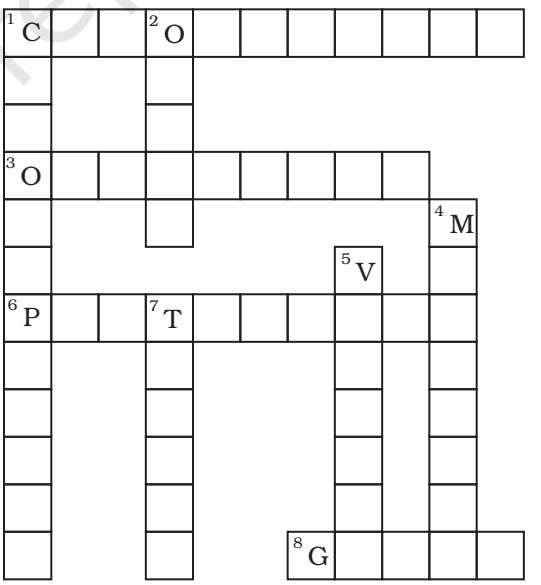
1. Indicate whether the following statements are True (T) or False (F).
   (a) Unicellular organisms have one-celled body. (T/F)
   (b) Muscle cells are branched. (T/F)
   (c) The basic living unit of an organism is an organ. (T/F)
   (d) *Amoeba* has irregular shape. (T/F)
2. Make a sketch of the human nerve cell. What function do nerve cells perform?.
3. Write short notes on the following.
   (a) Cytoplasm
   (b) Nucleus of a cell
4. Which part of the cell contains organelles?
5. Make sketches of animal and plant cells. State three differences between them.
6. State the difference between eukaryotes and prokaryotes.
7. Where are chromosomes found in a cell? State their function.
8. 'Cells are the basic structural units of living organisms'. Explain.
9. Explain why chloroplasts are found only in plant cells?
10. Complete the crossword with the help of clues given below.

**Across**

1. This is necessary for photosynthesis.
3. Term for component present in the cytoplasm.
6. The living substance in the cell.
8. Units of inheritance present on the chromosomes.

**Down**

1. Green plastids.
2. Formed by collection of tissues.
4. It separates the contents of the cell from the surrounding medium.
5. Empty structure in the cytoplasm.
7. A group of cells.



## Extended Learning — Activities and Projects

1. Visit a laboratory for senior secondary students in your school or in a neighbouring school. Learn about the functioning of a microscope in the laboratory. Also observe how a slide is observed under the microscope.
2. Talk to the senior biology teacher in your school or a neighbouring school. Find out if there are diseases which are passed on from parents to the offspring. Find out how these are carried and also if these diseases can be treated. For this you can also visit a doctor.
3. Visit an agriculture extension centre in your area. Find out about genetically modified (GM) crops. Prepare a short speech for your class on this topic.
4. Find out about *Bt* cotton from an agriculture expert. Prepare a short note on its advantages/disadvantages.

## Did You Know?

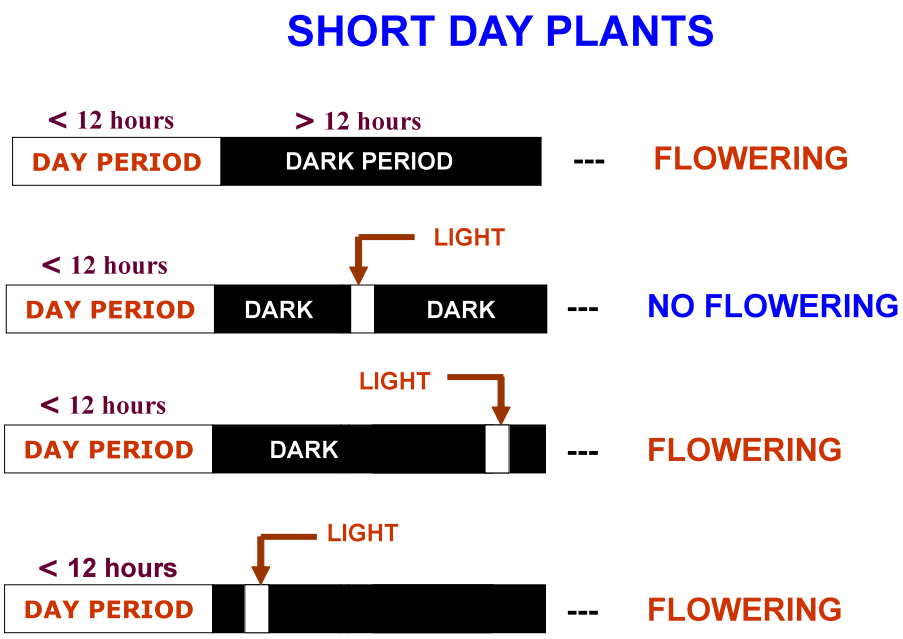
The cells in the outermost layer of our skin are dead. An average adult carries around about 2 kg of dead skin. Billions of tiny fragments of the skin are lost every day. Every time you run your finger on a dusty table, you shed a lot of old skin.

# 15. **PHOTOPERIODISM**

Photoperiodism is the phenomenon of physiological changes that occur in plants in response to relative length of day and night (i.e. photoperiod). The response of the plants to the photoperiod, expressed in the form of flowering is also called as photoperiodism. The phenomenon of photoperiodism was first discovered by Garner and Allard (1920).Depending upon the duration of photoperiod, the plants are classified into three categories.

1. Short day plants (SDP)
2. Long day plants (LDP)
3. Day neutral plants (DNP)

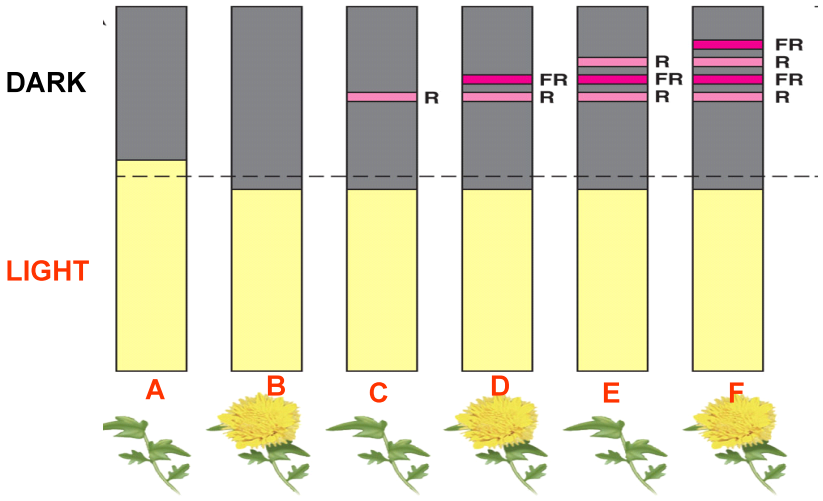
***1. Short day plants***




These plants require a relatively short day light period (usually 8-10 hours) and a continuous dark period of about 14-16 hours for subsequent flowering. These plants are also known as long-night plants

E.g. Rice, coffee, soybean, tobacco and chrysanthemum

- In short day plants, the dark period is critical and must be continuous. If this dark period is interrupted with a brief exposure of red light (660-665 nm wavelength), the short day plant will not flower.
- Maximum inhibition of flowering with red light occurs at about the middle of critical dark period.
- However, the inhibitory effect of red light can be overcome by a subsequent exposure with far-red light (730-735 mm wavelength)
- Interruption of the light period with red light does not have inhibitory effect on flowering in short day plants.
- Prolongation of the continuous dark period initiates early flowering.



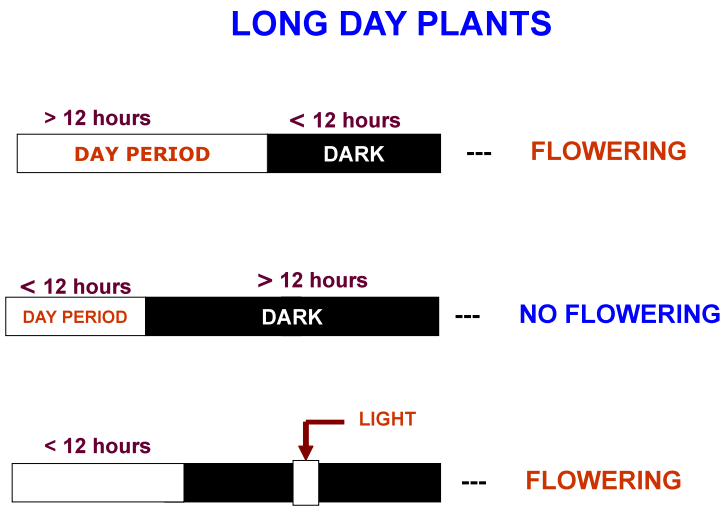

SHORT DAY PLANTS - FLOWERING

***2. Long day plants***

These plants require longer day light period (usually 14-16 hours) in a 24 hours cycle for subsequent flowering. These plants are also called as short night plants.

E.g. Wheat, radish, cabbage, sugar beet and spinach.

- In long day plants, light period is critical
- A brief exposure of red light in the dark period or the prolongation of light period stimulates flowering in long day plants.




***3. Day neutral plants***

These plants flower in all photoperiod ranging from 5 hours to 24 hours continuous exposure.

E.g. Tomato, cotton, sunflower, cucumber, peas and certain varieties of tobacco.

During recent years, intermediate categories of plants such as *long short day plants* and *short long day plants* have also been recognized.

*i. Long short day plants*

These are short day plants but must be exposed to long days during early periods of growth for subsequent flowering. E.g. Bryophyllum.

*ii. Short –long day plants*

These are long day plants but must be exposed to short day during early periods of growth for subsequent flowering. E.g. certain varieties of wheat and rye.

**Differences between short day and long day plants**

| | Short day plant | Long day plant |
|---|---|---|
| 1 | Plants flower when photoperiod is less than the critical day length | Plants flower when photoperiod is more than the critical day length |
| 2 | Interruption during light period with darkness does not inhibit flowering | Interruption during light period with darkness inhibit flowering |
| 3 | Flowering is inhibited if the long dark period is interrupted by a flash of light | Flowering occurs if the long dark period is interrupted by a flash of light |
| 4 | Long continuous and uninterrupted dark period is critical for flowering | Dark period is not critical for flowering |
| 5 | Flowering does not occur under alternating cycles of short day and short light period. | Flowering occurs under alternating cycles of short day followed by still shorter dark periods |

**Phytochrome**

It is observed that that a brief exposure with red light during critical dark period inhibits flowering in a short day plant and this inhibitory effect can be reversed by a subsequent exposure with far-red light. Similarly, prolongation of the critical light period or the interruption of the dark period stimulates flowering in long-day plants.

This inhibition of flowering in short day plant and stimulation of flowering in long day plants involves the operation of a proteinaceous pigment called *phytochrome*. It is present in the plasma membrane of cells and it has two components, chromophore and protein. Phytochrome is present in roots, coleoptiles, stems, hypocotyls, cotyledons, petioles, leaf blades, vegetative buds, flower tissues, seeds and developing fruits of higher plants.

The pigment, phytochrome exists in two different forms i.e., red light absorbing form which is designated as *Pr* and far red light absorbing form which is designated as *Pfr*. These two forms of the pigment are photo chemically inter convertible. When *Pr* form of the pigment absorbs red light (660-665 nm), it is converted into *Pfr* form. When *Pfr* form of the pigment absorbs far red light (730-735 nm), it is converted into *Pr* form. The *Pfr* form of pigment gradually changes into *Pr* form in dark.

It is considered that during day time, the *Pfr* form of the pigment is accumulated in the plants which are inhibitory to flowering in short day plants but is stimulatory in long day plants. During critical dark period in short day plants, this form gradually changes into *Pr* form resulting in flowering. A brief exposure with red light will convert this form again into *Pfr* form thus inhibiting flowering.

Reversal of the inhibitory effect of red light during critical dark period in SDP by subsequent far-red light exposure is because, the *Pfr* form after absorbing far-red light (730-354 nm) will again be converted back into *Pr* form.

Prolongation of critical light period or the interruption of the dark period by red- light in long day plants will result in further accumulation of the *Pfr* form of the pigment, thus stimulating flowering in long-day plants.

**Differences between Pr and Pfr forms of phytochrome**

| | **Pr form** | **Pfr form** |
|---|---|---|
| 1 | It is blue green in colour | It is light green in colour |
| 2 | It is an inactive form of phytochrome and it does not show phytochrome mediated responses | It is an active form of phytochrome and hence shows phytochrome mediated responses |
| 3 | It has maximum absorption in red region (about 680nm) | It has maximum absorption in far-red region (about 730nm) |
| 4 | It can be converted into Pfr form in red region (660-665nm) | It can be converted into Pr form in far red region (730-735nm) |
| 5 | It is found diffused throughout the cytosol | It is found in discrete areas of cytosol |
| 6 | The Pr form contains many double bonds in pyrrole rings | The Pfr form contains rearranged double bonds in all pyrrole rings |

**Significance of photoperiodism**

Photoperiodism is an example for *physiological preconditioning*. The stimulus is given at one time and the response is observed after months. Exposure to longer photoperiods hastens flowering (E.g). In wheat, the earing is hastened. During long light exposure, *Pr* form

is converted into *Pfr* form and flowering is initiated. If dark period is greater, *Pfr* is converted into *Pfr* form that inhibits flowering.

The important phytochrome mediated photo responses in plants include photoperiodism, seed germination, sex expression, bud dormancy, rhizome formation, leaf abscission, epinasty, flower induction, protein synthesis, pigment synthesis, auxin catabolism, respiration and stomatal differentiation.

# 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).

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.

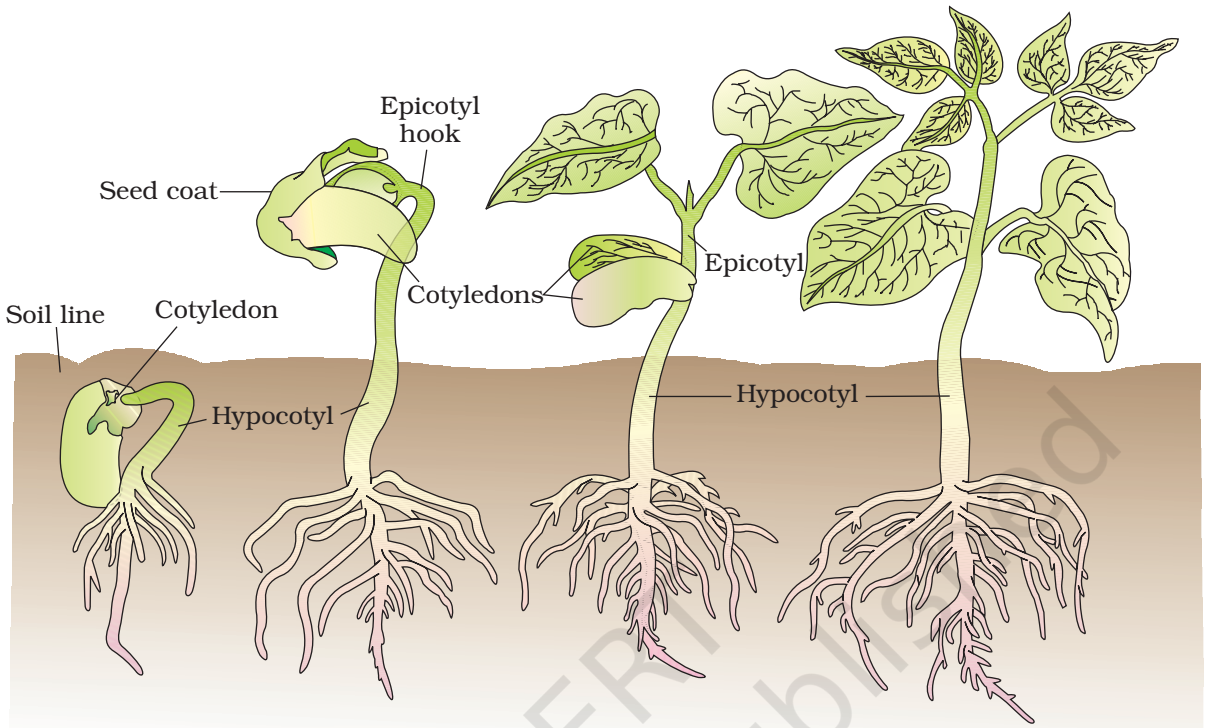



**Figure 15.1** Germination and seedling development in bean

## 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.

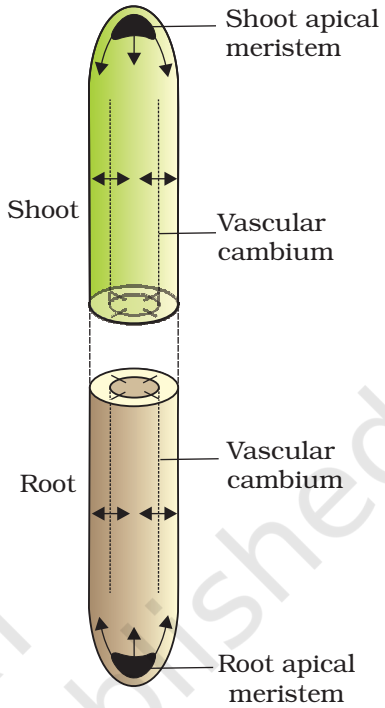



**Figure 15.2** Diagrammatic representation of locations of root apical meristem, shoot aplical meristem and vascular cambium. Arrows exhibit the direction of growth of cells and organ

### 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

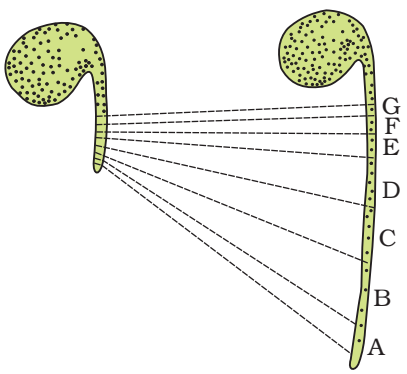



**Figure 15.3** Detection of zones of elongation by the parallel line technique. Zones A, B, C, D immediately behind the apex have elongated most.

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.

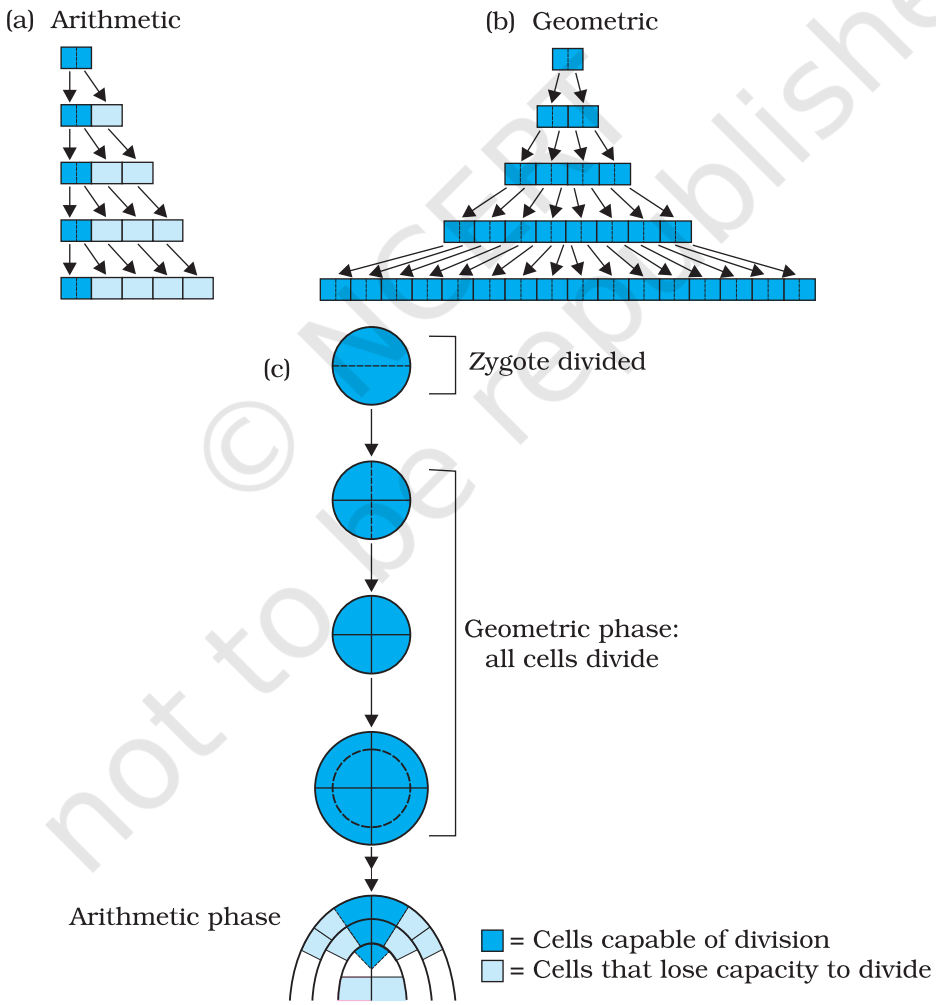



**Figure 15.4** Diagrammatic representation of : (a) Arithmetic (b) Geometric growth and (c) Stages during embryo development showing geometric and arithematic phases

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, $W_0$.

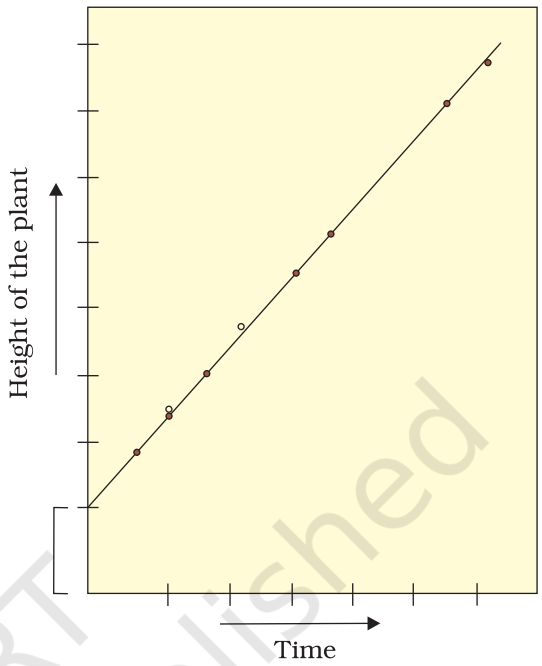



**Figure 15.5** Constant linear growth, a plot of length L against time t

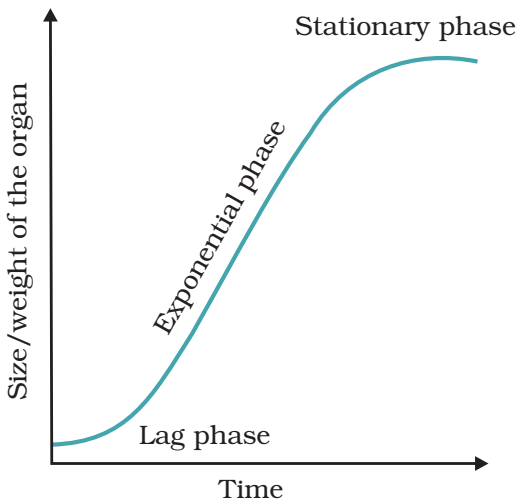



**Figure 15.6** An idealised sigmoid growth curve typical of cells in culture, and many higher plants and plant organs

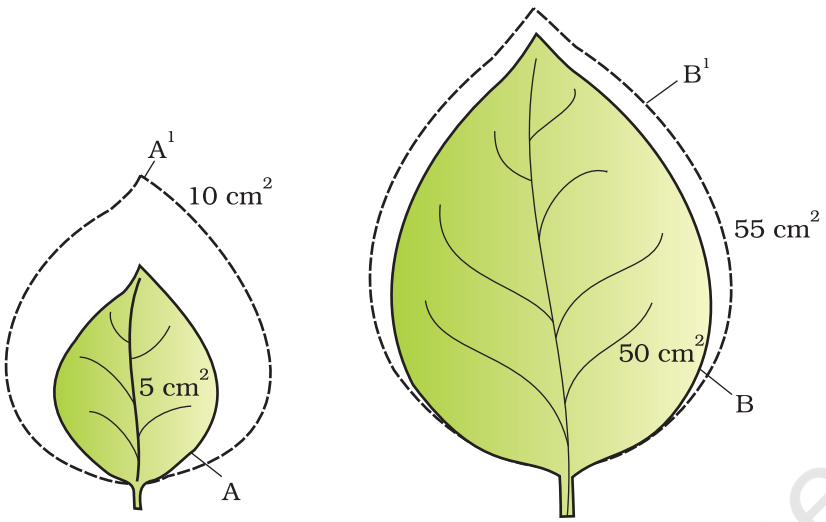



**Figure15.7** Diagrammatic comparison of absolute and relative growth rates. Both leaves A and B have increased their area by 5 $cm^2$ in a given time to produce $A^1$, $B^1$ leaves.

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.

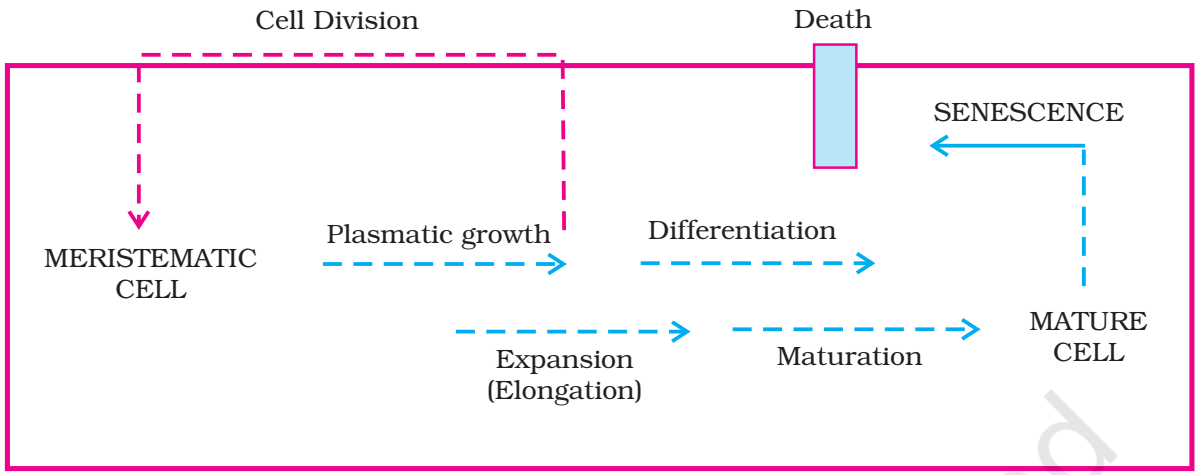



**Figure 15.8** Sequence of the developmental process in a plant cell

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.

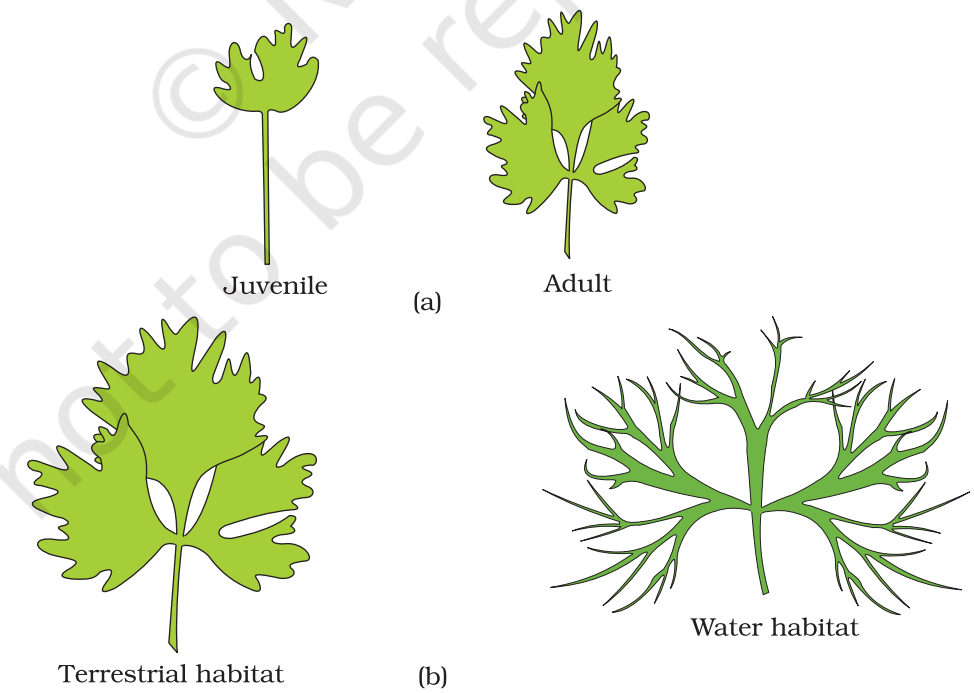



**Figure 15.9** Heterophylly in (a) larkspur and (b) buttercup

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.

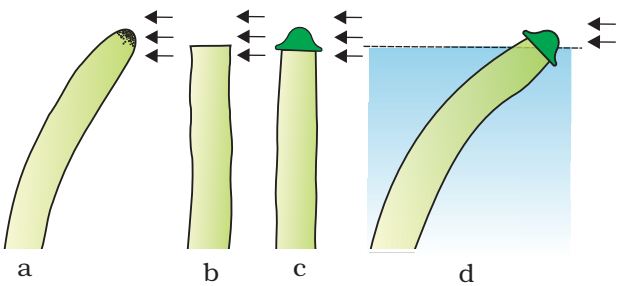



**Figure 15.10** Experiment used to demonstrate that tip of the coleoptile is the source of auxin. Arrows indicate direction of light

The 'bakane' (foolish seedling) disease of rice seedlings, was caused by a fungal pathogen *Gibberella fujikuroi*. E. Kurosawa reported the appearance of symptoms of the disease in uninfected 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.

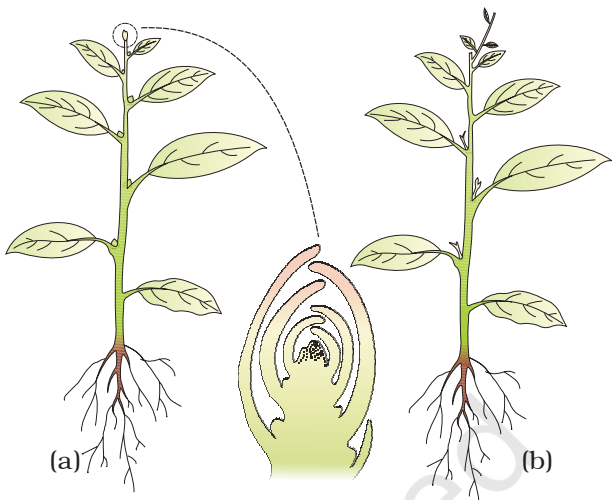



**Figure 15.11** Apical dominance in plants : (a) A plant with apical bud intact (b) A plant with apical bud removed Note the growth of lateral buds into branches after decapitation.

#### *15.4.3.2 Gibberellins*

Gibberellins are another kind of promotery 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

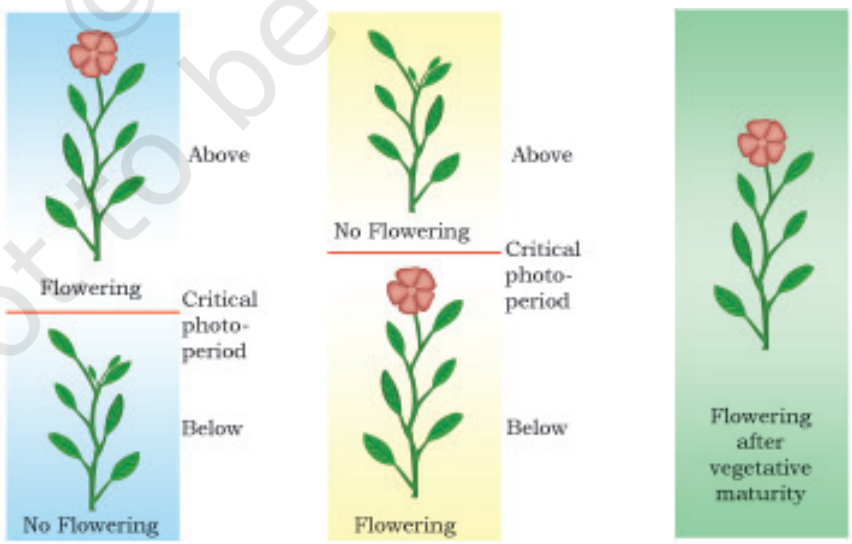



**Figure 15.12** Photoperiodism : Long day, short day and day neutral plants

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.



## 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.

# 10

# NITROGEN METABOLISM

All the living organisms are basically composed of carbon, hydrogen, oxygen, nitrogen and many other forms of chemical elements. These elements contribute to finally organize various biomolecules present in a cell. Nitrogen is next to carbon in importance in living organisms. In a living cell, nitrogen is an important constituent of amino acids, proteins, enzymes, vitamins, alkaloids and some growth hormones. Therefore, study of nitrogen metabolism is absolutely essential because the entire life process is dependent on these nitrogen-containing molecules. In this lesson, you will learn about various aspects of nitrogen metabolism including nitrogen fixation and nitrogen assimilation in plants.

## OBJECTIVES

After completing this lesson, you will be able to:

- *describe the modes of nitrogen fixation (both biological and abiological);*
- *explain the steps involved in nitrogen fixation by free living organisms;*
- *explain the mode of symbiotic nitrogen fixation in leguminous plants;*
- *describe the assimilation of nitrate and ammonia by plants;*
- *describe amino acid synthesis in plants.*

## 10.1 MOLECULAR NITROGEN

Nitrogen is primarily present in the atmosphere freely as dinitrogen or nitrogen gas. It is present in the combined form as Chile saltpetre or sodium nitrate and Chile in South America is the major source of this nitrate nitrogen.

Molecular Nitrogen or diatomic nitrogen ($N_2$) is highly stable as it is triple bonded (N≡N). Because of this stability, molecular nitrogen as such is not very reactive in the atmosphere under normal conditions. In the atmosphere molecular nitrogen is 78.03% by volume and it has a very low boiling point (-195.8$^{o}$C) which is even lower than oxygen. Proteins present in living organisms contain about 16% nitrogen.

## INTEXT QUESTIONS 10.1

1. What is the percent by volume of nitrogen gas in the atmosphere?

   .....

2. Name two biomolecules that contain nitrogen in plants.

   .....

3. Why nitrogen is a stable molecule?

   .....

4. What is the percentage of nitrogen in protein?

   .....

5. What is the boiling point of nitrogen?

   .....

## 10.2 NITROGEN FIXATION (BIOLOGICAL AND ABIOLOGICAL)

The conversion of molecular nitrogen into compounds of nitrogen especially ammonia is called **nitrogen fixation**. Nitrogen fixation, is a reductive process i.e., nitrogen fixation will stop if there is no reducing condition or if oxygen is present. This nitrogen fixation may take place by two different methods – abiological and biological.

### 10.2.1 Abiological nitrogen fixation

In abiological nitrogen fixation the nitrogen is reduced to ammonia without involving any living cell. Abiological fixation can be of two types : industrial and natural. For example, in the Haber's process, synthetic ammonia is produced by passing a mixture of nitrogen and hydrogen through a bed of catalyst (iron oxides) at a very high temperature and pressure.

$$N_2 + 3H_2 \xrightarrow[1000 \text{ atmosphere}]{500^\circ C} 2NH_3$$

This is industrial fixation and nitrogen reduced to ammonia.

In natural process nitrogen can be fixed especially during electrical discharges in the atmosphere. It may occur during lightning storms and nitrogen in the atmosphere can combine with oxygen to form oxides of nitrogen

$$N_2 + O_2 \longrightarrow 2NO \xrightarrow{O_2} 2NO_2$$

These oxides of nitrogen may be hydrated and trickle down to earth as combined nitrite and nitrate.

### 10.2.2 Biological nitrogen fixation

Chemically, this process is same as abiological. Biological nitrogen fixation is reduction of molecular nitrogen to ammonia by a living cell in the presence of an enzyme nitrogenase.

## INTEXT QUESTIONS 10.2

1. Define nitrogen fixation.

   .....

2. Which industrial process is utilized for converting nitrogen to ammonia?

   .....

3. Distinguish between biological and a biological nitrogen fixation.

   .....

4. Name the enzyme that helps in nitrogen fixation in lining cells.

   .....

5. Which gas prevents nitrogen fixation?

   .....

## 10.3 NITROGEN FIXATION BY FREE LIVING ORGANISMS AND SYMBIOTIC NITROGEN FIXATION

Nitrogen fixation is a distinctive property possessed by a select group of organisms, because of the presence of the enzyme nitrogenase in them.

The process of nitrogen fixation is primarily confined to microbial cells like bacteria and cyanobacteria. These microorganisms may be independent and free living (Table 10.1).

**Table 10.1 :** Some free living microbes which fix nitrogen

| Organisms | Status |
|---|---|
| *Clostridium* | Anaerobic bacteria (Non photosynthetic) |
| *Klebsiella* | Facultative bacteria (Non photosynthetic) |
| *Azotobacter* | Aerobic bacteria (Non photosynthetic) |
| *Rhodospirillum* | Purple, non-sulphur bacteria (Photosynthetic) |
| *Anabaena* | Cyanobacteria (Photosynthetic) |

Some microbes may become associated with other oragnisms and fix nitrogen. The host organism may be a lower plant or higher plant. The host organism and the

nitrogen fixing microbes establish a special relationship called **symbiosis** and this results in symbiotic nitrogen fixation (Table 10.2).

**Table 10.2 :** Some symbiotic nitrogen fixing organisms

| System | Symbionts |
|---|---|
| Lichens | Cyanobacteria and Fungus. |
| Bryophyte | Cyanobacteria and *Anthoceros.* |
| Pteridophyte | Cyanobacteria and *Azolla.* |
| Gymnosperm | Cyanobacteria and *Cycas.* |
| Angiosperms | Legumes and *Rhizobium.* |
| Angiosperms | Non leguminous and actinomycete (Such as *Alnus*, *Myrica*, *Purshia*). |
| Angiosperm | Brazilian grass (*Digitaria*), Corn and *Azospirillum.* |

### 10.3.1 Mechanism of Biological Fixation of Nitrogen

Nitrogen fixation requires

(i) the molecular nitrogen –

(ii) a strong reducing power to reduce nitrogen like FAD (Flavin adenine dinucleotide)

(iii) a source of energy (ATP) to transfer hydrogen atoms to dinitrogen and

(iv) enzyme nitrogenase

(v) compound for trapping the ammonia formed since it is toxic to cells.

The reducing agent and ATP are provided by photosynthesis and respiration.

The overall **biochemical process** involves stepwise reduction of nitrogen to ammonia. The enzyme nitrogenase is a Mo-Fe containing protein and binds with molecule of nitrogen ($N_2$) at its binding site. This molecule of nitrogen is then acted upon by hydrogen (from the reduced coenzymes) and reduced in a stepwise manner. It first produces dimide ($N_2H_2$) then hydrazime ($N_2H_4$) and finally ammonia ($2NH_3$).

$NH_3$ is not liberated by the nitrogen fixers. It is toxic to the cells and therefore these fixers combine $NH_3$ with organic acids in the cell and form amino acids.

The general equation for nitrogen fixation may be described as follows:

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

Molecular nitrogen is a very stable molecule. Therefore, sufficeient amount of cell energy in the form of ATP is required for stepwise reduction of nitrogen to ammonia.

In legumes, nitrogen fixation occurs in specialized bodies called **nodules**. The nodules develop due to interaction between the bacteria *Rhizobium* and the legume roots (see diagram 6.4c). The biochemical steps for nitrogen fixation are same. However, legume nodules possess special protein called LEGHEMOGLOBIN. The synthesis of leghemoglobin is the result of symbiosis because neither bacteria alone nor legume plant alone possess the protein. Recently it has been shown that a number of host genes are involved to achieve this. In addition to leghemoglobin, a group of proteins called **nodulins** are also synthesized which help in establishing symbiosis and maintaining nodule functioning.

Leghemoglobin is produced as a result of interaction between the bacterium and legume roots. Apparently, Rhizobium gene codes for Heme part and legume root cell gene codes for Globin moiety. Both the coded products together consitute the final protein leghemoglobin.

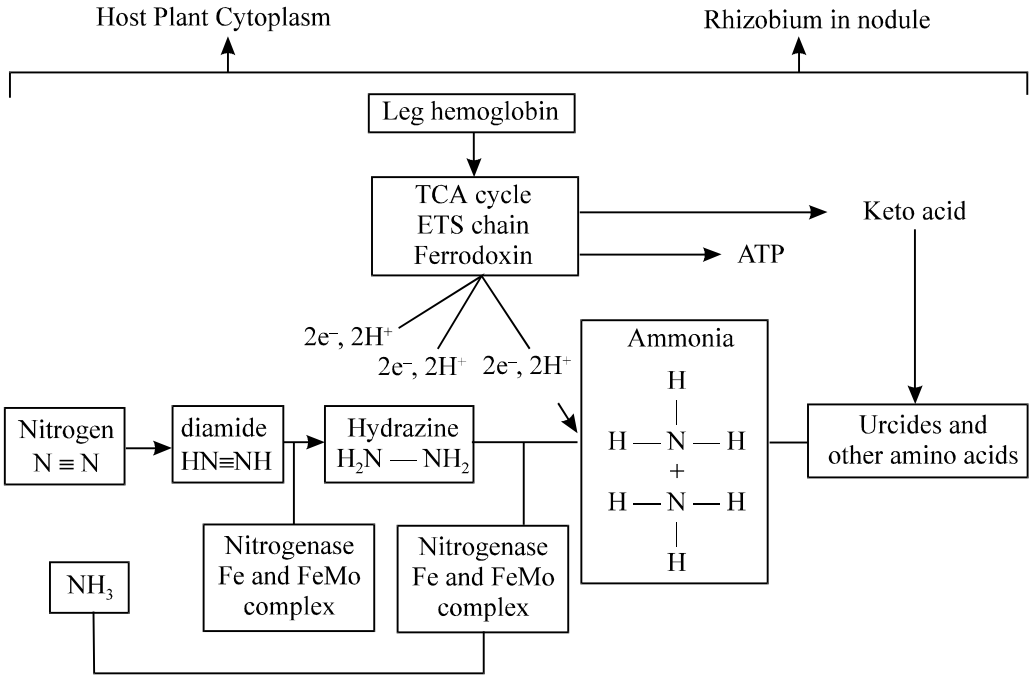



**Fig. 10.1** Simplified flowsheet of biochemical steps for nitrogen fixation

Leghemoglobin is considered to lower down the partial pressure of oxygen and helps in nitrogen fixation. However, this function is specific for legumes only because free living microbes do not possess nitrogen fixing leghemoglobin. Moreover, it has also not been found in cyanobacterial symbiosis with other plants.

## INTEXT QUESTIONS 10.3

1. Match the following:

| | A | B |
|---|---|---|
| (i) | *Azotobacter* | (a) anaerobic nitrogen fixer. |
| (ii) | *Clostridium* | (b) aerobic nitrogen fixer |
| (iii) | *Lichens* | (c) nitrogen fixing cyanobacterium |
| (iv) | *Anabaena* | (d) symbiotic nitrogen fixer. |

2. Which Gymnospermous plant fixes nitrogen?

   .....

3. Is there any other gas evovled during nitrogen fixation? If yes, name the gas evolved.

   .....

4. How many ATP molecules are required to reduce a single molecule of nitrogen?

   .....

5. What is the major source of electrons for reduction of nitrogen?

   .....

6. Match the following:

| | A | B |
|---|---|---|
| (i) | Leghemoglobin | (a) cyanobacterium |
| (ii) | *Anabaena* | (b) Legumes |
| (iii) | Reductive process | (c) nitrogen fixation |

7. Name the proteins that help in establishing symbiosis and maintain nodule functioning.

   .....

## 10.4 NITRATE AND AMMONIA ASSIMILATION BY PLANTS

As pointed in the previous section, nitrogen fixation is confined to selected microbes and plants. But all plants do require nitrogen because it has a role to play in the general metabolism. Therefore, plants which do not fix nitrogen, use other combined nitrogen sources such as nitrate and ammonia for carrying on metabolic activity.

Nitrate is absorbed by most plants and reduced to ammonia with the help of two different enzymes. The first step conversion of nitrate to nitrite is catalyzed by an enzyme called nitrate reductase. This enzyme has several other important constituents including FAD, cytochrome, NADPH or NADH and molybdenum.

$$NO_3^- + NADH + H^+ \xrightarrow{\text{Nitrate reductase}} NO_2^- + NAD^+ + H_2O$$

The overall process of nitrate reduction take place in the cytosol and is an energy dependent reaction.

The enzyme nitrate reductase has been studied in many plants and it is observed that the enzyme is continuously and synthesized and degraded. The enzyme nitrate reductase is inducible. This means that increase in nitrate concentration in the cytosol induces more of nitrate redutase to be synthesized. However, when excess $NH_4^+$ is produced then it has a negative effect on the synthesis of nitrate reductase. In plants, it has also been observed that light also increase nitrate reductase when nitrate is available.

In the second step the nitrite so formed is further reduced to ammonia and this is catalyzed by the enzyme nitrite reductase. Nitrite present in the cytosol is transported into chloroplast or plastids where it is reduced to ammonia.

$$NO_2^- + 3NADPH + 3H^+ \xrightarrow{\text{Nitrite reductase}} NH_3 + 3NADP^+ + H_2O + OH^-$$

The enzyme nitrite reductase is able to accept electrons from sources such as NADH, NADPH or $FADH_2$. Besides, reduced ferredoxin has also been shown to provide electrons to nitrite reductase for reducing nitrite to ammonia. Ammonia so formed has to be utilized quickly by plants because accumulation of ammonia has a toxic effect. Some plants including algae leach out excess ammonia which can further be oxidized to nitrite and nitrate by microorganisms in the soil or water.

## INTEXT QUESTIONS 10.4

1. Which is the most reduced form of inorganic nitrogen?

   .....

2. Match the following:

| | A | | B |
|---|---|---|---|
| (*i*) | Nitrate reductase | (a) | nitrogen fixation |
| (*ii*) | Nitrite reductase | b) | nitrate reduction |
| (*iii*) | Nitrogenase | c) | nitrite reduction |

3. In which pat of the cell, reduction of nitrate to nitrite occurs?

   .....

4 Which is the most oxidized form of inorganic nitrogen?

   .....

5 In which plant organelle reduction of nitrite to ammonia is catlyzed by the enzyme?

   .....

## 10.5 AMINO ACID SYSTHESIS BY PLANTS

As you have noticed that ammonia formation is achieved by plants either by (i.) nitrogen fixation or (ii) by reduction of nitrate to nitrite. Ammonium ($NH_4^+$) is the

most reduced form of inorganic combined nitrogen. This ammonium now becomes the major source for the production of amino acids, which are the building blocks of enzymes and proteins. Amino acids have two important chemical groups. (i) amino group (NH) and (ii) carboxy1 group (-COOH).

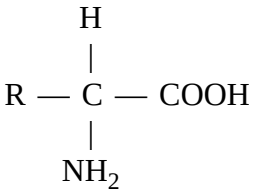


**Fig. 10.2** A typical amino acid with functional groups. R represents alkyl group.

Ammonium so produced is the major source of amino group. However, the carboxyl group has to be provided by other organic molecule synthesized by the plants. There are two major reactions for amino acid biosynthesis in plants:

### 10.5.1 Reductive amination reaction:

In this reaction, ammonia combines with a keto acid. The most important keto acid is the alpha ketoglutaric acid produced during the operation of Krebs cycle (see lesson 12 Plant Respiration). The keto acid then undergoes enzymatic reductive amination to produce an amino acid.

$$\underset{\text{(keto acid)}}{\alpha\text{-ketoglutaric acid}} + NH_3 \xrightarrow{\text{glutamate dehydrogenase}} \underset{\text{(amino acid)}}{\text{Glutamic acid}}$$

Similarly another amino acid called aspartic acid is produced by reductive amination of oxaloacetic acid.

> It has been noted that reductive amination respresents the major 'port of entry' for ammonia into the metabolic stream in plants. This initiates synthesis of glutamic acid followed by other amino acids.

### 10.5.2 Transamination reaction

This is another very important reaction for amino acid biosynthesis. The reaction involves transfer of amino group, from already synthesized amino acid, to the keto acid.

$$\underset{\text{(Keto acid)}}{\alpha\text{-Ketoglutaric acid}} + \underset{\text{(Amino acid)}}{\text{Aspartic acid}} \xrightarrow{\text{Transaminase}} \underset{\text{(Amino acid)}}{\text{Glutamic acid}} + \underset{\text{(Keto acid)}}{\text{Oxaloacetic acid}}$$

In the above reaction, aspartic acid has transferred its amino group ($NH_2$) to the α-ketoglutaric acid to synthesize glutamic acid and release keto acid. The reaction is catalyzed by enzymes called **transaminases**. A large number of amino acids are synthesized by this transamination reaction. Amino acids are organic molecule containing nitorgen. The incorporation of amino group, from ammonium, into keto acids represents the major step for synthesis of nitrogenous organic biomolecules.

## INTEXT QUESTIONS 10.5

1. Match the following:

| | A | B |
|---|---|---|
| (i) | Amino acid | (a) keto acid |
| (ii) | Glutamic acid | (b) amino group and carboxyl group |
| (iii) | α-ketoglutaric acid | (c) amino acid |

2. Name two biochemical reactions for biosynthesis of amino acids in plants.

   .....

3. Which group of enzymes catalyzes transamination reaction?

   .....

4. What is the source of amino group for amino acid synthesis in reductive amination reaction?

   .....

5. Which keto acid is the source for synthesis of glutamic acid?

   .....

## WHAT YOU HAVE LEARNT

- Nitrogen is an important constituent of several biomolecules such as amino acids, proteins and enzymes.
- Molecules such as vitamins, alkaloids, nucleic acids, pigments and some growth hormones also contain nitrogen.
- Molecular nitrogen is triple bonded and stable.
- Nitrogen fixation is the reduction of nitrogen to ammonia.
- Abiological nitrogen fixation is an industrial process (Haber's process)
- Biological nitrogen fixation takes place in a living cell.
- The enzyme that catalyzes nitrogen fixation is Nitrogenase.
- Nitrogen fixation may take place in free living organisms or in symbiotic systems.
- There are many symbiotic nitrogen fixation systems such as Lichens, Pteridophytes, Bryophytes, Gymnosperms and Legumes.
- Cyanobacteria is the symbiotic component in Lichens, Bryophytes, Pteridophytes and Gymnosperms.
- In Legumes, the symbiont is a species of bacterium *Rhizobium*.
- Source of electrons and energy for nitrogen fixation is generally pyruvic acid.

- Hydrogen gas evolution may also accompany nitrogen fixation process.
- Nitrate is the most oxidized form and ammonium is the most reduced form of nitrogen.
- Nitrate is reduced to nitrite by an enzyme nitrate reductase.
- Amino acids have two functional groups including amino group and carboxyl group.
- Amino acids may be produced by reductive amination of keto acids.
- Amino acids may be produced by transamination reaction.
- Reductive amination reactions are catalyzed by dehydrogenase..
- Transamination reactions are catalyzed by transaminases.

## TERMINAL EXERCISES

1. Define nitrogen fixation.
2. Which form of combined nitrogen may be formed during lightening storms?
3. Name three biomolecules other than enzymes and proteins, which contain nitrogen.
4. Name one aerobic and one anaerobic bacterium, which fixes nitrogen.
5. Which amino acid is synthesized due to reductive amination of α-ketoglutaric acid?
6. Differentiate between biological and abiological nitrogen fixation.
7. What is required for biological nitrogen fixation?
8. How does human hemoglobin differ from leghemoglobin?
9. What is the function of leghemoglobin?
10. What are the functional differences between nitrate reductase and nitrite reductase?
11. What is the difference between nitrogen fixation and nitrogen assimilation? Describe in brief the process of abiological nitrogen fixation.
12. Describe in brief various steps involved in biological nitrogen fixation.
13. Enumerate various free living and symbiotic nitrogen fixing system with suitable examples.
14. What are the major differences between free living and leguminous nitrogen fixing organisms?
15. Describe in brief nitrate and nitrite reduction in plants..
17. Describe in brief the reductive amination reactions for synthesis of amino acids in plants.
18. Describe the transamination reaction for synthesis of amino acids in plants. How does this differ from reductive amination?

## ANSWER TO INTEXT QUESTIONS

**10.1** 1. 78.03 percent 2. proteins and enzymes.
3. Because it is triple bonded. 4. 16 percent.
5. –195.8°C.

**10.2** 1. Conversion of molecular nitrogen to ammonia.
2. Haber's process.
3. Biological nitrogen fixation takes place in a living cell and abiological fixation without a living cell.
4. Nitrogenase. 5. Oxygen.

**10.3** 1. (i) b (ii) a (iii) d (iv) c
2. Cycas. 3. Yes, Hydrogen gas.
4. 16 ATP 5. Reduced coenzymes such as Ferrodoxin
6. (i) b (ii) a (iii) c 7. Nodulins.

**10.4** 1. NH
2. (i) b (ii) c (iii) a
3. Cytosol. 4. Nitrate. 5. Chloroplast.

**10.5** 1. (i) b (ii) c (iii) a
2. Reductive amination and transamination.
3. Transaminases. 4. Ammonia.
5. Alpha ketoglutaric acid.

# Enzymes

***Syllabus*** : . The protein nature of enzymes. The role of enzymes as catalysts in lowering activation energy through the formation of enzyme-substrate complex. The concept of active site and enzyme specificity. The induced-fit model of enzyme action.
The effects of temperature, pH, enzyme concentration and substrate concentration on the rate of enzyme reactions.
The effects of cofactors, reversible inhibitors (competitive and non-competitive) and irreversible inhibitors on the rate of enzymatic reactions. End-product inhibition.
The application of enzymes, e.g. biological washing powder and meat tenderiser.

## Introduction :

The chemical reactions which occur within organisms are collectively known as metabolism. It is estimated that over 1000 different reactions occur in an individual cell. There are two types : anabolism and catabolism.

Comparison between anabolism and catabolism :

| *Anabolism* | *Catabolism* |
|---|---|
| Building up processes in cell<br>i.e. $A + B \rightarrow C$<br>e.g. building of protein molecules from different amino acids | Breaking down process in cell<br>i.e. $X \rightarrow Y + Z$<br>e.g. deamination |
| Energy required, i.e. endogenic | Energy is released, i.e. exogenic |
| Both processes need enzymes to speed up the reaction | |

## Enzymes as organic catalysts :

Enzymes are organic catalyst produced within the living organisms which speed up chemical reactions, by lowering the activation energy, in the living organisms but themselves remain unchanged at he end of the reaction.

[Note] Activation energy is the energy required to make the substances react.

As heat is often the source of activation energy, enzymes often dispense with the need for this heat and so allow reactions to take place at lower temperature

In molecular terms, the enzyme combines with the substrate molecules to form an enzyme-substrate complex. In such close contact the substrate molecules may be distorted and hence easily react to form an enzyme-product complex which then split to release the product molecule and the enzyme . In this way, the activation energy is lowered.

Since they are not altered by the reactions they catalyse, so that they can be reused. In other words they are effective in very small amount.

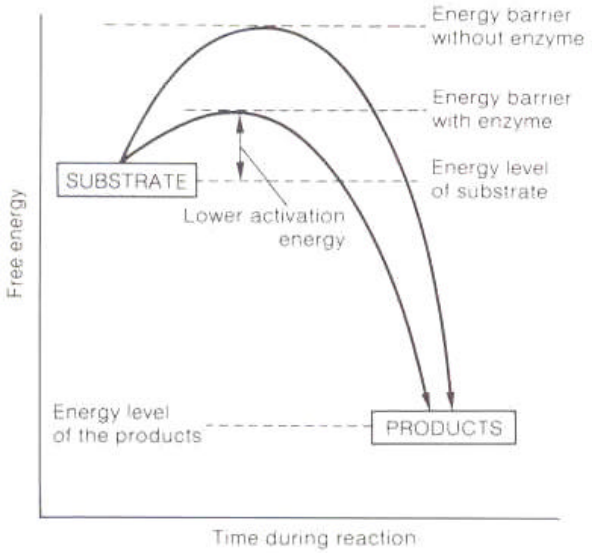



**Fig. 53** How enzyme lower the activation energy.

Enzymes are normally larger than the substrate molecules they act upon. Only a small part of the enzyme molecule actually comes into contact with the substrate. This region is called active site.

## Mechanism of Enzymatic Actions :

### Lock and Key Hypothesis :

It was suggested by Fischer in 1890.

On the surface of the enzyme molecule is specific site called the active site which has a distinctive configuration due to the unusual folding of the protein molecule and position of different chemical groups within it.Only the specific substrate molecules with the right shape can fit into the active site to form enzyme-substrate complex, thus the substrate and enzyme molecules act as the lock and key respectively.

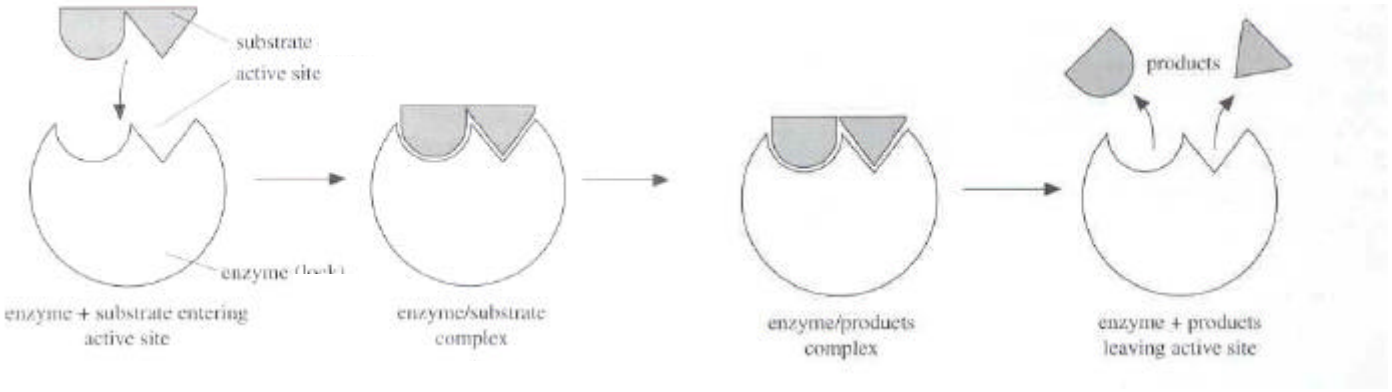



**Fig. 54** The lock and key hypothesis of enzyme action.

### Induced Fit Theory :

This is modified from lock and key hypothesis and was suggested by Koshland in 1959.

In the presence of substrate the active site of the enzyme may change shape to fit the substrate i.e. the enzyme is flexible and moulds to fit the substrate molecule. This theory is stated based on the nature of enzyme--- protein molecule is flexible enough to allow conformational changes.

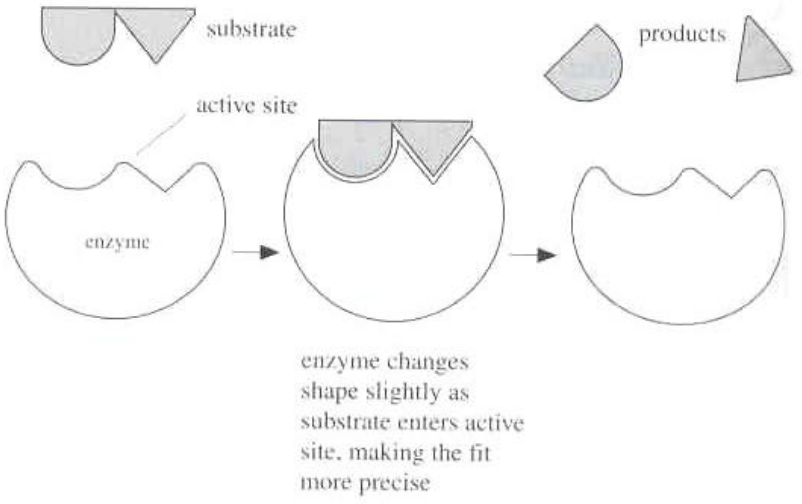



**Fig. 55** The induced fit theory of enzyme action.

## Properties of enzyme :

1. It speeds up chemical reactions but remain undestroyed at the end of the reaction. i.e. it has the catalytic properties.
2. It works in either direction. i.e. it catalyses the forward and backward reaction to the same extent. The direction in which the reaction goes depends on the relative amounts of substrate and products present. The products are continuously removed to maintain the reaction in living organism. e.g. $A + B \leftrightarrow C + D$
3. An enzyme changes the rate only at which chemical equilibrium is reached; it does not affect the position of the equilibrium.
4. An enzyme speeds up the rate of reaction by lowering the activation energy barrier.
5. It works rapidly and therefore is required in small quantity.
6. It is soluble in water and works in aqueous solution in living cells.
7. All enzymes operate only on specific substrates. Only substrates of particular shape will fit the active site of an enzyme.
8. All enzymes are proteins, some may have other associated molecules.
9. Enzyme may be denatured by excessive heat, extreme pH or various chemicals. This is because enzymes are soluble proteins. Such extremities or chemicals cause enzymes to coagulate by breaking down bonds in the molecule making them insoluble. The molecular configuration of the active site is changed. Hence the enzyme can no longer carry its catalytic function and is said to be denatured. Such changed are irreversible. The phenomenon is known as denaturation.
10. The activity of an enzyme is affected by temperature.
11. Enzyme activity is affected by pH of the medium. It worked best at an optimum pH.
12. Some enzymes work efficiently only in the presence of appropriate co-factors.

**Co-factors :**

It is a non-protein substance which is essential for some enzymes to function efficiently. There are three types :

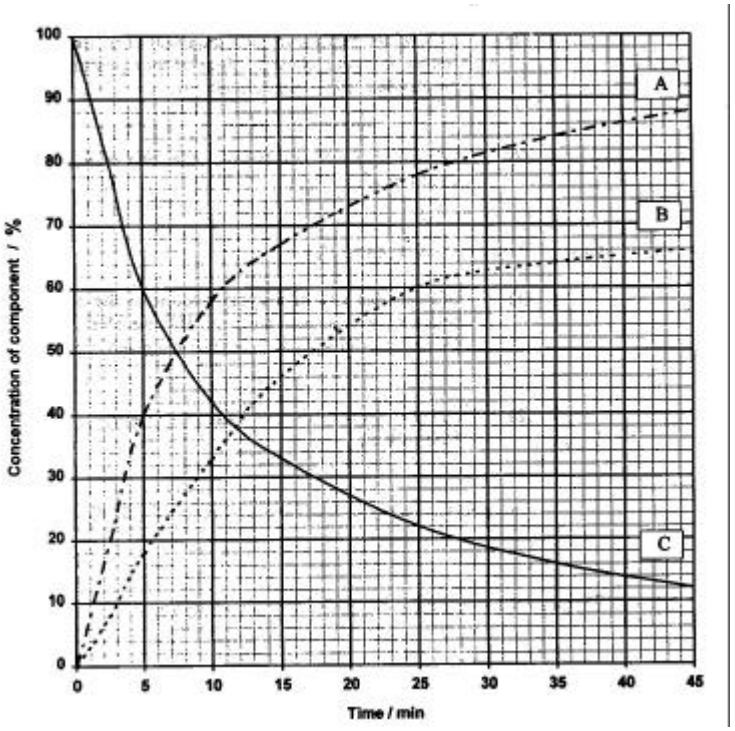
a) Activators : are substances which are necessary for the functioning of certain enzymes. They may assist in forming the E-S complex by moulding either the enzyme or substrate molecule into a more suitable shape.
   e.g. i) Enzyme thrombokinase, which converts prothrombin into thrombin during blood clotting, is activated by Calcium ions ;
   ii) Salivary amylase requires the presence of chloride ions before it converts starch into maltose.

b) Coenzymes : are non-protein organic substances which are essential to the efficient functioning of some enzymes, but are not themselves bound to the enzyme. Many coenzymes are derived from vitamins.
   e.g. Nicotinamide adenine dinucleotide (NAD) act as a coenzyme to dehydrogenases by acting as a hydrogen acceptor in the Krebs Cycle.

c) Prosthetic groups : are organic , non-protein molecules and bound to the enzyme themselves.
   e.g. Haem is an iron-containing prosthetic group. it may function as electron carrier and oxygen carrier in haemoglobin. It is also found in catalases and peroxidases, which catalyse the decomposition of hydrogen peroxide to water and oxygen.

**Exercise : (96 I 10)**

(a) A student performed an experiment to monitor the progress of a simple enzyme-catalysed reaction involving one substrate and one product. He prepared replicate reaction tubes. In each tube, the concentration of only one reaction component, either the substrate or product, was measured. The results of three selected tubes are shown as curves A, B and C below :




(i) Identify which measured component, substrate or product, is represented by each curve. [1½ marks]

(ii) It was realised that the reaction mixture in one of the three tubes had been wrongly prepared. This resulted in a different reaction condition.

(1) State which curve represents the result of an error in the preparation. Give a reason for your choice. Suggest TWO possible mistakes in the student' s preparation of this reaction mixture. [3½ marks]

(2) What evidence shows that the other two curves represent identical reaction conditions ? [2½ marks]

(iii) With reference to curve C,

(1) calculate the rate of enzyme reaction at the 3rd minute, given that 100 % concentration is equivalent to 100 mmole of the measured component (Show the readings you take from the graph in your calculation.) ; [2 marks]

(2) compare and explain the difference in enzyme reaction rate at the 3rd and 15th minute. [2½ marks]

(iv) Curve A will finally level off and will not reach 100 %. Explain this phenomenon. [1½ marks]

(b) Explain what is meant by the ' active site' of an enzyme. [1½ marks]

## Factors affecting the rate of enzymatic reaction :

**1. Temperature**

a) If temperature is reduced to near or below freezing point, enzymes are inactivated only, not denatured. They will regain their catalytic influence when higher temperature are restored.

b) As the temperature increase, the kinetic energy of the substrate and enzyme molecules increases and so they move faster. The faster these molecules move, the more often they collide with one another and the greater the rate of reaction.

c) As the temperature increases further, the more the atoms which make up the enzyme molecules vibrate. This breaks the hydrogen bonds and other forces which hold the molecules on their precise shape. So the shape of the active site altered and no longer fit the substrate. The enzyme is said to be denatured and loses its catalytic activity forever.

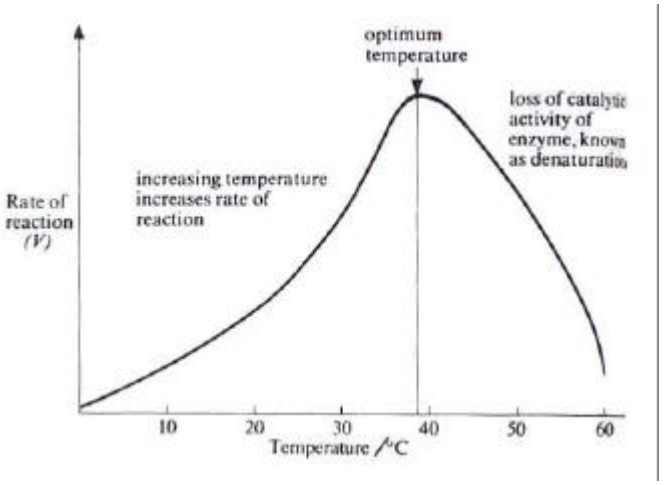



**Fig. 56** Effect of temperature on the rate of an enzyme controlled reaction.

**Exercise : (95 I 8a)**

An experiment was conducted to investigate the effect of temperature on the activities of amylases I and II. These enzymes were obtained from the same type of tissue of vertebrate animals I and II respectively. Similar pH, enzyme concentration and substrate concentration were used. The experimental data obtained are recorded in the following table.

| Temperature / 0C | Time for complete digestion of 1 unit of substrate / minute | |
|---|---|---|
| | Amylase I | Amylase II |
| 10 | 2.0 | 5.0 |
| 15 | 1.6 | 3.5 |
| 20 | 1.5 | 2.5 |
| 25 | 1.6 | 2.0 |
| 30 | 2.0 | 1.6 |
| 35 | 4.0 | 1.4 |
| 40 | 10.0 | 1.5 |

(i) Plot a graph to demonstrate the effect of temperature on the activities of amylases I and II. [5 marks]

(ii) Explain the results for amylase I. [2 marks]

(iii) What are the optimum temperatures for amylase I and amylase II ? What thermoregulatory ability would the data for amylase I suggest for vertebrate animal I ? [2 marks]

## 2. pH

- Changes in pH alter the ionic charge of the acidic and basic groups that help to maintain the specific shape of the enzyme i.e. break the hydrogen bonds. The pH change leads to an alternation in enzyme shape, particularly at its active site. i.e. enzyme denatured
- Under constant temperature and pressure, every enzyme has a narrow pH range within which it will function effectively. As the pH of reacting medium increases above or falls below the optimum pH, enzyme activity decreases. At either extreme of pH, the enzyme is denatured.
- Different enzymes may have different optimum pH

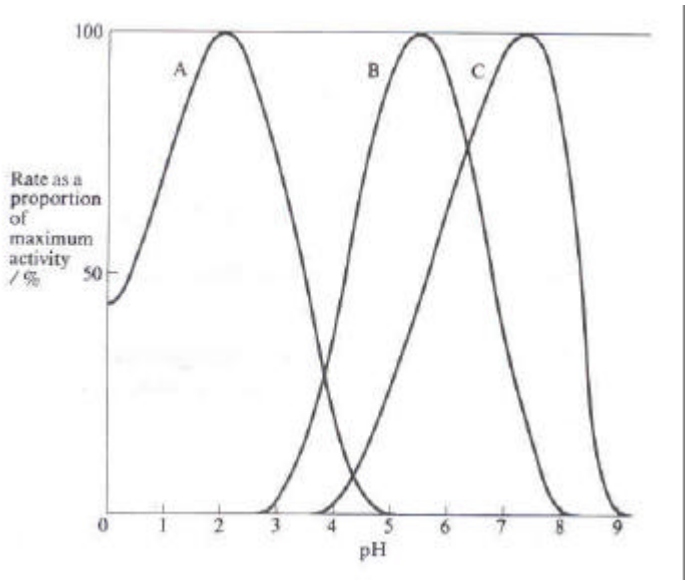



**Fig. 57** Effect of pH on the activity of three enzymes A, B and C.

## 3. Enzyme Concentration

- The active site of an enzyme may be used again and again, thus enzymes work efficiently at very low concentrations.
- One enzyme molecule can deal with only one substrate molecule at a particular instant of time. Thus, the more the enzyme molecules present in the same period of time, the larger the number of substrate molecules can be converted to products provided that there is an unlimited supply of substrate..

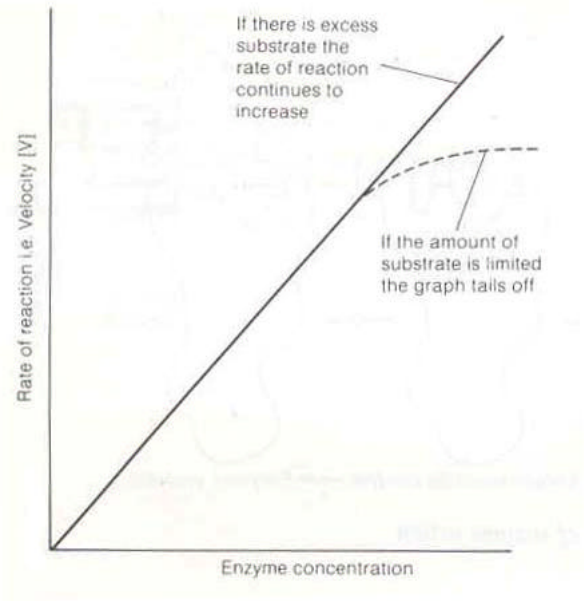



**Fig. 58** Effect of enzyme concentration on the rate of an enzyme controlled reaction.

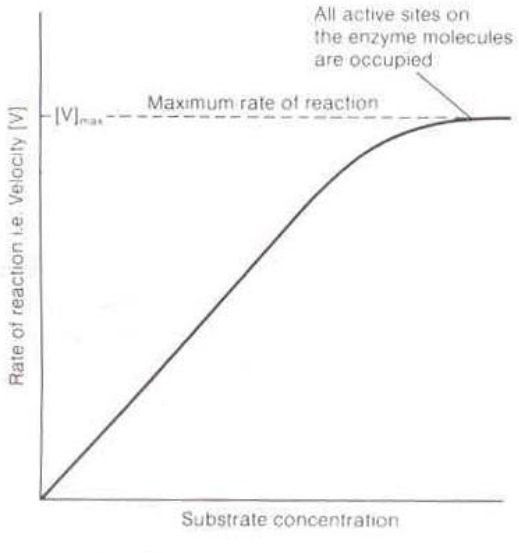



**Fig. 59** Effect of substrate concentration on the rate of an enzyme controlled reaction.

**4. Substrate Concentration**

- At low substrate concentrations, the active sites of the enzyme molecules are not all used because there are not enough substrate molecules to occupy them all.
- As substrate concentration is increased, more and more sites come into use.
- When over the saturation point, a point where all active sites are being occupied by substrates, increasing the substrate concentration cannot increase the rate of reaction. This is because any extra substrate has to wait for the E-S complex dissociated into products and free enzyme.
- Therefore at high substrate levels, both enzyme concentration and the dissociation time are the limiting factors.

**5. Concentration of End-products**

- As the end-products accumulate, the pH of the medium may change so that the rate of reaction may also be changed.
- In some enzymatic reactions, the enzyme itself combines with one of the end product i.e. those end-products will inhibit the activity of the enzymes-- negative feedback. this may be due to the shape of the product similar to that of the substrate and therefore can fit into the active sites of enzymes.
- So, rate of enzymatic reaction decreases with the accumulation of the end-products.

**6. Inhibition**

A variety of small molecules exist which can reduce the rate of an enzyme-controlled reaction. They are called enzyme inhibitors. Inhibition may be reversible or irreversible.

I./ Reversible inhibition

The inhibitor can be easily removed from the enzyme and cause no permanent damage.

a) Competitive inhibitors

- compete with the substrates for the active sites of enzyme molecules as they are structurally similar to the substrate
- while remain in the active sites, they prevent access of the true substrate
- if the substrate concentration is increased, the rate of reaction will be increased

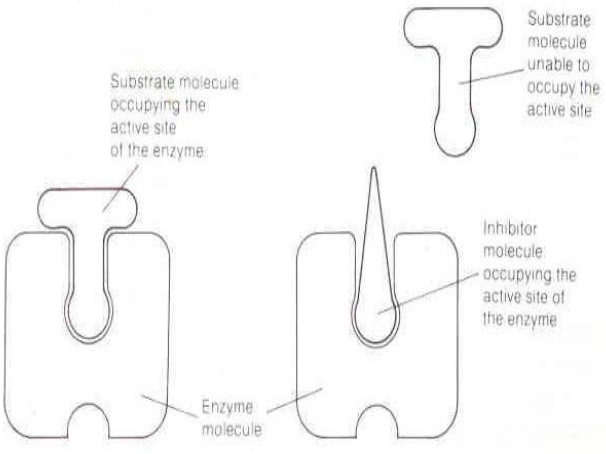



**Fig. 60** Competitive inhibition.

For example :

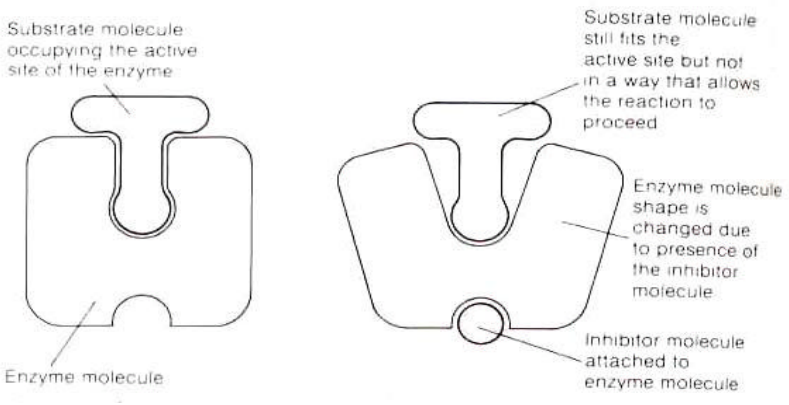
i) Malonic acid --- It compete with succinate for the active sites of succinic dehydrogenase, an important enzyme in the Krebs Cycle.

ii) sulphonamides --- during the Second World War, sulphonamides were used extensively to prevent the spread of microbial infection.

The sulphonamides are similar in structure to paraaminobenzoate (PAB), a substance essential to the growth of many pathogenic bacteria. The bacteria require PAB for the production of folic acid, an important enzyme cofactor. Sulphonamides acts by interfering with the synthesis of folic acid from PAB.

- this type of inhibitor has no real structural similarity to the substrate and forms an enzyme-inhibitor complex at a point on the enzyme other than its active site

- they alter the shape of the enzyme in such a way that the active site can no longer properly accommodate the substrate
- increase the substrate concentration will not therefore reduce the effect of the inhibitor as they are not competing the same site.
- but increase the inhibitor concentration will decrease the reaction rate




For example :
Cyanide --- attaches itself to copper prosthetic group of cytochrome oxidase, thereby inhibiting respiration.

**Fig. 60** Non-competitive inhibition.

II./ Irreversible inhibition :

The inhibitor combines with the enzyme permanently and so the enzyme unable to carry out its catalytic function forever. Heavy metal ions such as mercury and silver cause disulphide bonds to break. These bonds help to maintain the shape of the enzyme molecule. Once broken the enzyme molecule's structure becomes irreversibly altered with the permanent loss of its catalytic properties.

For example: a) DFP (the nerve gas used in Second World War) --- forms enzyme-inhibitor complex with the amino acid serine at the active site of enzyme acetylcholinesterase. This enzyme deactivates the chemical transmitter substance acetylcholine so that a normal transmission of nerve impulse can be propagated. If this enzyme is inhibited, paralysis or death will be the end result.

b) Some insecticides (such as parathion) --- have similar effects as the nerve gas.

## The application of enzymes :

There re three main advantages of using enzymes in industrial processes.

1. They are specific in their action and are therefore less likely to produce unwanted by-products.
2. They are biodegradable and therefore cause les environmental pollution.
3. They work in mild conditions, i.e. low temperatures, neutral pH and normal atmospheric pressure, and are therefore energy-saving.

Commerical enzymes are produced by micro-organisms such as yeasts and bacteria. Sometimes naturally occurring strains are usued, but increasingly nowadays special strains are developed by genetic engineering to produce particular enzymes.

Common use of the enzymes :

(i) Biological washing powders :

- contain enzymes, usually proteases
- remove 'biological' stains such as food, blood and so on
- as to reduce allergic reactions to man, the enzymes are encapsulated in wax from which they are released only when in the wash

(ii) Meat tenderizers :

- contain protease, made by *Bacillus subtilis*
- as the main component of meat is protein, so the protease may digest some peptide bonds of the meat, this tenderizes the meat

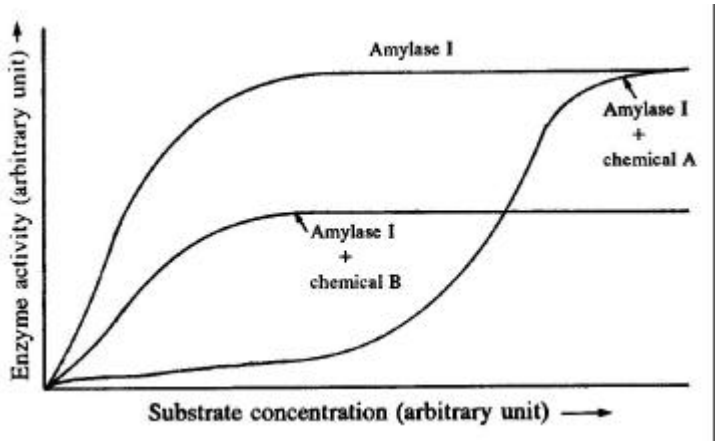
(iii) Other applications :

| *Applications* | *Enzymes used* |
|---|---|
| Biological detergents | Primarily proteases, produced in extracellular form from bacteria. |
| Baking industry | α-amylases are used to improve flour, which destroyed during backing process |
| Brewing industry | α-amylases are used to breakdown of starch in beer production |
| Sweetener | Glucose isomerase is used to make the soft drinks and cake fillings tastes sweet |
| Dairy industry | Lipase used in flavour development of cheese;<br>Lactose used to sweeten the milk |
| Photographic industry | Protease (ficin) used to digest the protein coat on the film when developing the image |
| Paper industry | Amylases used to remove the starch from the raw materials |

**Exercise :**

**(95 I 8b)**

The graph below shows the effects of two chemicals, A and B, on the activity of amylase I. Similar pH, temperature, enzyme concentration and chemical concentration were used in the experiment.




(i) Compare and contrast the effects of chemical A and chemical B on the activity of amylase I. [3 marks]

(ii) Explain how each chemical, A and B, exerts its effect on amylase I. [3 marks]

**(97 I 2)**

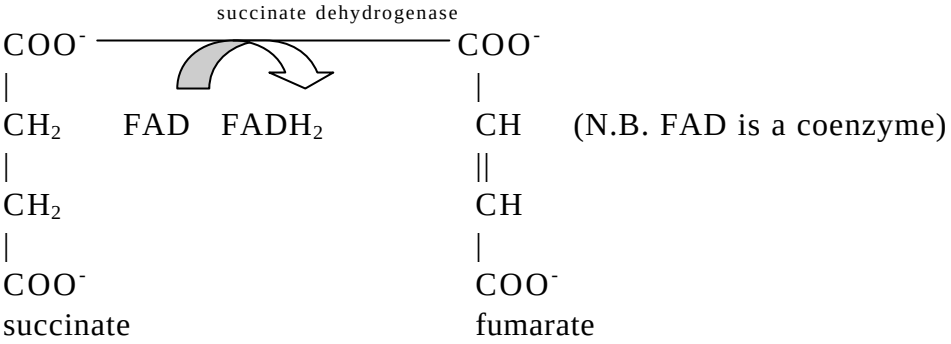
Compare and contrast the characteristics of competitive and non-competitive inhibitors on enzyme activity. [4 marks]

**(98 I 6)**

Give ONE application of enzyme and explain its effect. [2½ marks]

**(99 I 12)**

The following reaction is catalysed by the enzyme succinate dehydrogenase :

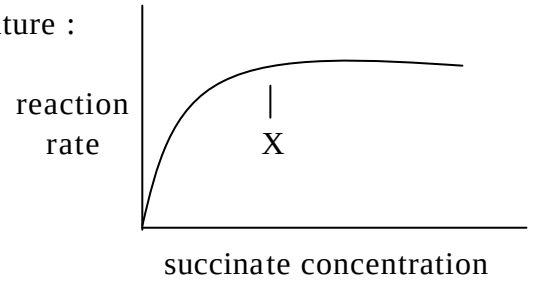



(N.B. FAD is a coenzyme)

(a) State THREE parameters that can be used to determine the rate of this reaction. [3 marks]

(b) The graph below shows the effect of succinate concentration on the rate of reaction under optimum pH and temperature :

(c) The graph below shows the effect of succinate concentration on the rate of reaction under optimum pH and temperature :



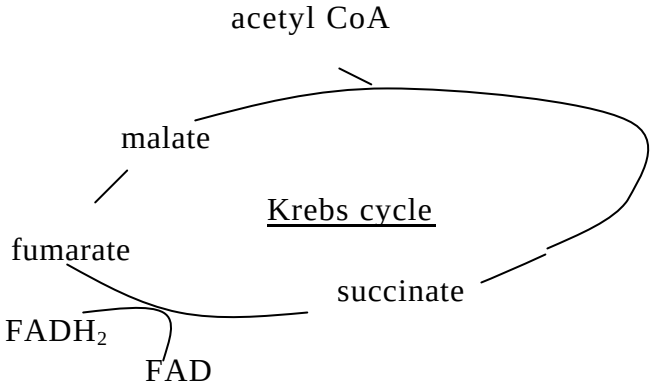

The curve flattens out at X. Give TWO explanations for this observation based on the mechanism of enzymatic reaction. [2 marks]

(c) Malate and succinate are metabolic intermediates in the Krebs cycle. They have the following structural formulae :

| malate | succinate |
|---|---|
| COO⁻ | COO⁻ |
| CHOH | $CH_2$ |
| $CH_2$ | $CH_2$ |
| COO⁻ | COO⁻ |

(i) Suggest what effect malate might have on the rate of reaction catalused by succinate dehydrogenase. [1 mark]

(ii) The positions of malate, succinate and fumarate in the Krebs cycle are shown in the following flowchart :




Malate can play a role in regulating the rate of reactions in the Krebs cycle. Explain the possible mechanism by which malate can achieve such a control.

[4 ½ marks]

# 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. 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 one that was exposed to light. On testing these leaves for starch it was clear that photosynthesis occurred only in the green parts of the leaves in the presence of light.

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

## 13.2 EARLY EXPERIMENTS

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

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

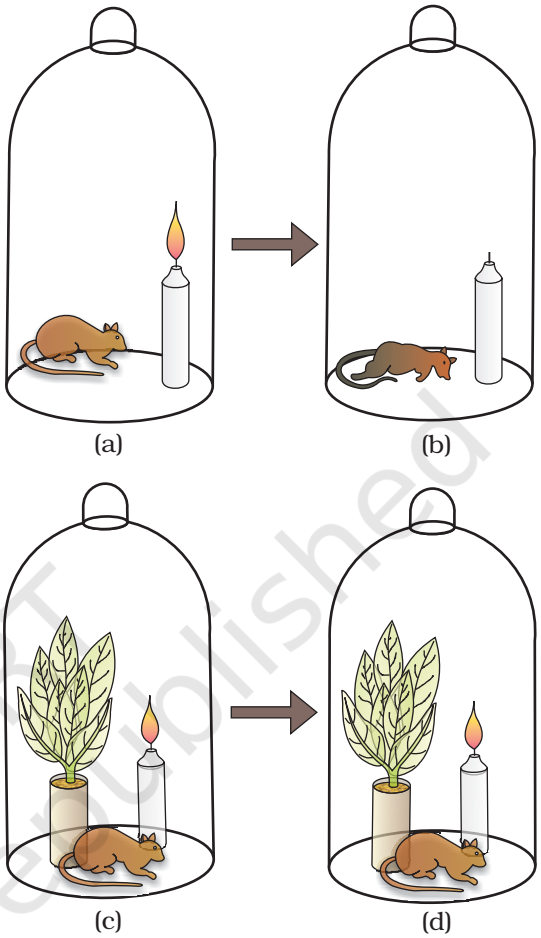



**Figure 13.1** Priestley's experiment

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

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

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

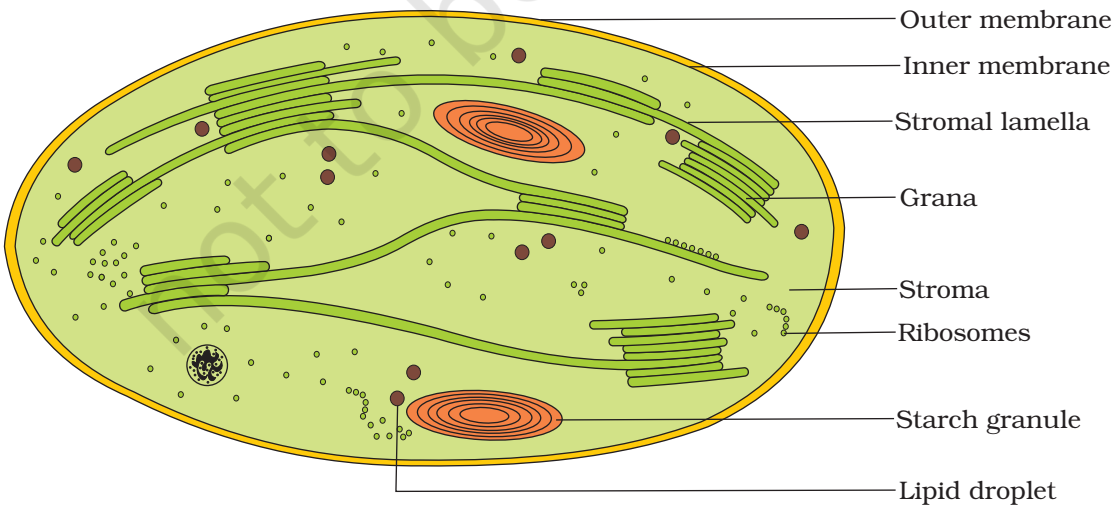



**Figure 13.2** Diagrammatic representation of an electron micrograph of a section of chloroplast

## 13.4 How many Pigments are Involved in Photosynthesis?

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?*

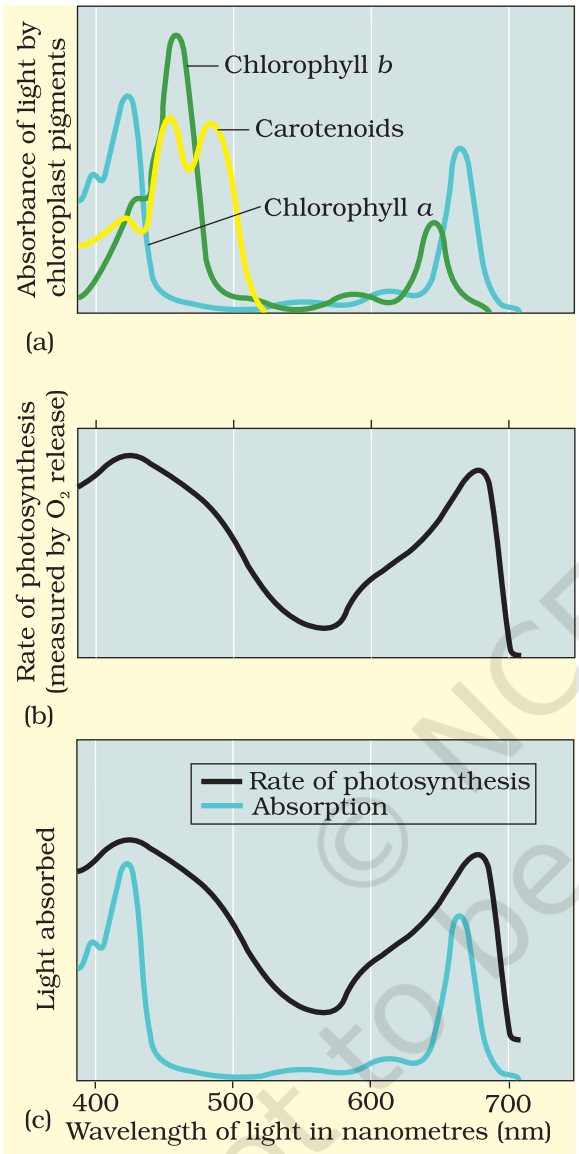



**Figure 13.3a** Graph showing the absorption spectrum of chlorophyll *a*, *b* and the carotenoids

**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*

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 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**.

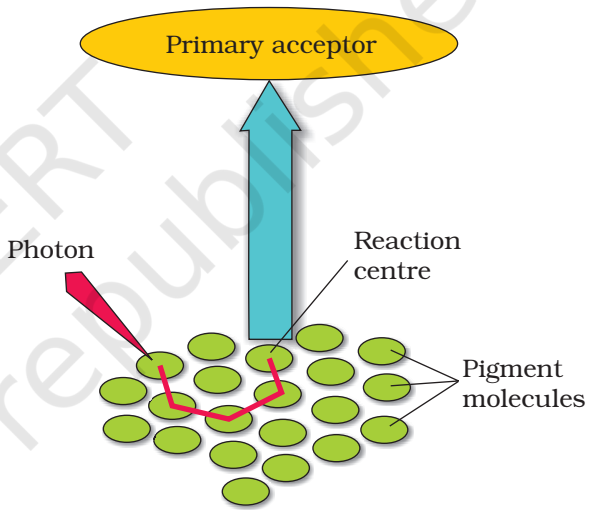



**Figure 13.4** The light harvesting complex

## 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**

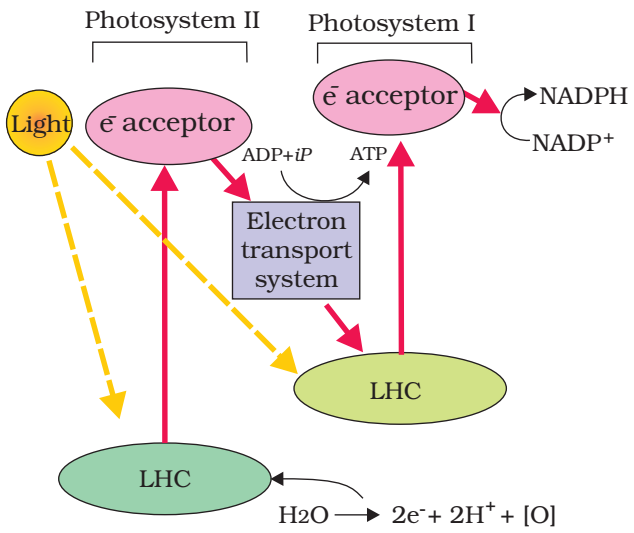



**Figure 13.5** Z scheme of light reaction

**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 $NADP^+$ causing it to be reduced 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 $H^+$, [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).

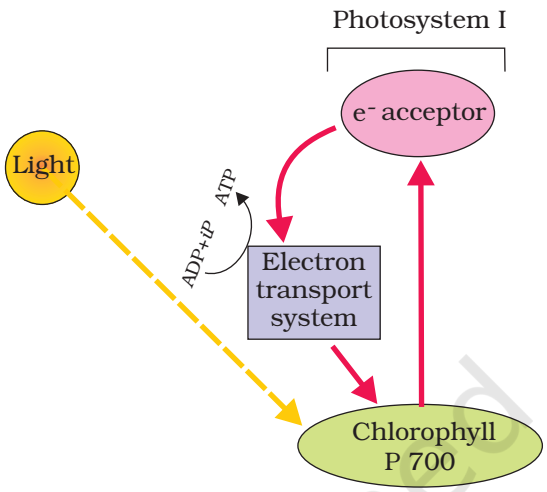



**Figure 13.6** Cyclic photophosphorylation

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 membranes of the 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.

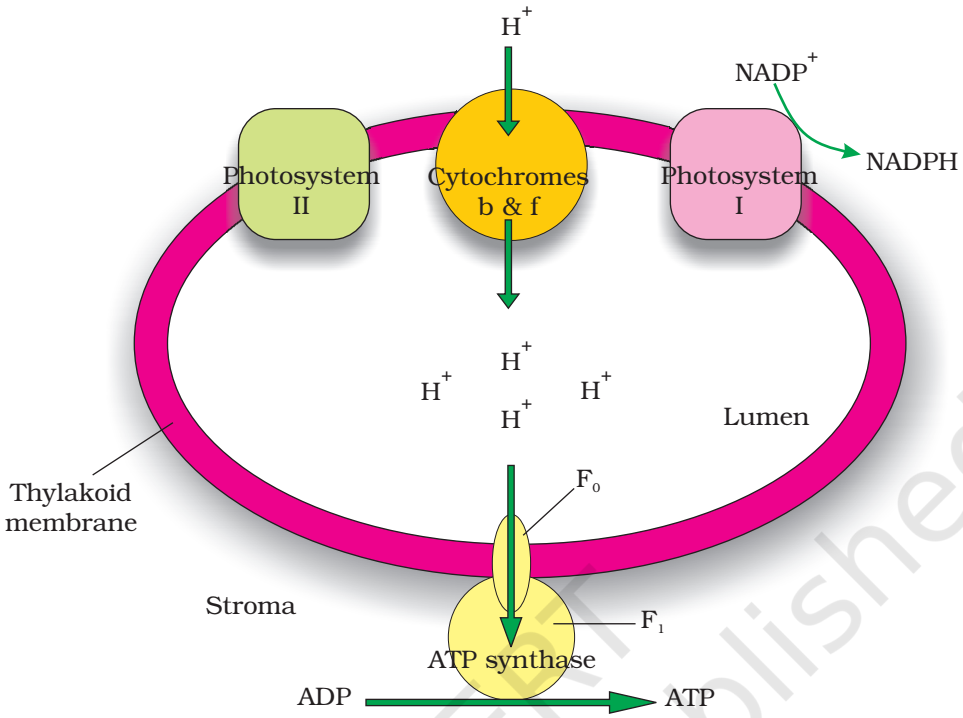



**Figure 13.7** ATP synthesis through chemiosmosis

(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 release of energy. The gradient is broken down due to the movement of protons across the membrane to the stroma through the transmembrane channel

of the $F_0$ of the ATPase. The ATPase enzyme consists of two parts: one called the $F_0$ is embedded in the membrane and forms a transmembrane channel that carries out facilitated diffusion of protons across the membrane. The other portion is called $F_1$ 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 $F_1$ particle of the ATPase, which makes the enzyme synthesise several molecules of energy-packed ATP.

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

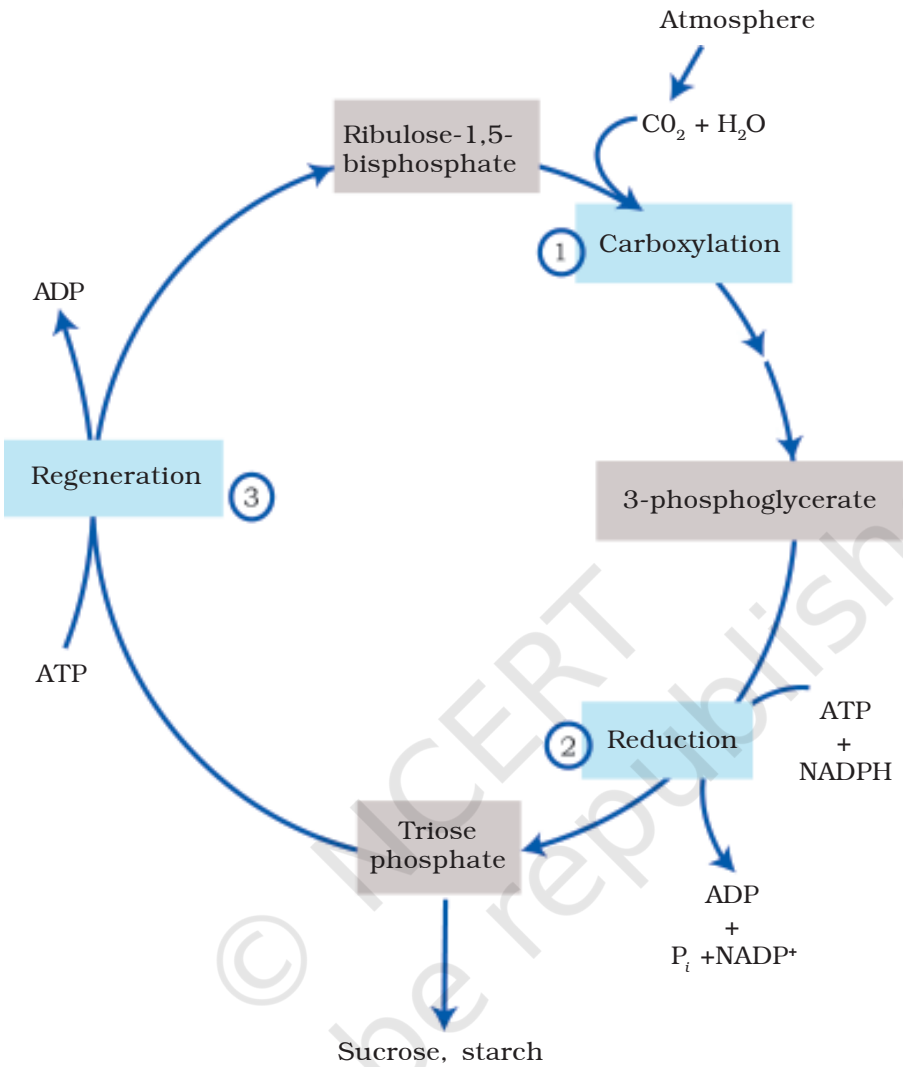



**Figure 13.8** The Calvin cycle proceeds in three stages : (1) carboxylation, during which $CO_2$ combines with ribulose-1,5-bisphosphate; (2) reduction, during which carbohydrate is formed at the expense of the photochemically made ATP and NADPH; and (3) regeneration during which the $CO_2$ acceptor 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 removal 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 $C_4$ pathway 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

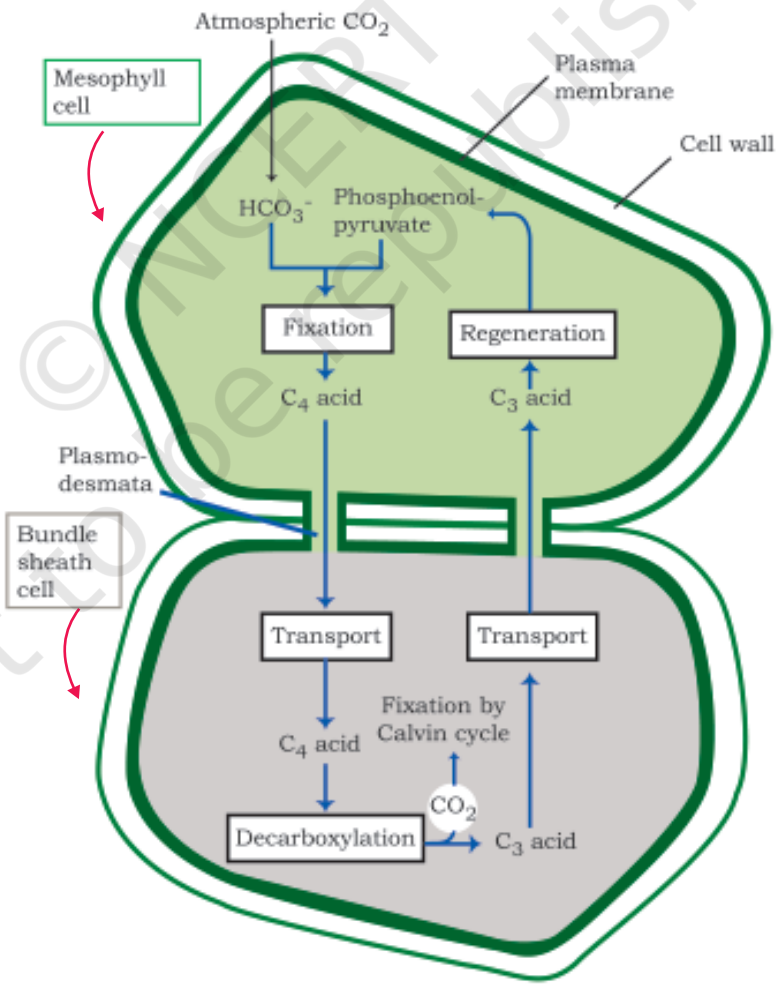



**Figure 13.9** Diagrammatic representation of the Hatch and Slack Pathway

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 $CO_2$ 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 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. Therefore, photorespiration is a wasteful process.

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.

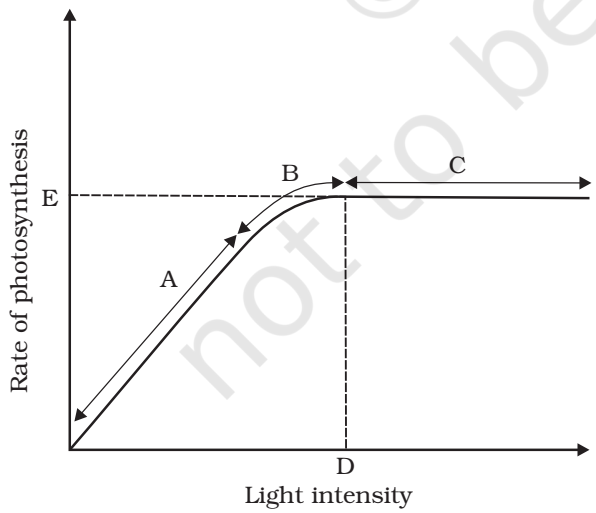



**Figure 13.10** Graph of light intensity on the rate of photosynthesis

### 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.

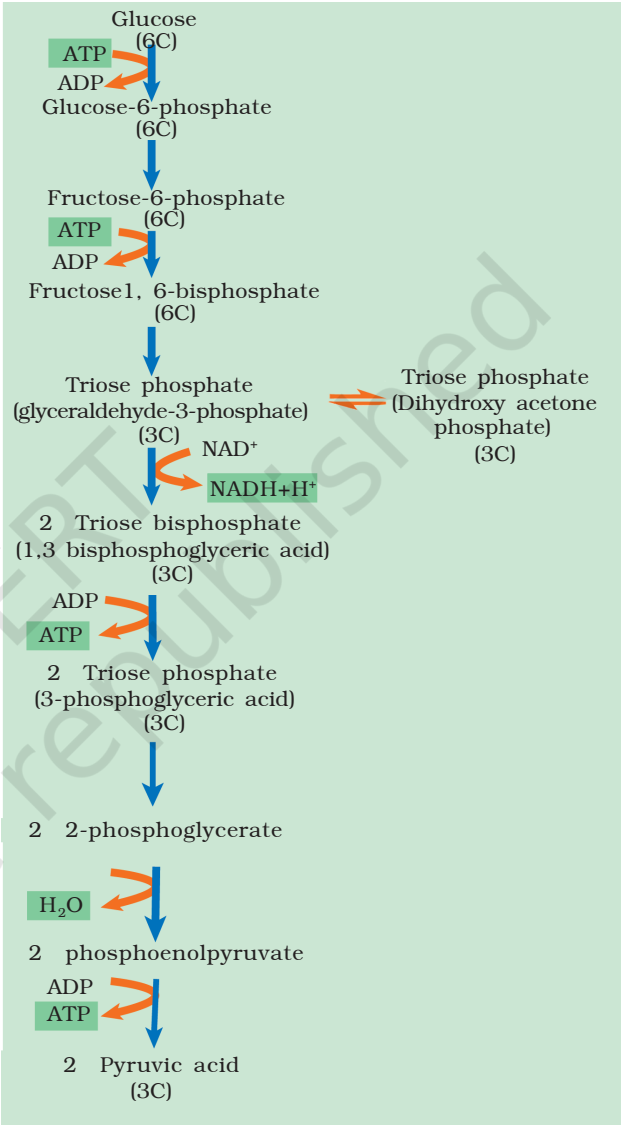



**Figure 14.1** Steps of glycolysis

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.

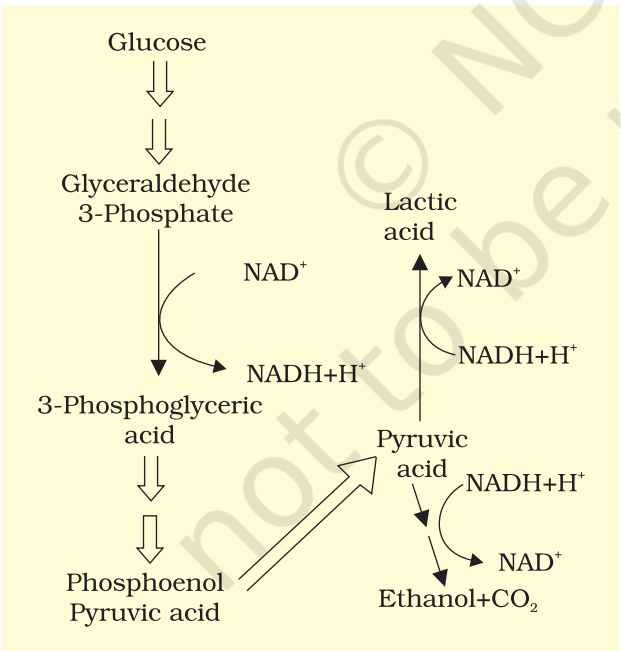



**Figure 14.2** Major pathways of anaerobic respiration

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

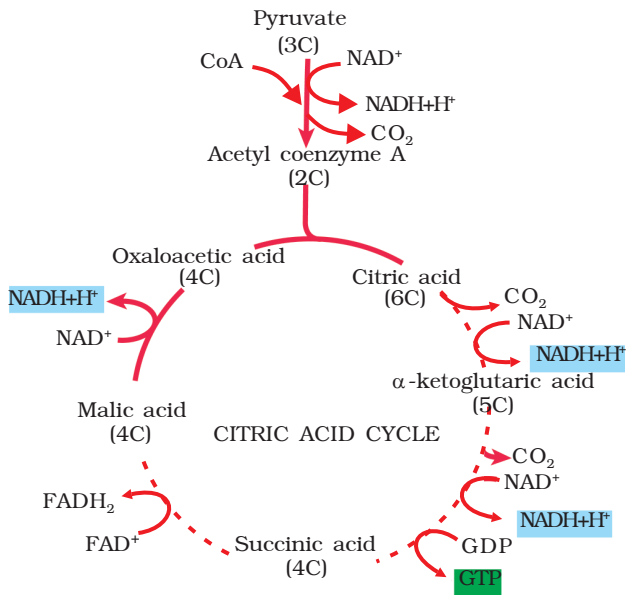



**Figure 14.3** The Citric acid cycle

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.

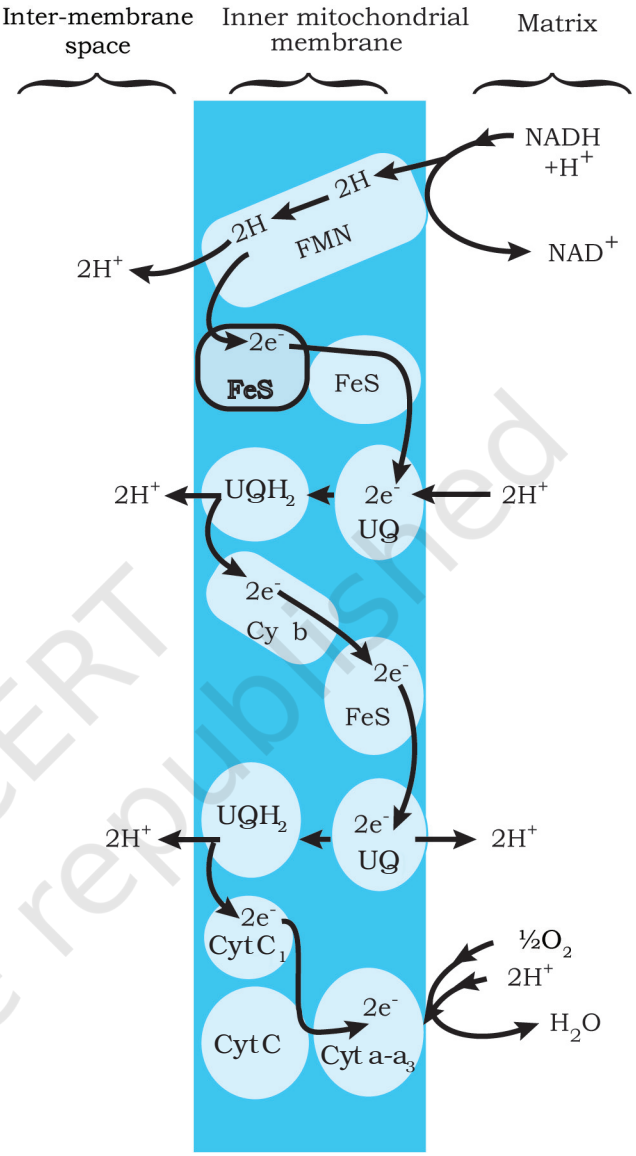



**Figure 14.4** Electron Transport System (ETS)

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

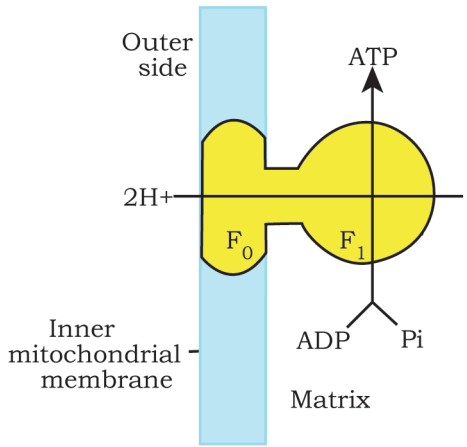



**Figure 14.5** Diagramatic presentation of ATP synthesis in mitochondria

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 36 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.

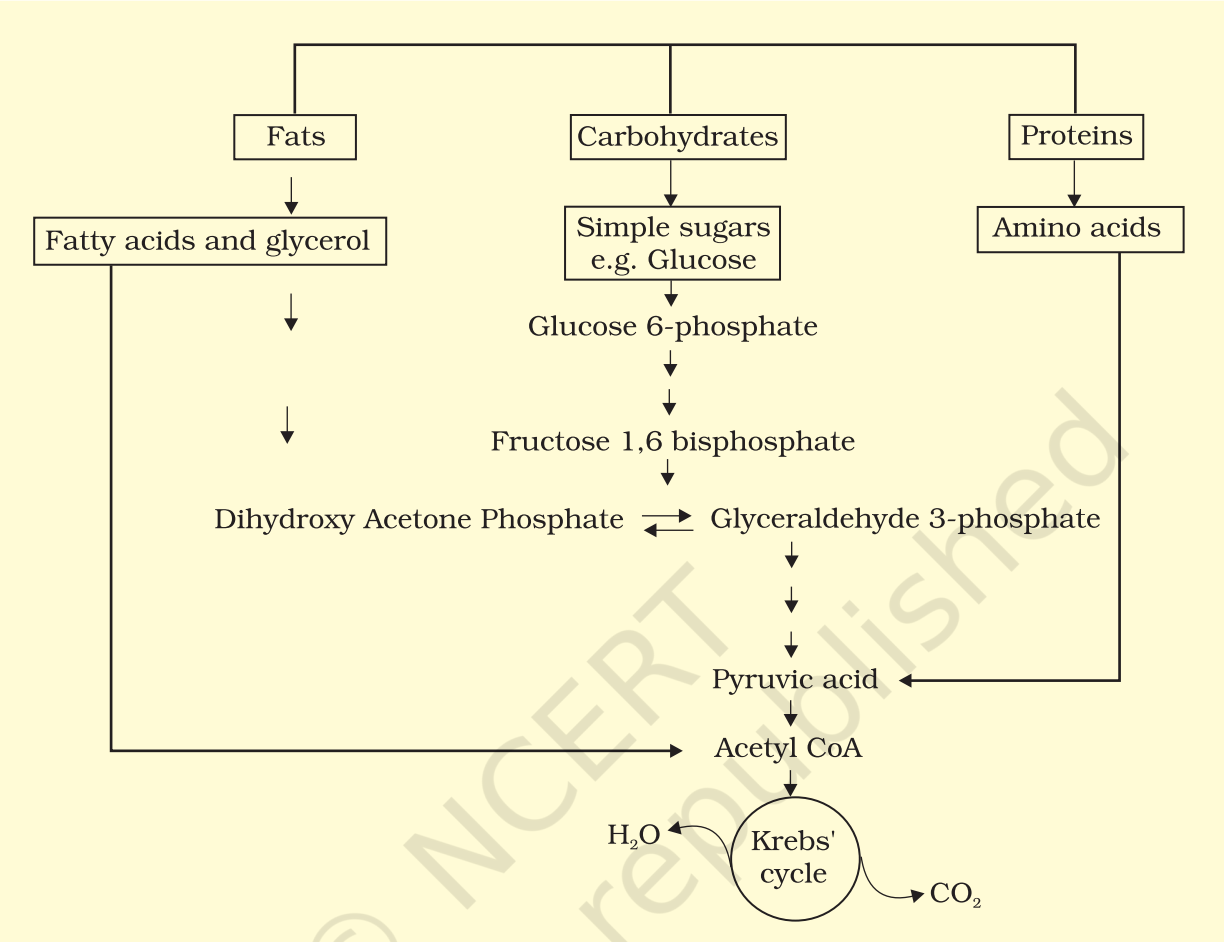



**Figure 14.6** Interrelationship among metabolic pathways showing respiration mediated breakdown of different organic molecules to $CO_2$ and $H_20$

## 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 5 - Phloem transport

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*This Chapter is updated from a previous version written by John W Patrick, Ian Wardlaw and Tina Offler for Plants in Action 1st Edition.*

Contents:


A plant is a coordinated network of assimilatory regions (sources) linked to regions of resource utilisation (sinks). The phloem vascular system provides a path for assimilate transport from source to sink.

The phloem conduits distribute the sugars made in the leaves to growing tissues and organs that cannot carry out photosynthesis. These 'sinks' include shoot and root apices, flower buds, and developing fruit and seed.

Early development of a pineapple. Phloem conduits from the leaves distribute sugars to the growing inflorescence, with flower buds arranged in spirals, which will later develop into the large juicy fruit.

Xylem conduits are responsible for delivery of water, inorganic nutrients and organic forms of nitrogen to transpiring leaves (Chapters 3 and 4).

Section 5.1 describes the pathway of the distribution of sugars made in chloroplasts, as well as nitrogen assimilates made in the leaves, to growing organs and other non-photosynthetic tissues. Section 5.2 describes the composition of phloem sap and how to collect it. Quantitative information is presented on the speed of phloem transport from sources to sinks, and the controls of long-distance transport.

Cellular and regulatory mechanisms of phloem loading in leaves are shown in Section 5.3, and mechanisms of phloem unloading at sinks in Section 5.4 with particular reference to developing seeds.

The focus of this chapter is on the transport of sugars. The transport of amino acids and other nitrogen-containing compounds is equally important, and the same general principles apply to nitrogen-containing or phosphorus-containing compounds that are synthesised in the leaf.

# 5.1 - Distribution of photoassimilates within plants

$CO_2$ fixed by photosynthesis in chloroplasts has several possible fates, but most ends up as sucrose or starch. Starch is stored in chloroplasts, and sucrose is stored in vacuoles of mesophyll cells. Both starch and vacuolar sucrose serve as temporary storage pools from which the cytoplasmic sucrose pool is replenished. Sucrose, along with amino acids and mineral nutrients, is loaded into the phloem tissue which consists of sieve element—companion cell (se—cc) complexes for long-distance transport to growing tissues and other non-photosynthetic sinks. These solutes are exchanged reversibly between se-cc complexes and short- and long-term storage pools along the axial pathway. Short-term storage pools include phloem apoplasm, and the protoplasm of non-transport cells provides a long-term storage pool. At the end of the pathway, sucrose and other transported solutes are consumed in respiration and growth, or are stored as solutes in vacuoles or polymers in amyloplasts (starch) or protein bodies.

The overall flow of photoassimilates throughout the plant can therefore be called a source–path–sink system (Figure 5.1).

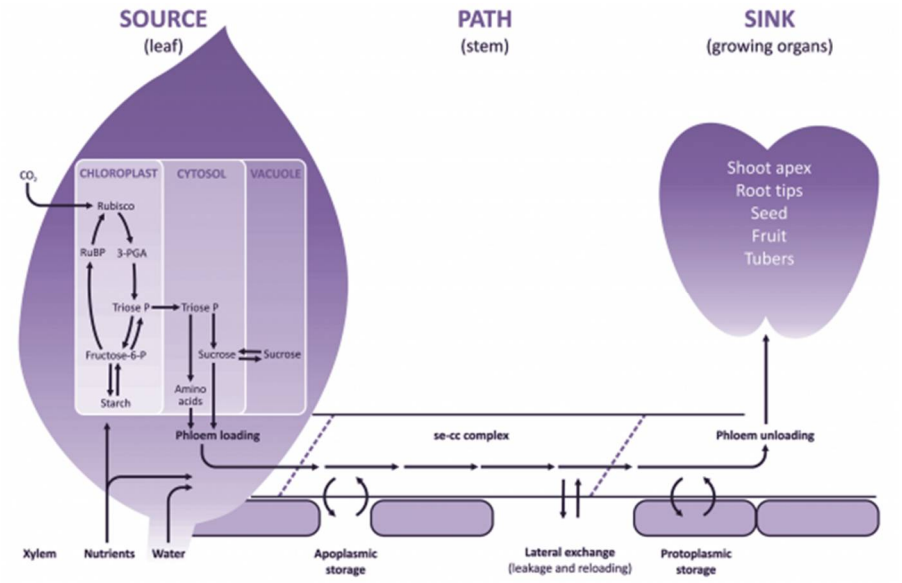



Figure 5.1 Schematic diagram of transfer and transport processes contributing to the flow of assimilates cquired from aerial or soil environments, through the source-path-sink system. $CO_2$ fixed by photosynthesis in chloroplasts gives rise to sucrose and starch. Sucrose, amino acids and mineral nutrients are loaded into sieve element—companion cell (se—cc) complexes of leaf phloem for long-distance transport to non-photosynthetic sinks. These solutes are exchanged reversibly between se-cc complexes and short- and long-term storage pools along the axial pathway. Short-term storage pools include phloem apoplasm, whereas the protoplasm of non-transport cells provides a long-term storage pool. In sink tissues, solutes are used for respiration, growth or storage.

# 5.1.1 - Source–path–sink transport processes

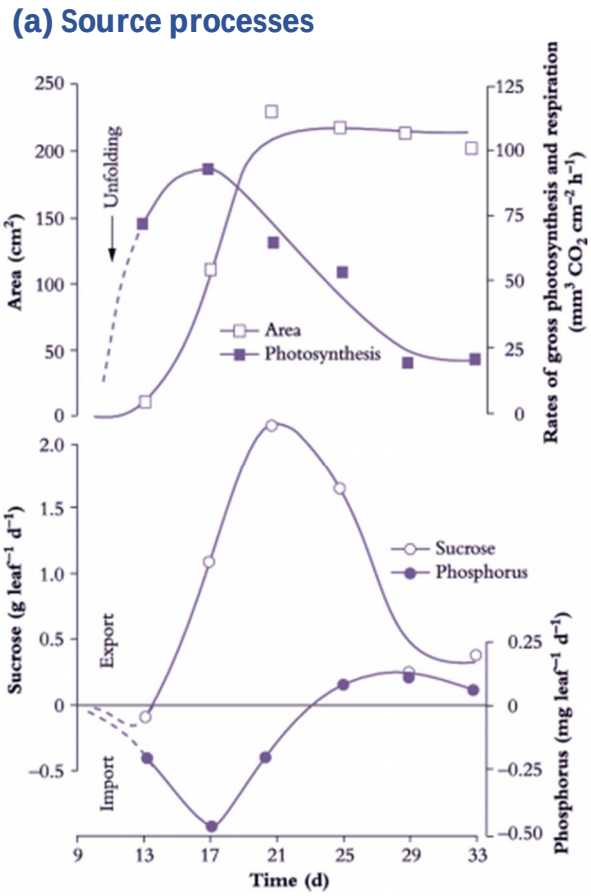



Figure 5.2 Time course of sucrose and phosphorus (P) net import and export from a leaf during its development. As a cucumber leaf expands, net sucrose export coincides with the rise in net leaf photosynthetic rate (O) to meet photoassimilate demands of young leaves. Once a leaf has reached some 30% of its final area, net photosynthesis by the whole leaf exceeds photoassimilate demand by growth and so excess sucrose can be exported. Thereafter, the rate of sucrose export closely follows photosynthetic rate, reaching a maximum when the leaf reaches its final size and gradually declining thereafter. Import of P (and other mineral nutrients) continues throughout leaf expansion and P export only starts once the leaf is fully expanded. Sucrose import and export were calculated from the difference between rates of whole-leaf photosynthesis and dry matter gain (Based on Hopkinson 1964)

Net export of photoassimilates occurs from fully expanded leaves (Figure 5.2) and long-term storage pools located along the axial transport pathway. Chloroplasts of $C_3$ plants (Chapters 1 and 2) partition photoassimilates between the photosynthetic oxidative cycle and starch biosynthesis or release them immediately to the cytosol as triose phosphate for sucrose synthesis. In non-starch-forming leaves, high concentrations of sugars can be accumulated in the vacuoles of mesophyll cells or made available for immediate loading into the phloem and export. Leaves also serve as secondary sources for nutrients and amino acids previously delivered in the transpiration stream. Nutrients and amino acids can be exported in the phloem immediately, or after accumulation in short-term storage pools.

An additional source of photoassimilates is located along the axial phloem path (petioles, stems, peduncles, pedicels and roots) as a result of leakage from the vascular tissues. Leaked photoassimilates accumulate in short- or long-term storage pools which serve as secondary sources to buffer photo-assimilate supplies to the sinks against shifts in export rates from the primary photoassimilate sources.

### (b) Path processes

Assimilates including sucrose, amino acids are transferred into sieve elements of fully expanded leaves against significant concentration and electrochemical gradients. This process is referred to as phloem loading. The cellular pathways of phloem loading, and hence transport mechanisms and controls, vary between plant species. Longitudinal transport of assimilates through sieve elements is achieved by mass flow and is termed phloem translocation. Mass flow is driven by a pressure gradient generated osmotically at either end of the phloem pathway, with a high concentration of solutes at the source end and a lower concentration at the sink end. At the sink, assimilates exit the sieve elements and move into recipient sink cells where they are used for growth or storage. Movement from sieve elements to recipient sink cells is called phloem unloading. The cellular pathway of phloem unloading, and hence transport mechanisms and controls, vary depending upon sink function.

### (c) Sink processes

Many sink organs are characterised by low rates of transpiration (an exception is a developing leaf) so that most assimilates are delivered by the phloem. Having reached the sink cell cytoplasm through the post-sieve-element transport pathway, assimilates are either metabolised to satisfy the energy, maintenance and growth requirements of sink cells or are compartmented into polymer or vacuolar storage. Collectively, metabolism and compartmentation create a demand for assimilates which is ultimately responsible for driving phloem import.

# 5.1.2 - Photoassimilate transport and biomass production

### (a) Whole-plant growth

Sink and source strength must be in balance at a whole-plant level. Thus, an increase in whole-plant sink strength must be matched by an equal increase in source strength, either through increases in source activity or source size. Prior to canopy closure in a crop, much of the increase in source strength comes from increased source size, source activity remaining relatively constant. Significantly, until a leaf has reached some 30% of its final size, photoassimilates for leaf production are exclusively imported through the phloem from fully expanded leaves (Figure 5.2).

### (b) Photoassimilate transport and crop yield

During domestication of crop plants, plant breeders selected for crop yield via maximum investment into harvested organs (mostly seeds). Total plant biomass production of advanced wheat is the same as its wild progenitors yet grain yield has increased some 30-fold through breeding. That is, whole-plant source and sink strength have not changed. Increases in wheat yield are associated with a diversion of photoassimilates from vegetative organs to the developing grain, as illustrated by the relative accumulation of $^{14}C$ photoassimilates exported from the flag leaf.

Final grain yield is not only determined by partitioning of current photoassimilates, but also depends upon remobilisation of non-structural carbohydrates stored in stems, particularly under conditions where environmental stress impairs leaf photosynthesis (Wardlaw 1990). In fact, remobilisation of reserves affects yield in many food plants. For example, deciduous fruit trees depend entirely on remobilised photo-assimilates to support flowering and fruit set as do early stages of pasture regrowth following grazing.

# 5.1.3 - Whole-plant distribution of photoassimilate

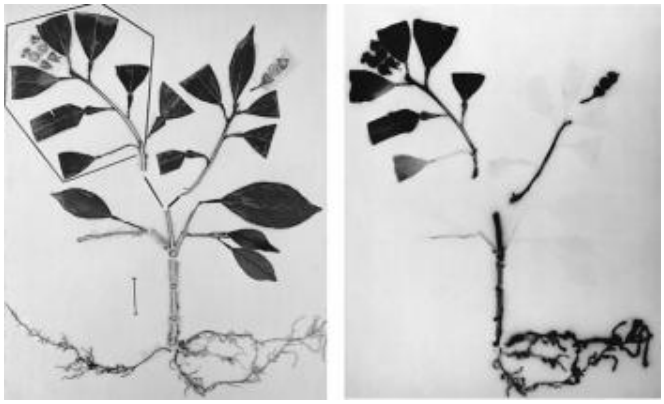


Figure 5.3 Photoassimilate distribution in a rooted cutting of Washington Navel orange (mounted specimen shown on left; matching autoradiograph on right). $^{14}CO_2$ was supplied to source leaves (boxed area top left) for a day, and movement of $^{14}C$-labelled assimilate followed by autoradiography of harvested plant material. $^{14}C$ photosynthates were distributed widely via vascular conduits to sinks including some roots and a fruit on an adjacent shoot (note stem labelling between sources and sinks). Nearby mature leaves failed to import; they were additional sources of photosynthate. Scale bar = 2 cm (Unpublished material courtesy P.E. Kriedemann)

Photoassimilate transport to harvestable organs plays a central role in crop yield brought about by greater harvest indices. This raises questions about transport and transfer processes that collectively influence photoassimilate partitioning between competing sinks.

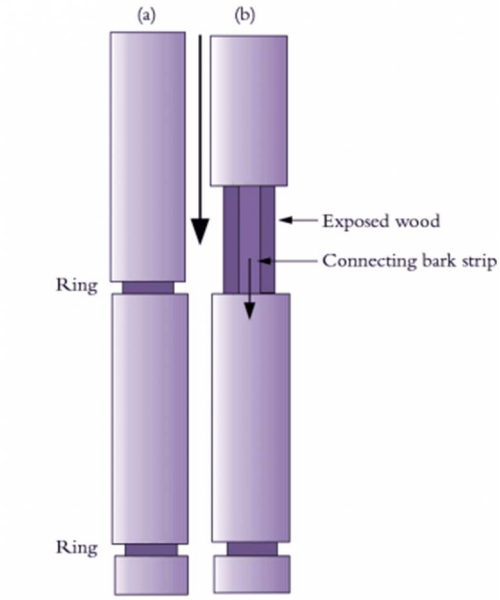
Historically, these questions were elucidated by observing partitioning patterns of photoassimilates exported from specified source leaves labelled with $^{14}C$ supplied as a pulse of $^{14}CO_2$. Following a chase period, in which $^{14}C$ photoassimilates are transported to and accumulated by recipient sink organs, the plant is harvested. The pattern of photoassimilate

partitioning operating during the pulse is deduced from $^{14}C$ activity accumulated by sinks (Figure 5.3).

Photoassimilates are partitioned from source leaves to sinks in characteristic and reproducible patterns. For instance, in a vegetative plant, lower leaves are the principal suppliers of photoassimilate to roots, whereas upper leaves are the principal suppliers to the shoot apex. Leaves in an intermediate position export equal quantities of photoassimilates in either direction. However, the pattern of photoassimilate partitioning is not static, it changes with plant development. In vegetative plants, the direction of flow from a leaf changes as more leaves above it become net exporters. Furthermore, at the onset of reproductive development, growing fruits or seeds become dominant shoot sinks for photoassimilates at the expense of vegetative apices.

Photoassimilate partitioning patterns can be altered experimentally by removal of selected sources (e.g. leaves) or sinks (e.g. fruits). These manipulative experiments demonstrate that photoassimilate partitioning reflects the relative strengths of individual sources and sinks. Properties of the phloem pathway connecting sources with sinks are shown in the following Section 5.2.

# 5.2 - Phloem transport




| Width of bark strip (% of intact stem) | 0 | 10 | 33 | 87 |
|---|---|---|---|---|
| Carbohydrate transported to the lower part of the stem in 24 h (mg) | 0 | 437 | 609 | 744 |

Figure 5.4 The role of bark (phloem) in sugar movement in plants. Mason and Maskell (1928) demonstrated that removing a complete ring of bark (a) while leaving the wood (xylem) intact prevented downward movement of sugars. When a strip of bark was retained between upper and lower stem parts (b), sugars flowed downwards in direct proportion to the width of the remaining bark.

Photoassimilate, mainly in the form of sucrose, is loaded into phloem of photosynthetically active leaves for long distance transport to nonphotosynthetic sink tissues. Figure 5.4 shows that assimilate transport occurs in phloem but not xylem. Key characteristics of phloem transport along with its chemical composition and regulation are described below.

# 5.2.1 - Phloem structure and function

## (a) Phloem structure

In most plant species, phloem is made up of phloem fibres, phloem parenchyma, sieve cells (sieve elements) and their accompanying companion cells (Figure 5.5a). Sieve elements are ideally suited for rapid transport of substances at high rates over long distances. They are elongated and are arranged end to end in files referred to as sieve tubes (Figure 5.16b). Abutting sieve elements are interconnected through membrane-lined pores (sieve pores) with large diameters (1 to 15µm). These pores collectively form sieve plates (Figure 5.16c). The transport capacity of sieve tubes is dependent on a developmentally programmed degeneration of the sieve element protoplasm (cell contents) leaving an open, membrane-bound tube. In mature conducting sieve elements, the protoplast is limited to a functional plasma membrane enclosing a sparse cytoplasm containing low densities of plastids, mitochondria and smooth endo-plasmic reticulum distributed along the lateral walls (Figure 5.16d). These relatively empty sieve tubes provide a longitudinal network which conducts phloem sap (Figure 5.5b).

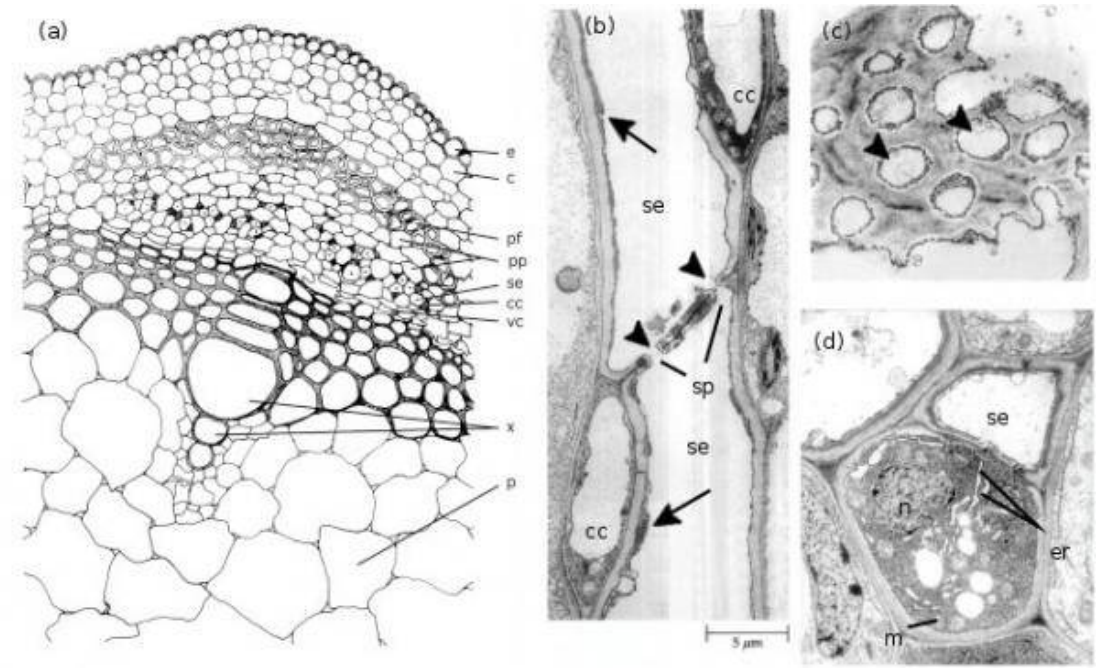



Figure 5.5 (a) spatial arrangement of cell types in a vascular strand from the primary stem of *Phaseolus vulgaris* (French bean); electron micrographs of stem phloem of *Curcurbita maxima* (b,c) and *P. vulgaris* (d) illustrating significant structural characteristics of sieve elements and companion cells. (a) Conducting cells of the phloem (sieve elements) and accompanying companion cells form groups of cells that are separated by phloem parenchyma cells. This mosaic of cells is located between the cortex and xylem and capped by phloem fibres. Scale bar = 7.3 µm. (b) A longitudinal section through two sieve elements arranged end to end to form

part of a sieve tube. Companion cells can also be seen. The abutting wall (sieve plates) displays characteristic membrane-lined sieve pores (arrowheads). Cytoplasm of the sieve elements has largely degenerated leaving only endoplasmic reticulum (arrows) and a few plastids around the mature sieve element. Scale bar = 5 μm. (c) A face view of part of a sieve plate showing sieve pores (arrowheads). Scale bar = 0.5 μm. (d) Transverse section through a sieve element and its accompanying companion cell illustrating the sparse cytoplasm and low density of organelles in the sieve element contrasting with the dense ribosome-rich cytoplasm of the nucleated companion cell. Note the mitochondria and rough endoplasmic reticulum. Scale bar = 1.0 μm. c, cortex; cc, companion cell; e, epidermis; er, endoplasmic reticulum; m, mitochondrion; n, nucleus; p, pith; pf, phloem libres; pp, phloem parenchyma; se, sieve element; sp, sieve plates; vc, vascular cambium; x, xylem

Sieve elements are closely associated with one or more companion cells, forming a sieve element–companion cell (se–cc) complex (Figure 5.5d) that plays an important role in transport. These distinct cell types result from division of a common procambial mother cell. In mature se–cc complexes, relatively open sieve elements contrast with adjacent companion cells containing dense, ribosome-rich cytoplasm with a prominent nucleus and abundant mitochondria and rough endoplasmic reticulum (Figure 5.5d). High densities of extensively branched plasmodesmata in contiguous walls of sieve elements and companion cells (Figure 5.6) account for intense intercellular coupling in se–cc complexes (van Bel 1993). Thus, companion cells are considered to perform the metabolic functions surrendered by, but required for, maintenance of viable sieve elements. This functional coupling has led to the concept of se–cc complexes being responsible for phloem transport.

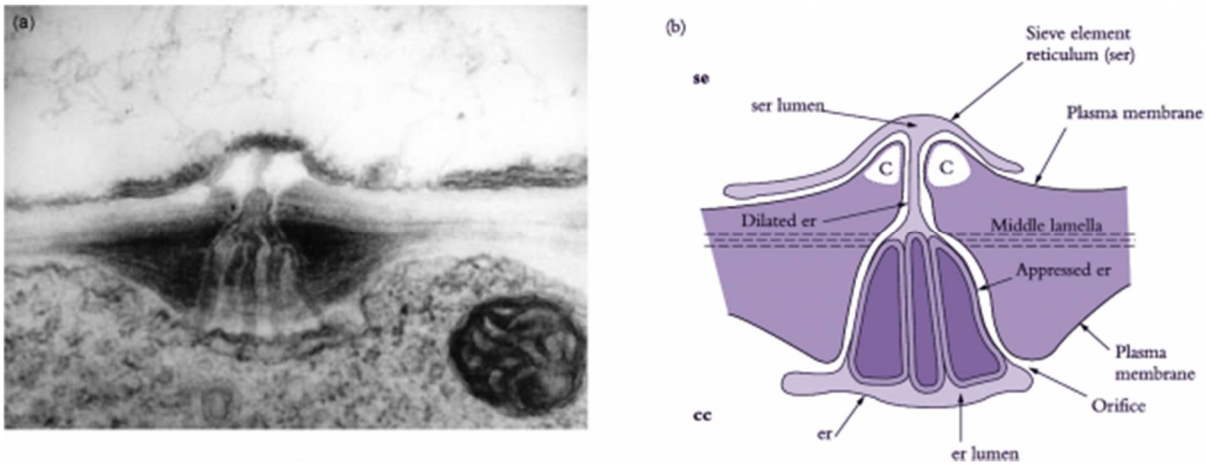



Figure 5.6 (a) Electron micrograph and (b) diagrammatic interpretation of a secondary plasmodesma interconnecting a mature sieve element and its companion cell in a tobacco leaf. Note the characteristic branching of the plasmodesma within the wall of a companion cell. Scale bar = 0.2 μm. c, callose; other symbols as for Figure 5.16 (Based on Ding *et al*. 1993)

## (b) Visualising the translocation stream

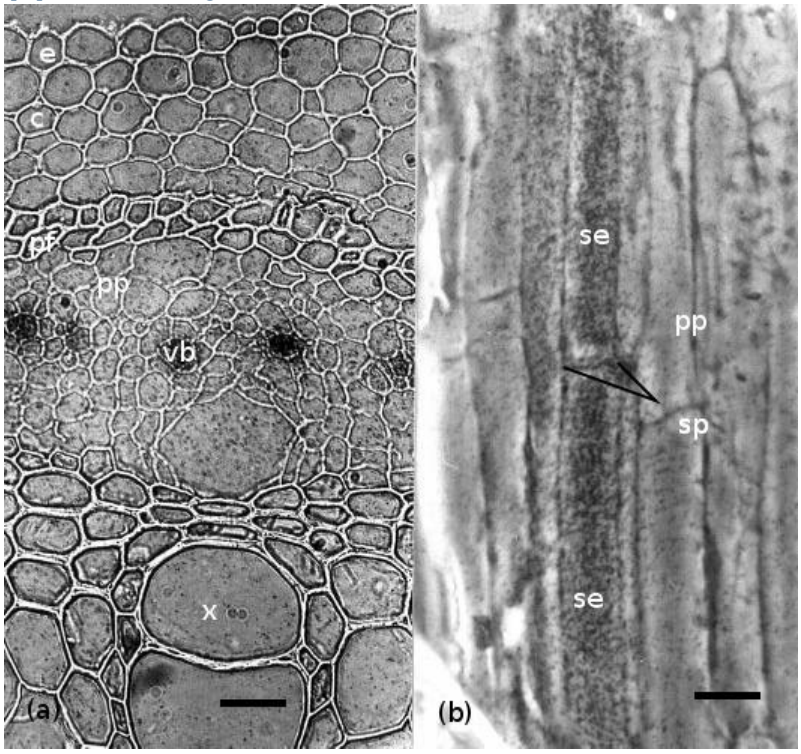



Figure 5.7 Microautoradiographs of (a) transverse and (b) longitudinal sections of *Phaseolus vulgaris*stem tissue illustrating localisation of $^{14}C$-labelled photosynthate in sieve tubes. These sections are obtained by snap freezing plant tissue and removing frozen water by sublimation (e.g. freeze-drying or freeze substitution). $^{14}C$-labelled compounds do not move during preparation. Tissues are embedded in absolute dryness and thin sections are cut, mounted dry on microscope slides and overlain with a thin film of photographic emulsion. Silver grains are visible in the emulsion where $^{14}C$, an ideal radioisotope for these experiments, irradiates the film. Abbreviations: se, sieve element; pp, phloem parenchyma; vb, vascular bundle; other symbols as for Figure 5.6. Scale bar in (a) = 20μm; in (b) = 10 μm

Transport of radioactively labelled substances through phloem has been demonstated using microautoradiography (Figure 5.7), providing irrefutable evidence that sieve elements are conduits for transport of phloem sap. Experimentally, a pulse of $^{14}CO_2$ is fixed photosynthetically and $^{14}C$-labelled sugars are given time to reach the stem, which is then excised and processed for microautoradiography. As $^{14}C$ first moves through the stem, most of the isotope is confined to the transport pathway and very little has had time to move laterally into storage pools. High densities of $^{14}C$-labelled sugars are found in sieve elements (Figure 5.7), demonstrating that these cells constitute a transport pathway.

## (c) Phloem sealing mechanisms

Herbivory or environmental factors causing physical damage could pose a threat to transport through sieve tubes and has undoubtedly imposed strong selection pressure for the evolution of an efficient and rapid sealing mechanism for damaged sieve tubes. Since sieve tube contents are under a high turgor pressure (*P*), severing would cause phloem contents to surge from the cut site, incurring excessive assimilate loss in the absence of a sealing mechanism. For dicotyledonous species, an abundant phloem-specific protein (P-protein) provides an almost instantaneous seal. P-protein is swept into sieve pores where it becomes entrapped, thus sealing off the damaged sieve tubes. Production of callose (β-1,3 glucan) in response to wounding or high-temperature stress is another strategy to seal off damaged sieve tubes.

Callose also seals off sieve pores during overwintering in deciduous plants. Callose is deposited between the plasma membrane and cell wall, eventually blocking sieve pores. Whether deposited in response to damage or overwintering, callose can be degraded by β-1,3 glucanase, allowing sieve tubes to regain transport capacity.

# 5.2.2 - Techniques to collect phloem sap

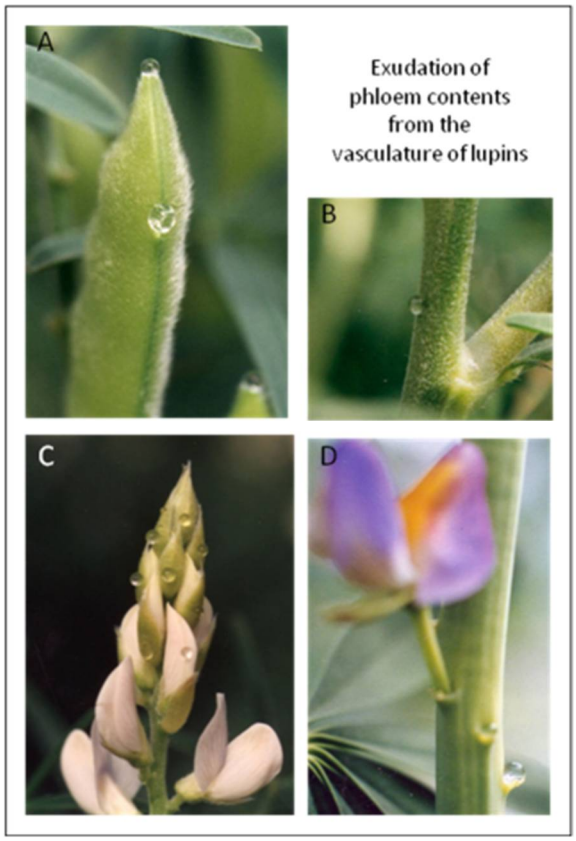



Figure 5.9. Exudation of phloem contents from lupins. A, following incision of the vasculature at the stylar tip and of the ventral suture of fruits of *Lupinus angustifolius.* B, following incision of the vasculature of a stem of *L. angustifolius*. C, following incisions to the vasculature of pre and post anthesis stage flowers on the inflorescence of *L. angustifolius.* D. exudation at the abscission zone following abscission of two flowers 5 minutes earlier on the raceme of *L. mutabilis*. Photographs courtesy Craig. Atkins.

Since phloem translocation is confined to sieve elements embedded within a tissue matrix, it is difficult to obtain uncontaminated samples of translocated sap. The least equivocal approach has been to take advantage of the high *P* of sieve tube contents. Puncturing or severing sieve tubes should cause exudation of phloem sap provided a sealing mechanism is not activated.

For some plant species, sieve-pore sealing develops slowly, or can be experimentally down-regulated by massage or repeated excisions (Milburn and Kallarackal 1989) or slowed by puncturing the vasculature while it is snap frozen in liquid N2 (Pate et al 1984). Carefully placed incisions that do not disturb the underlying xylem, which in any case is more likely to be under tension, permit collection of relatively pure phloem exudate through the severed

sieve tubes. Nevertheless, contamination with the contents of cells other than sieve tubes damaged at the site of incision is inevitable. For the major solutes of phloem such as sugars or amino acids that are present in high concentrations this problem is minimal but for less abundant molecules like hormones or other signals, particularly proteins or nucleic acids, conclusions about the origin and functions of these must be made with caution. The 'natural hemophiliacs' of the plant world are few and include a number of cucurbits, some brassicas, castor bean, species of the genus *Yucca* and some species of lupin (*Lupinus albus, L. angustifolius, L. mutabilis* and *L. cosentinii*). The excision technique has been expanded to plant species that do not readily exude, by chemically inhibiting the sealing mechanism. Callose production is blocked when wounded surfaces are exposed to the chelating agent ethylenediaminetetraacetic acid (EDTA) by complexing with calcium, a cofactor for callose synthase. Immersing whole, excised organs in EDTA solution, which is essential to inhibit blockage, risks contaminating sap with solutes lost from the apoplast as well as non-conducting cells. This is not an ideal technique.

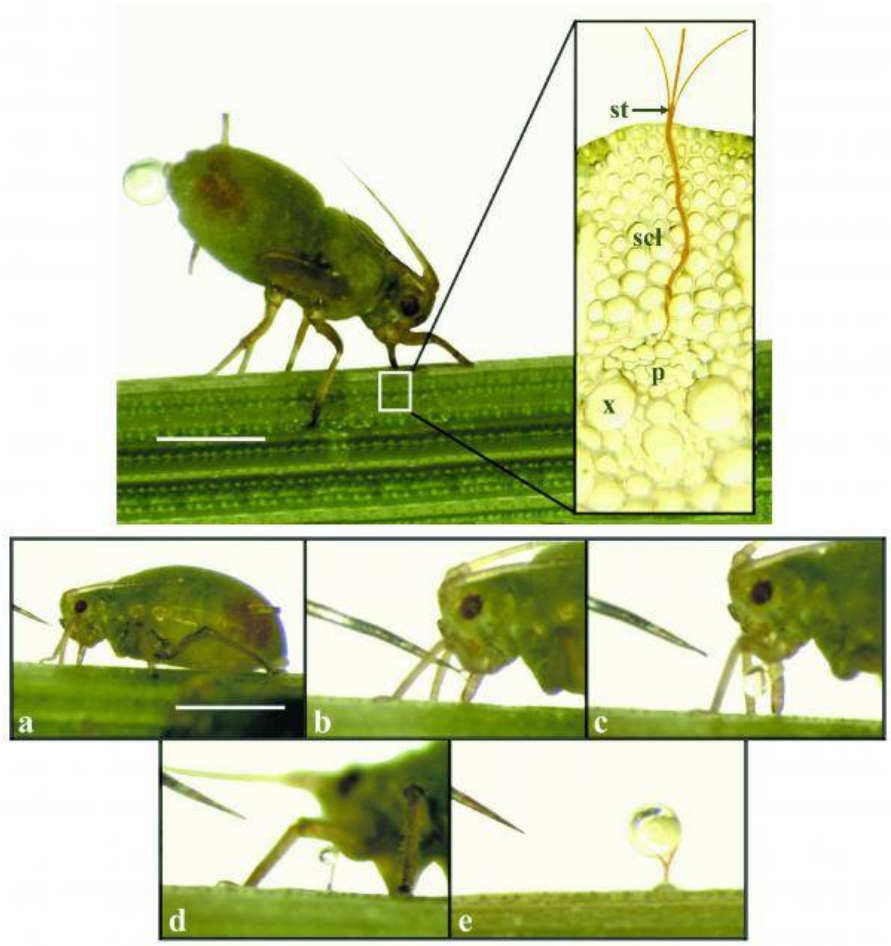



Figure 5.8 Aphids can be used to collect phloem sap. Top photograph: a feeding aphid with its stylet embedded in a sieve tube (see insert); scl, sclerenchyma; st, stylet; x, xylem; p, phloem. Note the drop of 'honeydew' being excreted from the aphid's body. Plates (a) to (e) show a sequence of stylet cutting with an RF microcautery unit at about 3-5 s intervals (a to d) followed by a two-minute interval (d to e) which allowed exudate to accumulate. The stylet has just been cut in (b); droplets of hemolymph (aphid origin) are visible in (b) and (c); once the aphid moves to one side the first exudate appears (d), and within minutes a droplet (e) is available for microanalysis. Scale bars: top = 1 mm, bottom = 1.5 mm (Courtesy D. Fischer)

Enlisting sap-sucking aphids or leaf hoppers to sample sap has been more successful. Aphids can guide a long syringe-like mouthpart (a stylet) into conducting sieve elements (Figure

5.8). Pressure normally forces sieve-tube sap through the stylet into the aphid's gut where it becomes food or is excreted as 'honeydew'. By detaching the aphid from its mouthpart pure phloem contents can be collected from the cut end of the implanted stylet. Detaching the aphid body can be achieved by surgery following rapid anesthesia in high CO2 or by severing the stylet using a laser. While stylectomy has been successful with a number of monocotyledons (rice, wheat and barley) the technique has proved more difficult to use with dicotyledons, yielding at best a few microlitres of phloem contents. On the other hand collection of milliliter volumes of exudate from one of the natural hemophiliacs is possible permitting extensive analysis of solutes and macromolecules. In the case of lupins, exudation occurs readily at many sites on the plant so that solutes translocated from source tissues as well as entering sinks can be collected and analysed (Figure 5.9).

# 5.2.3 - Chemical nature of translocated material

### (a) Chemical analysis of phloem exudate

Chemical analyses of phloem exudate collected from a wide range of plant species have led to a number of generalisations (e.g. Milburn and Baker 1989) about the contents of sieve tubes. Phloem exudate is a concentrated solution (10–12% dry matter), generating an osmotic pressure (Π) of 1.2 to 1.8MPa. pH is characteristically alkaline (pH 8.0 to 8.5). The principal organic solutes are non-reducing sugars (sucrose), amides (glutamine and asparagine), amino acids (glutamate and aspartate) and organic acids (malate). Of these solutes, non-reducing sugars generally occur in the highest concentrations (300–900 mM). Nitrogen is transported through the phloem as amides and amino acids; nitrate is absent and ammonium only occurs in trace amounts. Calcium, sulphur and iron are scarce in phloem exudate while other inorganic nutrients are present, particularly potassium which is commonly in the range of 60–120 mM. Physiological concentrations of auxins, gibberellins, cytokinins and abscisic acid have been detected in phloem exudate along with nucleotide phosphates. The principal macromolecule group is protein but low levels of peptides and nucleic acids are also present. While in cucurbits the protein in exudate is comprised largely of P-protein, a diverse array of proteins, many of them enzymes, have also been detected.

### (b) Significance of the chemical forms translocated

Phloem sap provides most inorganic and all organic substrates necessary to support plant growth. Non-transpiring tissues are particularly dependent on resources delivered in the phloem (Section 5.1). That translocated sugars represent the major chemical fraction of the phloem sap is consistent with the bulk of plant dry matter (90%) being composed of carbon, hydrogen and oxygen. Carbon transport is further augmented by transport of nitrogen in organic forms.

Carbohydrate is translocated as non-reducing sugars in which the metabolically reactive aldehyde or ketone group is reduced to an alcohol (mannitol, sorbitol) or combined with a similar group from another sugar to form an oligosaccharide. Apart from sucrose, transported oligosaccharides belong to the raffinose series. In this series, sucrose is bound with increasing

numbers of galactose residues to form raffinose, stachyose and verbascose respectively. However, sucrose is the most common sugar species transported. In a small number of plant families, other sugar species predominate. For example, the sugar alcohol sorbitol is the principal transport sugar in the Rosaceae (e.g. apple) and stachyose predominates in the Cucurbitaceae (e.g. pumpkin and squash). Exclusive transport of non-reducing sugars probably reflects packaging of carbohydrate in a chemical form which protects it from being metabolised. Metabolism of these transported sugars requires their conversion to an aldehyde or ketone by enzymes which are thought to be absent from sieve-tube contents.

Plant physiologists have long regarded the two long distance translocation streams of xylem and phloem as having functions additional to the distribution of nutrients and assimilates. Specifically, each serves as a means of communication between the source and sink organs such that systemic signals are thought to transmit molecular responses to endogenous and environmental cues. Furthermore, evidence is accumulating that some of these signals regulate gene expression as a consequence of their translocation (see below).

### (c) Macromolecule composition of phloem

Proteomic and transcriptomic analyses have demonstrated a widely diverse composition of proteins, peptides and nucleic acids, including mRNA and small RNAs, in phloem exudates. While the origin of each individual protein or nucleic acid remains to be verified the limited compositional data available from stylectomy confirms that indeed each group of macromolecules is present in phloem. In cucurbit phloem exudate some 1110 different proteins have been detected along with a large number of mRNAs and similar data have been obtained for exudates from other species (*Brassica napus*, *Ricinus communis* and *Lupinus albus*). Compositional data for phloem proteins of these species show a common complement that includes phloem-specific P proteins together with proteins involved in sugar metabolism and transport, protein turnover and transport, detoxification of reactive oxygen species, as well as proteins that provide defence against insect herbivores and pathogens (Figure 10). Some undoubtedly play a role in maintenance of the SE system while others, such as the Flowering Locus T (FT) protein associated with the flowering response (‘florigen’), appear to be systemic ‘signals’ (Rodriguez-Medina et al 2011) and there may be many more. Because sieve tubes are enucleate and lack ribosomes (5.2.2 a), proteins in the translocation stream are not formed in situ but are transported from sites of synthesis in the companion cells.

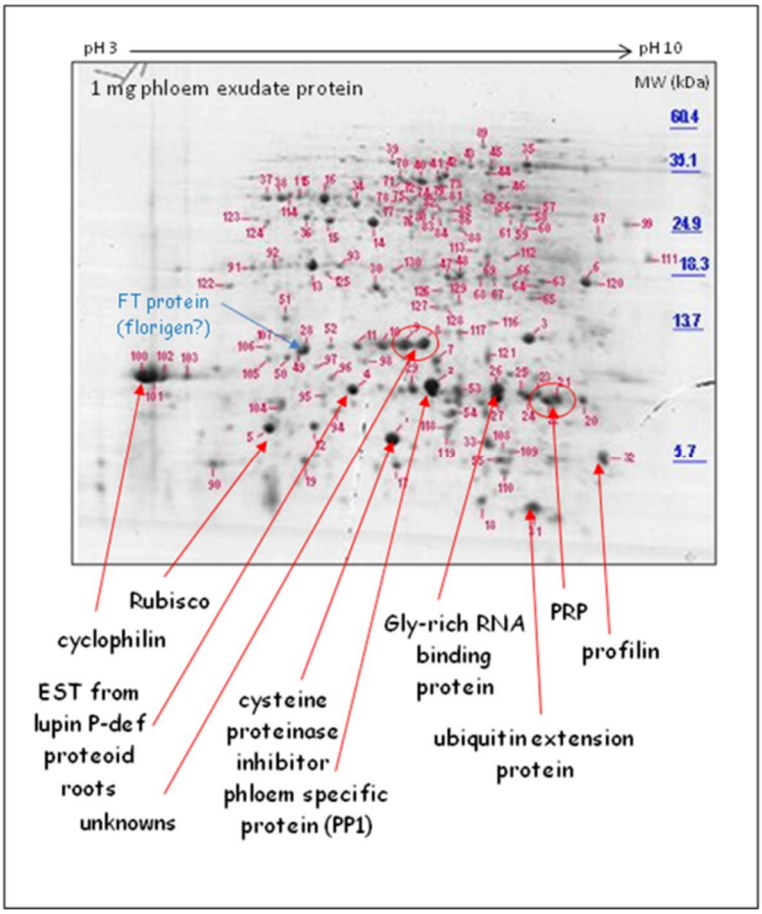



Figure 5.10. Two dimensional polyacrylamide gel electrophoresis separation of proteins in phloem exudate from *Lupinus albus*. The gel was developed in the first dimension by isoelectric focusing with a linear pH gradient of 3-10 followed by separation due to differences in molecular mass. The positions of mass standards are shown on the right hand side of the gel. After staining with Coomassie Blue to locate the spots they were excised for digestion with trypsin. The peptides were then analysed by partial sequence determination using MS/MS and identified using database searches. (Courtesy C. Atkins)

Functional analysis of the cDNA identified in transcriptome studies of phloem exudates revealed transcripts involved in a wide range of processes that include metabolism, plant responses to stresses, transport, DNA/RNA binding and protein turnover. The presence of transcripts in phloem exudate supports the idea of an RNA-based signalling network that is thought to function in control of processes associated with plant growth and development (Lough and Lucas, 2006). However, the functional role of transcripts in the contents of sieve tubes as well as their actual translocation is yet to be determined.

Small RNA molecules (18-25 nt) have been identified in phloem exudate collected from rape, white lupin, pumpkin, castor bean and *Yucca filamentosa* as well as in aphid stylet exudate collected from apple stems. The population includes both microRNAs (miRNA) and small interfering RNAs (siRNA) a large number of which target mRNA of transcription factors that themselves regulate genes expressions. miRNAs are also involved in mediating environmental responses, including responses to salinity, drought, nutrient limitations, as well as hormone interactions. Their small size and powerful functions in targeting mRNAs to regulate expression suggest that those in phloem exudate are likely to be systemic signals.

An important question that relates to the significance of macromolecules in the contents of sieve tubes is proof that they are translocated and that translocation is essential for their function at a sink. A diversity of studies that have exploited cucurbit root stocks and grafted scions has provided clear evidence that P proteins among others are graft transmissible. In a series of elegant experiments Aoki et al. (2005) labelled and injected two isolated pumpkin phloem proteins (CmPP16-1 and CmPP16-2) into the vasculature of intact rice plants through severed leaf hopper stylets and showed their translocation as well as some evidence for specificity in protein translocation. The Flowering Locus T (FT) protein formed in leaves mediates the flowering transition of shoot apical meristems and the evidence that it is translocated is compelling. The long distance movement of RNA molecules was first demonstrated for plant viruses and there is now good evidence for phloem translocation of a number of transcripts (Lough and Lucas 2006). A recent compilation identified 13 miRNAs involved in plant responses to drought/salt stress (Covarrubias and Reyes 2010). Eight of these were identified in lupin phloem exudate (Rodriguez-Medina et al. 2011) and, importantly, six were also recovered from PCR amplification of apple stylet exudate (Varkonyi-Gasic et al 2010). There is thus a possibility that the responses to drought and salinity are mediated through miRNAs translocated from sites where the stress is sensed to sites where a response is initiated.

The most convincing case for a translocated miRNA in phloem regulating gene expression relates to Pi homeostasis. While both local and systemic signals are involved, miR399 is phloem mobile and acts directly in roots to down regulate the expression of PHO2 (a ubiquitin conjugating enzyme) that results in greater expression of Pi transporters to increase Pi uptake under conditions of deficiency. Systemic signaling has also been implicated in homeostasis of other nutrients, including N, S and Cu with, in each case, miRNAs involved.

# 5.2.4 - Phloem flux

Phloem flux can be estimated in a number of ways. The simplest is to determine dry weight gain of a discrete organ connected to the remainder of a plant by a clearly definable axis of known phloem cross-sectional area. Developing fruits or tubers meet these criteria. Sequential harvests from a population of growing fruit or tubers provide measures of the organ's net gain of dry matter imported through the phloem. Net gains or losses of dry matter resulting from respiration or photosynthesis are incorporated into calculations to give gross gain in dry matter by the organ. Flux of dry matter through the phloem (specific mass transfer — SMT; Canny 1973) can then be computed on a phloem or preferably on a sieve-tube lumen cross-sectional area basis. Area estimates can be obtained from histological sections of the pedicel or stolon that connects a test organ to its parent plant. Expressed on a *phloem* cross-sectional area basis, SMT estimates are normally in the range of 2.8–11.1 g $m^{-2}$ phloem $s^{-1}$ (Canny 1973). Flux on the basis of *sieve-tube lumen* cross-sectional area is preferable but relies on identification of sieve tubes and the assumption that they are equally functional as transport conduits. Sieve tubes account for some 20% of phloem cross-sectional area, suggesting fluxes are about five-fold higher through a sieve-tube lumen.

Speed of phloem translocation can be determined from simultaneous measurements of SMT and phloem sap concentrations as shown in Equation 5.1 below:

$$\text{Speed}(m \cdot s^{-1}) = \text{SMT}(g \cdot m^{-2} \cdot s^{-1}) / \text{concentration}(g \cdot m^{-3}) \qquad (5.1)$$

For a sucrose concentration of 600 mM (or 2.16 x $10^5$ g $m^{-3}$) and the highest SMT values shown above, Equation 5.1 estimates that phloem sap can move at speeds of up to 56 × $10^{-5}$ m $s^{-1}$ or 200 cm $h^{-1}$. These estimates have been verified by following the movement of radioisotopes introduced into the phloem translocation stream.

These estimates of transport rates and speeds tacitly assume that phloem sap moves through sieve tubes by mass flow (water and dissolved substances travel at the same speed). Independent estimates of transport rate, concentration of phloem sap and translocation speed lend support to, but do not verify, the assumption that movement occurs as a mass flow.

A simple and direct test for mass flow is to determine experimentally whether water and dissolved substances move at the same speed. This test should be relatively easy to apply using radioactively labelled molecules. Unfortunately, in practice it turns out that different molecular species are not loaded into the sieve tubes at the same rates and the plasma membranes lining the sieve tubes are not equally permeable to each substance. Thus, the analysis is complicated by the necessity to use model-based corrections for rates of loading into and losses from the sieve tubes. Nevertheless, the speed estimates obtained from such experiments are found to be similar for dissimilar molecules, supporting the proposition that mass flow accounts for most transport through sieve tubes.

Phloem translocation is generally believed to be driven by pressure. Münch (1930) proposed that a passive mass flow of phloem sap through sieve tubes was driven by the osmotically generated pressure gradient between source and sink regions (Figure 5.11). At source regions, the principal osmotica of phloem sap are actively or passively loaded into sieve tubes from companion cells or mesophyll cells (see 5.3.2), thereby driving water towards the lower water potentials within sieve tubes. As water enters, *P* rises. Unloading of solutes from sieve tubes at sink regions reverses water potentials; water flows out of sieve tubes and *P* falls relative to that of sieve tubes in source regions.

The pressure-flow hypothesis can be modelled using the relationship that rate of mass flow ($F_f$) of a substance is given by the product of speed (*S*) of solution flow, path cross-sectional area (*A*) and its concentration (*C*). That is:

$$F_f = S \cdot A \cdot C \qquad (5.2)$$

Speed (m $s^{-1}$) has the same units as volume flux ($J_v$ — $m^3$ $m^{-2}$ $s^{-1}$) of solution passing through a transport conduit. Poisseuille's Law describes the volume flux ($J_v$) of a solution of a known viscosity (h) driven by a pressure difference (D*P*) applied over the length (*l*) of pathway of radius (*r*) as:

$$J_v = \pi r^4 \Delta P / 8 \eta l \qquad (5.3)$$

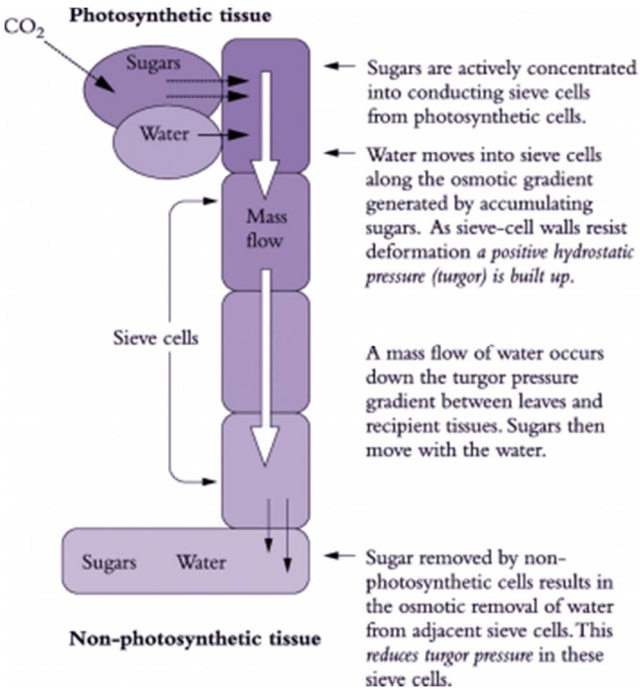



Figure 5.11 Scheme describing the pressure flow hypothesis of phloem transport (Based on Münch 1930)

The term $\pi r^4/8\eta l$ in Equation 5.3 provides an estimate of hydraulic conductivity ($L_p$) of the sieve-tube conduit which is set by the radius of the sieve pores. Raised to the fourth power, small changes in the sieve-pore radius will exert profound effects on the hydraulic conductivity of the sieve tubes (Section 5.2). The viscosity of sieve-tube sap is determined by the chemical species (particularly sugars) and their concentrations in the phloem sap.

Key features of the pressure-flow hypothesis are encapsulated in Equation 5.3. The central question is whether a pressure gradient exists in sieve tubes with the expected direction and of sufficient magnitude to support observed rates of sap flow. Indirect estimates of *P* in sieve tubes made through determination of intra- and extracellular P support the pressure-flow hypothesis. Direct measurements of sieve-tube *P* are technically challenging because of the inaccessibility of these small, highly turgid cells. They are, for instance, too small for pressure-probe measurements. However, manometric pressure measurements obtained using severed aphid stylets agree with indirect estimates (Wright and Fisher 1980). Experimental manipulation of the pressure gradient between the source and sink also results in alterations in phloem translocation rates consistent with the pressure-flow model.

Whether the pressure gradient is sufficiently steep is a more vexing question. The pressure gradient required to drive phloem translocation at observed rates is determined by the transport resistance of the phloem path, according to Ohm's Law. Dimensions of the sieve pores set a limiting radius for volume flux of transported sap (Equation 5.3) and hence transport resistance. If the sieve pores were open and unoccluded by P-protein, a number of studies have demon-strated that the measured pressure gradients are sufficient to support the observed rates of flow. However, the *in situ* radii of sieve pores remain unknown.

Overall, the pressure-flow hypothesis accounts for many observed features of phloem translocation, including distribution of resources. While conclusive evidence supporting this hypothesis is still sought, less attention is now focused on this issue with a growing appreciation that the phloem pathway has spare transport capacity. Evidence from

Kallarackal and Milburn (1984), for example, showed that the specific mass transfer (SMT – see preceding section) to an intact fruit of castor bean could be doubled on removal of competing fruits. Moreover, if *P* of sieve elements at the sink end of the phloem path was reduced to zero, by severing the pedicel and allowing exudation, SMT rose to an incredible 305 g $m^{-2}$ sieve-tube area $s^{-1}$! In another experiment, when half the conducting tissue was removed from the peduncle of sorghum or wheat plants, grain growth rate was not impaired (Wardlaw 1990). Together, these observations imply that phloem has excess carrying capacity in both dicotyledons and monocotyledons. Particularly in monocotyledonous plants, a strong selection pressure for spare transport capacity must exist because there is no vascular cambial activity to replace damaged sieve elements.

# 5.2.5 - Control of assimilate transport from source to sink

Loading of sugars, potassium and accompanying anions into sieve tubes at sources determines solute concentrations in phloem sap (Table 5.1). The osmotic pressure (Π) of these solutes influences *P* generated in sieve tubes. Thus, source output determines the total amount of assimilate available for phloem transport as well as the pressure head driving transport along the phloem path to recipient sinks. Withdrawal of assimilates from sieve tubes at the sink end of the phloem path, by the combined activities of phloem unloading and metabolism/compartmentation (Table 5.1), determines Π of phloem sap. Other sink-located membrane transport processes influence Π around sieve tubes. The difference between intra- and extracellular Π of sieve tubes is a characteristic property of each sink and determines *P* in sink sieve tubes.

**Table 5.1**
Properties and processes of the source, phloem path and sink which influence the driving variables for phloem transport and thereby determine pressure-driven assimilate flow through phloem.

| Transport component | Phloem transport variables | Property or process |
|---|---|---|
| **Source** | Assimilate concentration (*C*)<br>Turgor pressure ($P_{source}$) | Net photosynthetic rate; allocation to storage pools; phloem loading |
| **Phloem path** | Assimilate concentration (*C*)<br>Lumen cross-sectional area (A)<br>Hydraulic conductivity ($L_p$) | Unloading or reloading sieve-element number; sieve-pore radius |
| **Sink** | Turgor pressure ($P_{sink}$)<br>Compartmentation | Phloem unloading; metabolism |

The pressure difference between source and sink ends of the phloem pathway drives sap flow (Equation 5.3) and hence phloem translocation rate (Equation 5.2) from source to sink. The source and sink processes governing the pressure difference (Table 5.1) are metabolically dependent, thus rendering phloem translocation rates susceptible to cellular and environmental influences. The pressure-flow hypothesis predicts that the phloem path contribution to longitudinal transport is determined by the structural properties of sieve tubes (Table 5.1). Variables of particular importance are cross-sectional area (*A*) of the path (determined by numbers of sieve pores in a sieve plate and sieve-tube numbers) and radius of these pores (sets *r* in Equation 5.3). These quantities appear in Equations 5.2 and 5.3. Thus, the individual properties of each sink and those of the phloem path connecting that sink to its source will determine the potential rate of assimilate import to the sink (Figure 5.12).

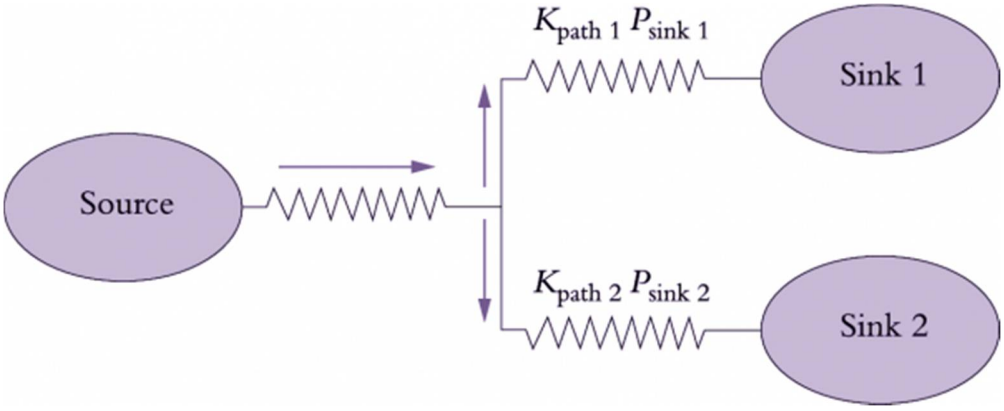



Figure 5.12. Scheme describing photoassimilate flow from a source leaf linked to two competing sinks, Sink 1 and Sink 2. Assimilate flows through alternative phloem paths (Path 1 and Path 2) each with its own conductance ($K_{path}$) and pressure difference (P) between source and sink. Hence Path 1 is distinguished by $K_{path1}$ and $P_{sink1}$ and Path 2 by $K_{path2}$and $P_{sink2}$

The transport rate (*R*) of assimilate along each phloem path, linking a source with each respective sink, can be predicted from the pressure-flow hypothesis (see Equations 5.2 and 5.3) as:

$$R = K_{path}\,(P_{source} - P_{sink})C \qquad (5.4)$$

where path conductance ($K_{path}$) is the product of path hydraulic conductivity ($L_p$) and cross-sectional area (*A*). Hence, the relative flows of assimilates between hypothetical sinks (*sink 1* and *sink 2*) shown in Figure 5.12 may be expressed by the following ratio:

$$\frac{K_{path1}\,(P_{source} - P_{sink1})C}{K_{path2}\,(P_{source} - P_{sink2})C} \qquad (5.5)$$

Partitioning of assimilates between two competing sinks is thus a function of path conductance and *P* at the sink end of the phloem path (Equation 5.5). Since phloem has spare capacity, any differences in the conductance of the inter-connecting paths (Figure 5.12) would exert little influence on the rate of phloem transport to the competing sinks. Assimilate partitioning between competing sinks would then be determined by the relative capacity of each sink to depress sieve-tube *P* at the sink end of the respective phloem path. Even when differences in path conductance are experi-mentally imposed, phloem transport rates are

sustained by adjustments to the pressure differences between the source and sink ends of the phloem path (Wardlaw 1990).

These conclusions have led to a shift in focus from phloem transport to phloem loading and unloading, which are instrumental in determining the amount of assimilate translocated and its partitioning between competing sinks, respectively.

# 5.3 - Phloem loading

Photoassimilates are loaded along the entire phloem transport pathway, from photosynthetic leaves to importing sinks. While most photoassimilate loading occurs in photosynthetically active leaves, root-produced metabolites, such as amino acids, move readily from xylem to phloem particularly at the stem nodes. Phloem loading also occurs in storage organs during periods when reserves are remobilised and exported. Indeed, the membrane transport events contributing to phloem loading were first examined using export of sucrose remobilised from the endosperm of germinating castor bean seed as an experimental model (Kriedemann and Beevers 1967).

This section focuses on phloem loading in the leaves. It analyses the cellular pathways for assimilate loading, and the regulatory controls.

# 5.3.1 - Pathway of phloem loading in source leaves

### (a) Delineating the transport path

*Phloem loading* is used variously to describe transport events outside, and inside, phloem tissues of leaves. The broader general application is adopted here — that is, phloem loading describes photoassimilate transport from the cytoplasm of photosynthetic mesophyll cells to se–cc complexes of leaf phloem.

Phloem loading commences in mesophyll cells and ends in the leaf vascular system. The se–cc complexes occur in a wide array of vascular bundle sizes. In dicotyledonous leaves, veins undergo repeated branching, forming the extensive minor vein network described in Section 5.2. For example, sugar beet leaves contain 70cm of minor veins $cm^{-2}$ of leaf blade, while the major veins contribute only 5.5cm $cm^{-2}$ of leaf blade (Geiger 1975). These observations and physiological studies (van Bel 1993) show that the principal site of phloem loading is in the minor vein network of dicotyledonous leaves. In contrast, the major veins transport loaded photoassimilates out of leaves.

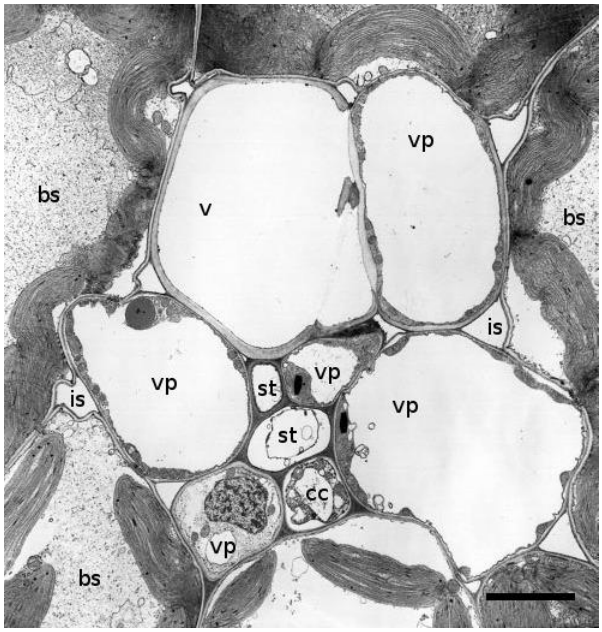



Figure 5.13. Transmission electron micrograph through a minor vein of a source leaf of maize (*Zea mays* L.) This vascular bundle consists of two sieve elements (st), one xylem vessel (v) and five vascular parenchuma cells (vp). These sieve elements are of two types, one thin walled and accompanied by a companion cell, the other thick walled and adjacent to the xylem vessel. Other symbols are: bs, bundle sheath; cc, companion cell; is, intracellular space; st, sieve tube. Scale bar - 4.2 µm (Based on Evert *et al*. 1978)

Minor veins usually comprise a single xylem element, vascular parenchyma cells and one to two sieve elements surrounded by one to four companion cells (Figure 5.13). The se–cc complex in minor veins bears similarities to that of stems (Figure 5.6). Companion cells have dense cytoplasm containing many mitochondria and are often considerably larger than the sieve elements they accompany. Companion cells are symplasmically connected to the sieve elements by branched plasmodesmata.

Cross-sectional areas of veins in monocotyledonous leaves reveal large and small parallel veins. Photoassimilates are loaded into the small veins and conducted through large veins. Fine transverse veins carry photoassimilates loaded into small veins across to large veins for export.

## (b) Cellular pathways — symplasmic versus apoplasmic

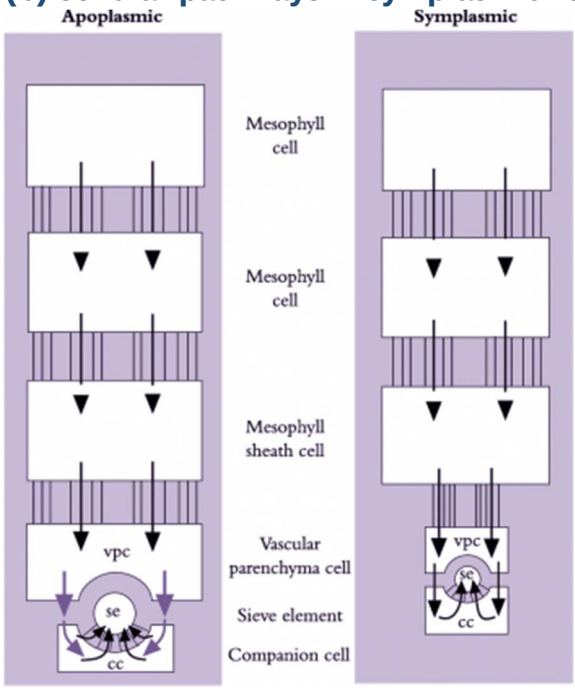



Figure 5.14. Scheme describing symplasmic and apoplasmic pathways of phloem loading. Lines without arrows joining boxes represent symplasmic continuity (i.e. plasmodesmata). Black arrows indicate symplasmic transport (i.e. through plasmdesmata); green arrows indicate apoplasmic transport requiring solutes to cross membranes. vpc, vascular parenchyma cell; se, sieve element; cc, companion cell (Based on van Bel 1993)

Photoassimilates could move intercellularly through interconnecting plasmodesmata from chloroplasts in mesophyll cells to the lumena of sieve elements (*symplasmic* phloem loading) or across plasma membranes, travelling part of the route through the cell wall continuum (*apoplasmic* phloem loading). These fundamentally different pathways are shown schematically in Figure 5.14. Debate persists over which cellular pathway of phloem loading prevails because experiments on transport from mesophyll cells to sieve elements are difficult.

Extraordinarily, the cellular pathway of phloem loading reflects evolutionary relationships. Species from ancient plant groups display symplasmic loading, while species of more modern plant groups appears to exhibit apoplasmic phloem loading (van Bel 1993). Evidence for respective routes of loading follows.

A symplasmic pathway depends upon development of extensive plasmodesmal interconnections between adjoining cells, forming a cytoplasmic continuum from mesophyll to se–cc complexes (Figure 5.14). Such symplastic continuity is found in leaves of plant families containing trees and shrubs as well as cucurbits such as squash (van Bel 1993). An abundance of plasmodesmal interconnections demonstrates potential for symplasmic transport but does not establish whether such transport actually occurs. Membrane-impermeant fluorescent dyes microinjected into mesophyll cells are transported to se–cc complexes, demonstrating that plasmodesmata can provide a route for photoassimilate transport. Furthermore, when leaves were fed $^{14}CO_2$ and treated with inhibitors that block sugar transport across plasma membranes, transport of $^{14}C$-labelled photo-assimilates continued unaffected along the enforced symplasmic unloading route (Figure 5.15; van Bel 1993). In this case, sugar levels are higher in the mesophyll than in the phloem and ions and

molecules diffuse through plasmodesmata at each interface, without a concentrating step (Turgeon 2010). Therefore, this is a passive symplasmic phloem loading.

Symplasmic phloem loading may also be an active process occurring in some herbaceous eudicots. This model of phloem loading, called polymer trap mechanism, depends on sucrose being biochemically converted to raffinose oligosaccharides (RFOs) in specialized CCs (intermediary cells - ICs) (Turgeon 2010). The biochemical synthesis of RFOs from sucrose requires metabolic energy. The synthesized RFOs exceed size exclusion limits of plasmodesmata linking mesophyll cells with ICs and therefore are trapped and accumulate to high concentrations in SE/IC complexes of minor veins for long distance transport (Turgeon 2010).

Plant species that load phloem from the leaf apoplasm are characterised by a low abundance of plasmodesmata between se–cc complexes and abutting vascular cells. However, as for symplasmic loaders, mesophyll cells of these species are interconnected by abundant plasmodesmata (Figure 5.23). Herbaceous and many crop species belong to this group of phloem loaders, including grasses (van Bel 1993). Conventional physiological observations are consistent with phloem loading in leaves of these species including a membrane transport event located somewhere between mesophyll cells and the se–cc complexes of minor veins (Figure 5.15).

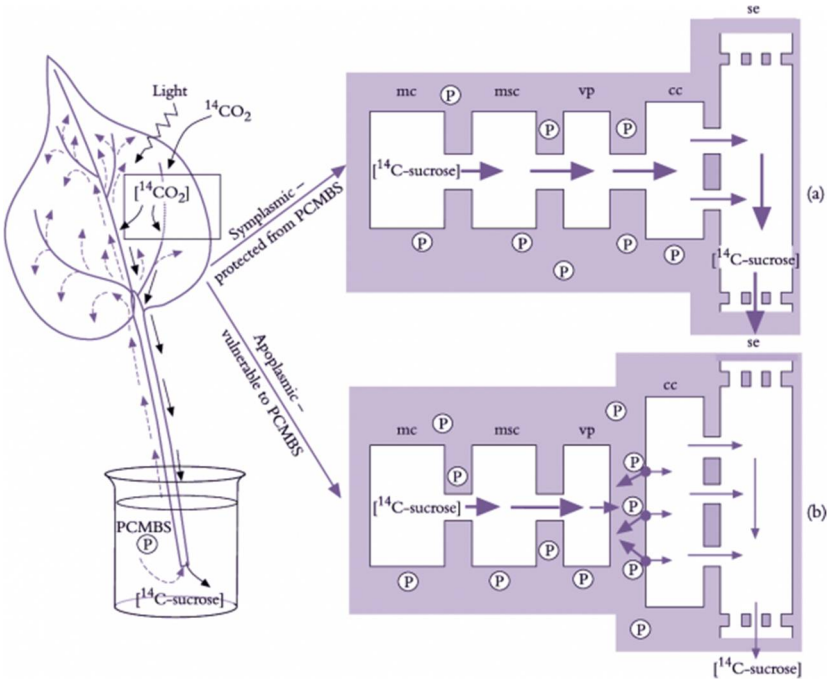



Figure 5.15. Testing whether photoassimilates move from mesophyll cells to se-cc complexes through (a) an entirely symplasmic route or (b) a route with an apoplasmic step. The approach is to use PCMBS as an inhibitor of membrane transport. PCMBS does not cross membranes but binds to the apoplasmic face of plasma membranes. Therefore, it blocks apoplasmic transport while symplasmic phloem loading is unaffected. PCMBS was introduced into the leaf apoplasm through the transpiration stream of excised leaves. Leaves were then exposed in a closed illuminated chamber to $^{14}CO_2$. The $^{14}C$ photoassimilate exported from labelled leaf blades was used to monitor phloem loading. PCMBS only reduced photoassimilate export (i.e. phloem loading) from those leaves with few plasmodesmata interconnecting se-cc complexes with surrounding cells. Thus, photoassimilate flow included a membrane transport step from the leaf apoplasm in certain plant species while others loaded via a symplasmic route. cc, companion cell; mc, mesophyll cell; msc, mesophyll sheath cell; PCMBS (para-chloromercuriben-zenesulphonic acid, also abbreviated to P); se, sieve element; vp, vascular parenchyma (Based on van Bel 1993)

Molecular biology has brought new insights to phloem loading. For instance, existence of an apoplasmic step demonstrated with PCMBS (Figure 5.15) has been elegantly confirmed using molecular biology to control activity of the sucrose/proton symporter responsible for sucrose uptake from phloem apoplasm into se–cc complexes. Specifically, potato plants were transformed with an antisense copy of the gene encoding the sucrose/proton symporter, producing a phenotype with low levels of the symporter in plasma membranes of se–cc complexes (Frommer *et al*. 1996). Excised leaves of transformed plants exported significantly less photoassimilates than wild-type plants, corroborating the inhibitory effect of PCMBS on apoplasmic phloem loading (Figure 5.15). This provides compelling evidence that passage of photoassimilates from mesophyll cells to se–cc complexes in potato leaves includes an apoplasmic step.

Vascular parenchyma cells are the most probable site for photoassimilate exchange to phloem apoplasm (van Bel 1993), ensuring direct delivery for loading into se–cc complexes. Furthermore, plasma membranes of se–cc complexes in minor veins have increased surface areas to support photoassimilate transfer from phloem apoplasm. Notably, the surface area of se–cc complexes in sugar beet leaves is surprisingly large—0.88 $cm^{-2}$of leaf blade surface. By implication, these large membrane surfaces are involved in phloem loading. Further support comes from cytochemical studies, demonstrating a great abundance of proteins associated with energy-coupled sucrose transport (Section 5.3.3(b)).

Leaf anatomies in some plant species suggest a potential for simultaneous phloem loading through apoplasmic and symplasmic pathways (van Bel 1993). Whether these pathways connect the same sieve element, different sieve elements in the same minor vein order or sieve elements in different vein orders is still unknown.

# 5.3.2 - Mechanisms of phloem loading

## (a) General characteristics

Any hypothesis of phloem loading must account for the following characteristics:

1. *Elevated solute concentration in se–cc complexes*. Estimated solute concentrations in sap of se–cc complexes is much higher than concentrations in sap of surrounding cell types, irrespective of whether phloem loading is by an apoplasmic or a symplasmic route.
2. *Selective loading of solutes into se–cc complexes*. Chemical analysis of phloem sap by techniques shown above in Section 5.2 reveals *relative solute concentrations* different from those in surrounding cells. Phloem loading is therefore a selective process.

### (b) Symplasmic loading

The above-described characteristics have been used to argue against loading of se–cc complexes through a symplasmic route on the grounds that plasmodesmata lack mechanisms for concentrating and selecting solutes. However, a contribution of plasmodesmata to concentrating and selecting solutes cannot be precluded from our current knowledge of plasmodesmal structure and function.

Plants that load se–cc complexes through a symplasmic route translocate 20–80% of sugars in the form of raffinose-related compounds such as raffinose, stachyose and verbascose (Section 5.2.3(c)). Grusak *et al.* (1996) proposed a model for symplasmic phloem loading that accounts for the general characteristics stated above. According to this model (Figure 5.16), sucrose diffuses from mesophyll and bundle sheath cells into intermediary (companion) cells through plasmodesmata. Within companion cells, sucrose is thought to be enzymatically converted to oligosaccharides (raffinose or stachyose) maintaining a diffusion gradient for sucrose from mesophyll cells into se–cc complexes. The molecular-size-exclusion limit of plasmodesmata interconnecting mesophyll and companion cells is such that it prevents back diffusion of stachyose and raffinose molecules, which are larger than sucrose. These oligosaccharides are able to diffuse through plasmodesmata with larger diameters linking companion cells with sieve elements (van Bel 1993). This model accounts for selective loading of sugars to achieve high photoassimilate concentrations in phloem elements.

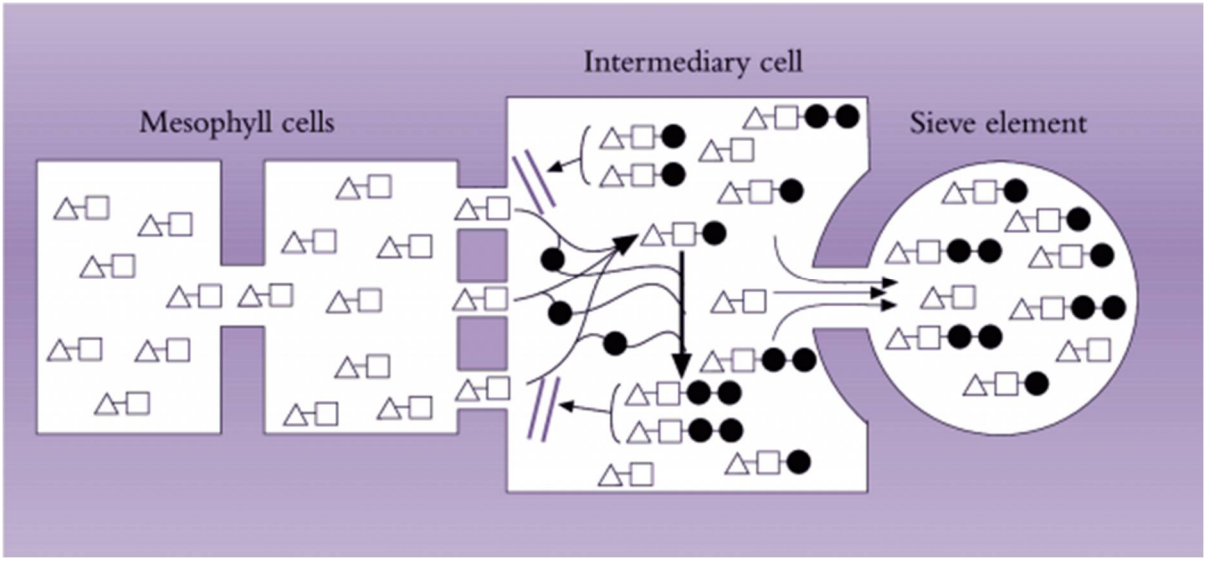



Figure 5.16. Model of the 'polymerisation trap mechanism' to explain symplasinic phloem loading against a solute concentration gradient. Sucrose moves through a symplasmic path from photosynthetic cells into intermediary (companion) cells of the minor veins. Sucrose movement is by diffusion down a concentration gradient maintained by the polymerisation of sucrose into oligosaccharides (raffinose and stachyose) in intermediary cells. Diffusion of these oligosaccharides into mesophyll cells is prevented, as their size exceeds the molecular exclusion limit of plasmodesmata joining mesophyll and intermediary cells. However, the larger-diametered plasmodesmata linking intermediary cells with sieve elements permit oligosaccharides to be loaded into sieve elements for export from the leaf. [], glucose; Δ, fructose; • galactinol (Based on Grusak et al. 1996)

### (c) Apoplasmic loading

Phloem loading with an apoplasmic step is an attractive model, explaining both how solutes become concentrated in se–cc complexes (energy-coupled membrane transport) and how they could be selected by specific membrane transporters (see van Bel 1993). Identifying transport mechanisms responsible for photoassimilate transport to and from the leaf apoplasm has proved challenging.

Based on estimates of sucrose fluxes and high sucrose concentrations in phloem sap, there is little doubt that sucrose loading into phloem is energy dependent. The demonstration that PCMBS blocks loading of photoassimilates in whole leaves of certain species (Section

5.3.2(b)) points to carrier-mediated transport across plasma membranes. Genes encoding sucrose porters have been cloned from leaf tissue (Frommer *et al*. 1996) and shown to be specifically expressed in leaf phloem. Complementation studies in yeast defective in sucrose transport suggest that the phloem-located sucrose porter catalyses sucrose/proton symport in a similar way to that illustrated in Figure 5.32. Antisense transformants of potato with low abundance of this symporter have impaired sucrose transport (Section 5.32(b)).

In contrast to photoassimilate *uptake* from phloem apoplasm, very little is known about the mechanism of sugar efflux into the apoplasm until very recently. Estimates of photoassimilate flux to phloem apoplasm, based on rates of sucrose export from leaves, suggest that this transport event must be facilitated by other transport processes (van Bel 1993). This is now confirmed by the recent cloning of sucrose efflux protein that sheds a light on the molecular mechanisms of phloem loading (Chen *et al*. 2012).

# 5.3.3 - Sink regulation of phloem loading

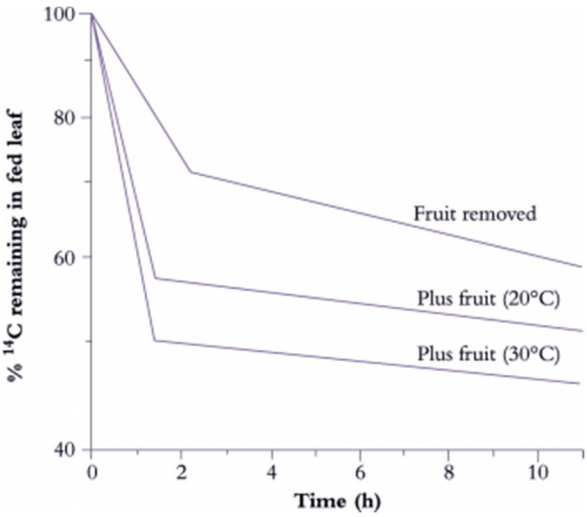



Figure 5.17. Time-course of photoassimilate export from source leaves of tomato plants. Control plants, in which fruits were a major sink for photoassimilates, were maintained at 20°C. Treatments involved (1) removing fruit or (2) exposing plants with fruits to 30°C. The proportion of $^{14}C$ label remaining in source leaves after a radioactive pulse was monitored through time to show that (1) presence of major sinks or (2) more rapid metabolism accelerated $^{14}C$ export from source leaves (Based on Moorby and Jarman 1975).

### (a) Sink effects on export

The response of photoassimilate export to changes in sink demand depends upon whether photoassimilate flow is source or sink limited (Wardlaw 1990). A source-limited system does not respond rapidly to an increase in sink demand, depending more on the capacity of leaves to increase the size of the transport pool. In contrast, alterations in sink demand in a sink-limited system elicit immediate effects on photoassimilate export. Figure 5.17 shows how the presence of fruits accelerates $^{14}C$ export, especially at high temperatures. For leaves that load the se–cc complexes from apoplasmic pools, changes in sink demand probably influence photoassimilate export by altering membrane transport properties. These changes in

membrane transport entrain a flow of adjustments in biochemical partitioning within the leaf through substrate feedback (see below).

### (b) Sink effects on membrane transport

Changes in the turgor pressure of phloem sap or altered phytohormone levels could serve as signals for sink demand.

Changes in the pressure of sink phloem sap are rapidly transmitted through sieve tubes to sources. Phloem loading in source tissues responds to this pressure signal by changes in solute transport rates mediated by membrane-associated porters (van Bel 1993). This is a proposed mechanism for phloem loading which would respond rapidly (within minutes) to changes in sink demand.

**Table 5.2**

Effect of phytohormones gibberellic acid (GA3) and indole-3-acetic acid (IAA) on rates of photoassimilate export and phloem loading of sucrose. Leaves of celery were pretreated for 1 hour with $10^{-6}$ M $GA_3$ or IAA. Subsequent rates of $^{14}C$ export from intact leaves, or *in vitro* $^{14}C$-sucrose uptake by phloem strands isolated from treated leaves (µmol g-1 fresh mass $h^{-1}$), were determined. Sucrose concentration in the bath medium was 5 mM.

| Hormone addition | $^{14}C$ export from leaf | $^{14}C$-sucrose uptake by phloem |
|---|---|---|
| **None** | 13 | 2.2 |
| **GA3** | 22 | 3.0 |
| **IAA** | 27 | 4.1 |

(Based on Daie *et al.* 1986)

Phytohormone levels in leaves respond to changes in the source/sink ratio. For instance, gibberellin levels in leaves proximal to developing inflorescences increase at fruit set. In contrast, abscisic acid levels in soybean and grape leaves are inversely related to alterations in sink demand (Brenner 1987). Therefore, changes in leaf phytohormone levels could serve to signal shifts in sink demand for photoassimilates. In this context, direct application of auxin and gibberellic acid to source leaves results in a rapid enhancement of photoassimilate export (Table 5.2). Gibberellic acid did not stimulate leaf photosynthesis or alter photoassimilate partitioning, appearing instead to upregulate phloem loading. This was confirmed by faster $^{14}C$ loading into isolated phloem strands (Table 5.2**).**

### (c) Sink influences on biochemical partitioning within source leaves

A substrate feedback response is elicited if the rate of photo-assimilate export from chloroplasts is limited by sink demand. If sucrose export from source pools is accelerated by phloem loading, substrate feedback inhibition of photoassimilate delivery is alleviated. A cascade of adjustments in the activity of key regulatory enzymes follows (see Section 2.3) with the final outcome of an increased flow of sucrose into transport pools. Conversely, if

photoassimilate flow is limited by photosynthetic rate, the activity of enzymes responsible for sucrose biosynthesis is not subject to feedback inhibition by substrates. As a consequence, responses to increased sink demand can only be mediated by increases in photosynthetic enzyme activity.

# 5.4 - Phloem unloading and sink utilisation

Photoassimilate removal from phloem and delivery to recipient sink cells (phloem unloading) is the final step in photoassimilate transport from source to sink. Within sink cells, cellular metabolism and compartmentation are the end-users of phloem-imported photoassimilates. Combined activities of these sink-located transport and transfer events determine the pattern of photoassimilate partitioning between competing sinks and hence contribute to crop yield.

*Phloem unloading* describes transport events responsible for assimilate movement from se–cc complexes to recipient sink cells. A distinction must be made between transport across the se–cc complex boundary and subsequent movement to recipient sink cells. The former transport event is termed *sieve element unloading*and the latter *post-sieve element transport*. On reaching the cytoplasm of recipient sink cells, imported photoassimilates can enter metabolic pathways or be compartmented into organelles (e.g. amyloplasts, protein bodies and vacuoles). Metabolic fates for photoassimilates include catabolism in respiratory pathways, biosynthesis (maintenance and growth) and storage as macromolecules (starch and fructans).

Compared with phloem loading, phloem unloading and subsequent sink utilisation of imported photoassimilates operate within a much broader range of configurations:

1. *morphological* (e.g. apices, stems, roots, vegetative storage organs, reproductive organs);
2. *anatomical* (e.g. provascular differentiating sieve elements, protophloem sieve elements lacking companion cells, metaphloem se–cc complexes);
3. *developmental* (e.g. cell division, cell expansion);
4. *metabolic* (e.g. storage of soluble compounds/polymers, growth sinks).

A correspondingly large range of strategies for phloem unloading and sink utilisation must be anticipated.

# 5.4.1 - Cellular pathways of phloem unloading

Most photoassimilates travel along one of three cellular pathways: apoplasmic, symplasmic or a combination of both with symplasmic transport interrupted by an apoplasmic step (Figure 5.19).

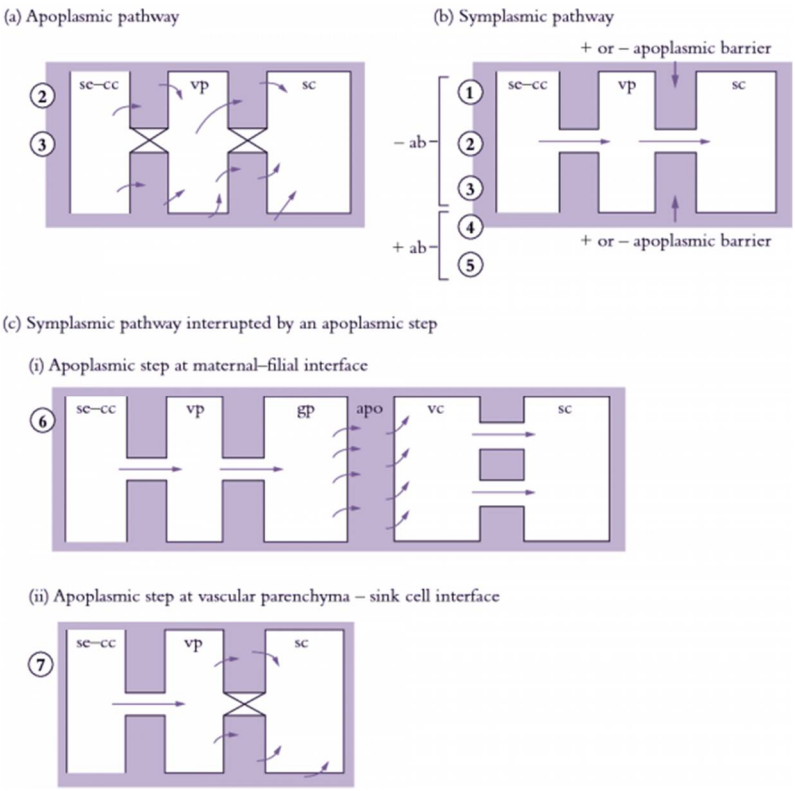



Figure 5.18. Scheme describing the cellular pathways of phloem unloading and their relationship with sink types. (a) Apoplasmic unloading showing direct transport of photoassimilates from se—cc complexes to the phloem apoplasm. (b) Symplasmic unloading pathway which may or may not have an apoplasmic barrier between sieve elements and recipient sink cells. (c) Symplasmic unloading with the intervention of an apoplasmic step at (i) the maternal-filial interface of developing seeds and (ii) the vascular parenchyma-sink cell interface. Circled numbers denote different sink types assigned to each pathway. 1, vegetative apex; 2, elongating axis of a dicotyledonous stem; 3, mature axis of a primary dicotyledonous stem i permanent storage; 4, mature axis of a primary monocotyledonous stem i permanent storage; 5, mature primary root; 6, fleshy fruit; 7, developing seed. ab, apoplasmic barrier; apo, apoplasm; gp, ground parenchyma; sc, sink cell; se—cc, sieve element—companion cell complex; vp, vascular parenchyma.

## (a) Apoplasmic pathways

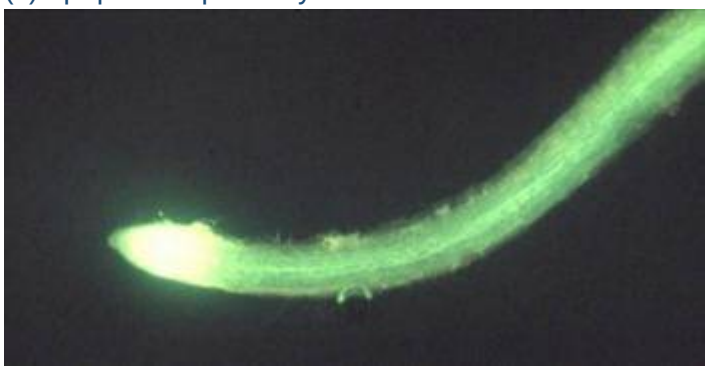


Figure 5.19. Fluorescent micrograph of the distribution of a membrane-impermeant fluorescent dye, carboxyfluorscein (CF), imported through the phloem into roots of French bean (*Phaseolus vulgaris*L.). The green/yellow fluorescence of CF is confined to the se-cc complexes of mature portions of roots as seen by the thin central band of fluorescence away from the root apex. In contrast, dye spreads through the apex itself apparently via the symplasm of young cells. Scale bar = 2 mm

Photoassimilates can move directly across plasma membranes of se–cc complexes to the surrounding apoplasm (Figure 5.19a). Apoplasmic unloading is important along the axial transport pathway of roots and stems where vascular parenchyma and ground tissues serve as reversible storage sinks.

### (b) Symplasmic pathways

An entirely symplasmic path of photoassimilate transport from sieve elements to recipient sink cells (Figure 5.18b) operates in a wide range of morphological and metabolic sink types. Terminal growth sinks such as root (Figure 5.31) and shoot apices, as well as vegetative storage sinks such as stems, roots and potato tubers, demonstrate symplasmic unloading.

In most sinks that exhibit symplasmic unloading, photo-assimilates are metabolised into polymeric forms within the recipient sink cells. Sugar cane is a notable exception because it stores sucrose unloaded symplasmically from sieve elements in parenchyma cells of stems. Stem sucrose reaches molar concentrations by this unloading route.

#### (c) Symplasmic pathway interrupted by an apoplasmic step

Symplasmic discontinuities exist at interfaces between tissues of differing genomes including biotrophic associations (e.g. mycorrhizas and mistletoes) and developing seeds (Figure 5.18c). In addition, within tissues of the same genome, plasmodesmata can close permanently or reversibly at points along the post-sieve-element pathway. This necessitates photoassimilate exchange between symplasmic and apoplasmic compartments (Figure 5.19c). For instance, photoassimilate exchange between apoplasm and symplasm has been detected in sinks that store high solute concentrations and have unrestricted apoplasmic transport between vascular and storage tissues. Developing seeds, particularly of cereals and large-seeded grain legumes (Patrick and Offler 1995), are another model for symplasmic/apoplasmic pathways.

The apoplasmic space between maternal (seed coat) and filial (embryo plus endosperm) tissues in seeds prevents symplasmic continuity in the unloading pathway (Patrick and Offler 1995). In these organs, photoassimilates are effluxed across membranes of maternal tissues and subsequently taken up across the membranes of filial tissues (Figure 5.18c). Photoassimilates are unloaded from sieve elements and transported symplasmically to effluxing cells where they are released to the seed apoplasm. Influx from the seed apoplasm by the filial generation is restricted to specialised cells located at the maternal–filial interface. The final transport of photoassimilates to the filial storage cells largely follows a symplasmic route.

### (d) Pathway linkage with sink function and pathway switching

The symplasm is the most frequently engaged cellular pathway of phloem unloading. Even where an apoplasmic step intervenes (e.g. developing seeds), photoassimilates travel predominantly through the sink symplasm (Figure 5.18c). Symplastic routes do not involve membrane transport and therefore offer lower resistances than apoplasmic routes.

Apoplasmic pathways are restricted to circumstances where (1) symplasmic transport compromises phloem translocation and (2) photoassimilate transport is between genetically distinct (e.g. maternal–filial) tissues. Phloem translocation would be compromised when

solutes accumulate to high concentrations in sink cells were it not avoided by symplasmic isolation of phloem from sinks. This is exemplified by the switch to an apoplasmic step during development of tomato fruit. In young fruit, imported sugars are converted into glucose or fructose to support cell division and excess photoassimilate is accumulated as starch. At this stage, phloem unloading of photoassimilates follows a symplasmic route (Figure 5.18b). However, once sugars commence accumulating during cell expansion, apoplasmic transport is engaged (Figure 5.18c). The apoplasmic path isolates pressure-driven phloem import from rising osmotic pressures (P) occurring in fruit storage parenchyma cells (Patrick and Offler 1996).

Radial photoassimilate unloading in mature roots and stems may switch between apoplasmic or symplasmic routes depending upon the prevailing source/sink ratio of the plant. At low source/sink ratios, photoassimilates remobilised from axial stores are loaded into the phloem for transport to growth sinks (Wardlaw 1990). Under these conditions, symplasmic unloading into axial stores might be blocked by plasmodesmal closure while photoassimilates are absorbed by se–cc complexes from the surrounding apoplasm. This would prevent futile unloading while stores are drawn upon. In contrast, net flow of photoassimilates into axial storage pools at high source/sink ratios would be facilitated by plasmodesmal opening.

# 5.4.2 - Mechanisms of phloem unloading

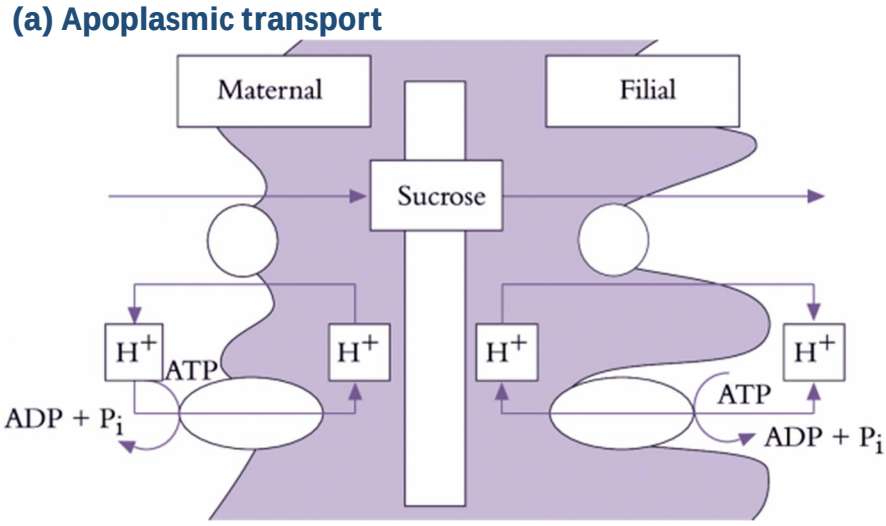



Figure 5.20. Mechanistic model for plasma membrane transport of sucrose from the coat and into the cotyledons of a developing legume seed. Plasma membrane ATPases vectorially pump protons to the seed apoplasm from both the opposing seed coat and cotyledon cells. The proton gradient is coupled to drive sucrose efflux from the seed coats through a sucrose/proton antiporter and sucrose influx into the cotyledons by a sucrose/proton symporter.

Se–cc complexes contain high sugar concentrations (Section 5.2.3(b)). Thus, a considerable transmembrane concentration gradient exists to drive a passive leakage of sugars to phloem apoplasm. Sugars leaked to phloem apoplasm are often retrieved by an active sucrose/proton symport mechanism (Figure 5.20). Thus, net efflux of sugars from se–cc complexes is determined by the balance between a passive leakage and sucrose/proton retrieval.

Passive *unloading* ($E_p$) of sucrose from se-cc complexes to the phloem apoplasm (Equation 5.6) is determined by the permeability coefficient ($P$) of se–cc complex plasma membranes and the transmembrane sucrose concentration ($C$) gradient between sieve element lumena (se) and surrounding phloem apoplasm (apo).

$$E_p = P(C_{se} - C_{apo}) \qquad (5.6)$$

Sinks containing extracellular invertase (e.g. developing tomato fruit, sugar beet tap roots, maize seeds) can hydrolyse sucrose, lowering $C_{apo}$ thereby enhancing sucrose unloading from se–cc complexes. Furthermore, hydrolysis of sucrose renders it unavailable for se–cc complex retrieval by sucrose/proton symport. The resulting hexoses can act as signals to promote cell division in many sinks such as developing seed of *Vicia faba*.

## (b) Symplasmic transport

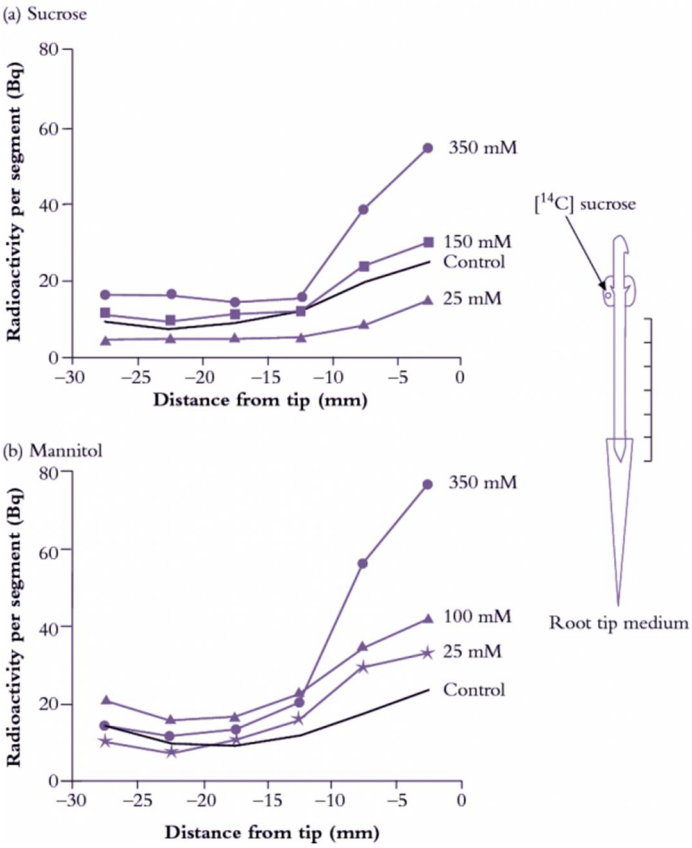



Figure 5.21. Externally supplied solutes have a marked effect on sucrose import into root tips of hydroponically grown pea seedlings. This was tested by immersing root tips in (a) sucrose or (b) mannitol solutions ranging up to 350 mM. Cotyledons, which supply these young roots with carbon, were fed 14C sucrose and 14C arriving in different root parts was measured (Bq per root segment). (a) Import of 14C into root tips was diminished when they were exposed to external sucrose concentrations of less than 100 mM but promoted by sucrose concentrations of 150 to 350 mM; Two effects operate. At low concentrations, sucrose might enter root tip cells and suppress phloem import by a feedback mechanism. At higher concentrations, sucrose might act mainly as an osmoticum (see (b)). Mannitol is not taken up or metabolised quickly and can therefore help answer these questions. (b) Import through the phloem was stimulated by exposing root tips to the slowly permeable sugar mannitol, at concentrations of from 13 to 350 mM. This demonstrates an osmotic dependence of import through the phloem pathway, presumably through progressively decreasing P of root tip cells as external solute concentrations rise (Based on Schultz 1994)

Symplasmic transport is mediated by cytoplasmic streaming in series with intercellular transport via plasmodesmata. Plasmodesmal transport is usually the overriding resistance determining transport rates between cells.

Root tips offer a useful experimental model to explore post-sieve-element symplasmic transport because of morphological simplicity and accessibility. Exposing pea root tips to low sucrose concentrations (<100mM) slowed photoassimilate accumulation (Figure 5.21a) by raising intracellular sucrose concentrations. This response to concentration gradients is consistent with a diffusion component to phloem unloading (Equation 5.7). When roots were bathed in much higher concentrations of either sucrose (Figure 5.22a) or a slowly permeating solute, mannitol (Figure 5.33b), turgor pressure ($P$) of sieve elements and surrounding tissues decreased and $^{14}C$ import rose. This is consistent with a hydraulically driven (bulk) flow of photoassimilates into the root apex. Thus, photoassimilate movement from phloem through a symplasmic path can be mediated by diffusion and/or bulk flow. The relative contribution of each transport mechanism depends on the magnitude of concentration and pressure gradients (Equations 5.6 and 5.8).

Physical laws can be used to model diffusion and bulk flow of sucrose through a symplasmic route. Sucrose *diffuses* through symplasm at a rate ($R_d$) defined by the product of plasmodesmal number in the path ($n$), plasmodesmal conductivity to diffusion ($K_d$) and sucrose concentration difference ($\Delta C$) between sieve elements and sink cell cytoplasm. That is:

$$R_d = n \cdot K_d \cdot \Delta C \qquad (5.7)$$

Transport by *bulk flow* ($R_f$) is determined by the product of flow speed ($S$), cross-sectional area of the plasmodesmal flow path ($A$) and concentration ($C$) of sucrose transported (Equation 5.2). Flow speed ($S$), in turn, is a product of hydraulic conductivity ($L_p$) of a plasmodesma and turgor pressure difference ($\Delta P$) between se–cc complexes and recipient sink cells (Equation 5.8). Flow over the entire pathway considers the number of interconnecting plasmodesmata ($n$). Thus, bulk flow rate ($R_f$) is given by:

$$R_f = n \cdot L_p \cdot \Delta P \cdot A \cdot C \qquad (5.8)$$

Equations 5.7 and 5.8 predict that sink control of symplasmic photoassimilate transport resides in plasmodesmal conductivity and/or sucrose metabolism/compartmentation.

Sucrose metabolism within sink cells influences cytoplasmic sucrose concentration and $\Pi_{sink}$. The difference between $\Pi_{sink}$ and $\Pi_{apo}$ determines $P$ (Section 4.3). Sucrose metabolism and compartmentation can affect sucrose concentration gradients and $\Delta P$, both driving forces for symplasmic transport from se–cc complexes to sink cells (Equations 5.6 and 5.8).

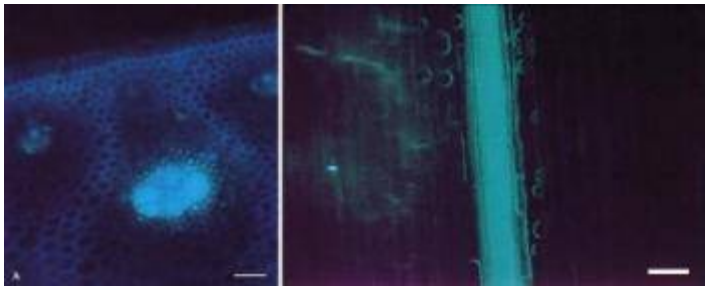


Figure 5.22. Cellular distribution of the apoplasmic tracer fluorescent dye 3-hydroxy—5,8,10—pyren—etrisulphonate (PTS) imported through the xylem in stem explants of sugar cane. (Left) Fluorescent micrograph of a longitudinal section of a stem with PTS (green fluorescence) localised to the vascular bundle. (Right) Fluorescent micrograph of a transverse section showing PTS confined to the vascular bundle. Retention of PTS in vascular bundles demonstrates that a barrier to lateral dye movement must be located in the walls of bundle sheath cells (bs). (Based on Jacobsen et al. 1992)

**Table 5.3**

Effect of a decrease in sucrose synthase activity in transgenic potato plants on final tuber dry weight and sugar content. The data are from one transgenic plant that exhibited the lowest activity, only 4% of the sucrose synthase activity in the wild-type plant. Growth and sucrose synthase activity were not affected in shoots of the transgenic plant.

| | Wild type tuber | Transgenic tuber |
|---|---|---|
| **Tuber dry weight (g)** | 4.8 | 2.4 |
| **Starch (% dry weight)** | 90 | 30 |
| **Sucrose % dry weight** | 1.5 | 2.5 |
| **Hexoses (% dry weight)** | 0.1 | 9.3 |

(After Zrenner *et al.* 1995; reproduced with permission of Blackwell Science)

Transgenic plants which under- or over-express key sugar metabolising enzymes have allowed definitive experiments to be carried out on the role of sucrose metabolism in symplasmic phloem unloading. For example, reduction of sucrose synthase activity (Section 5.4.4) in tubers of transformed potato to 5–30% of wild-type levels depressed dry weight of tubers and starch biosynthesis (Table 5.3). Tubers of transformed plants had very high hexose levels (hence high P) which might contribute to downregulation of photoassimilate import. As a corollary, plants with enhanced starch biosynthesis through overexpression of the key starch synthesising enzyme, ADP-glucose pyrophosphorylase (Section 5.4.5), also had higher rates of photoassimilate import.

For sinks that store sugars to high concentrations (e.g. sugar cane stems), gradients in Π, and hence *P*, between se–cc complexes and sink storage cells could become too small to sustain transport. Instead, P in the apoplasm of storage tissues increases as sucrose (hence Π) in the storage cell sap rises. This maintains a lower *P* in storage cells than in sieve elements and sustains transport. High sucrose concentrations in the apoplasm of storage cells is achieved through an apoplasmic barrier which isolates storage parenchyma cells from sieve elements (Figure 5.22).

## (c) Symplasmic transport interrupted by an apoplasmic step

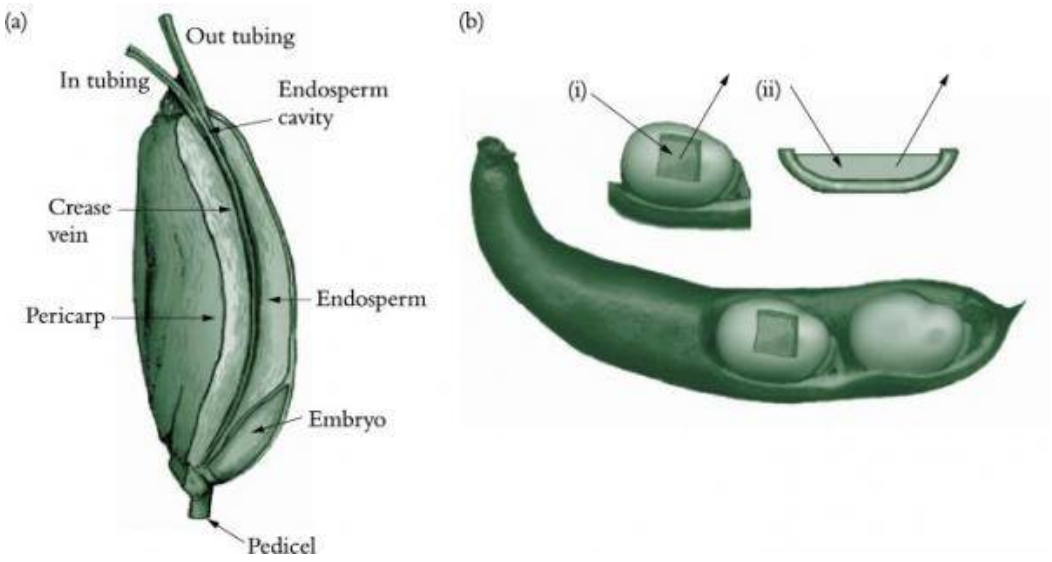



Figure 5.23. Experimental systems used to determine sucrose fluxes in (a) attached caryopses of wheat and (b) coats of developing legume seed. In (a), sucrose effluxed from the maternal tissues was collected by infusing the endosperm cavity of an attached wheat grain with solutions delivered and retrieved through micro-capillaries (Wang and Fisher 1994). In (b), embryos are surgically removed from the coats which may be (i) attached to or (ii) detached from the pod wall. The space vacated by the embryo is filled with a wash solution that is changed at frequent intervals. The wash solution is used to deliver treatments to the seed coat and as a trap to collect the effluxed sucrose.

Phloem unloading in legume seed pods is one case of symplasmic and apoplasmic transport operating in series; the pathway is described in Section 5.4.2(c). Whether sucrose efflux requires energy remains unknown since concentration gradients between seed coats and apoplasm might be steep enough to drive facilitated diffusion. Indeed, using an elegant infusion technique (Figure 5.23a), Wang and Fisher (1994) concluded that efflux from the nucellar projection cells of wheat grain was unlikely to be energy dependent. In contrast, sucrose efflux from coats (maternal tissue) of surgically modified legume seeds (Figure 5.23b) is inhibited by about 50% in the presence of PCMBS, a membrane transport inhibitor. Efflux from legume seed coat cells exhibits charac-teristics of a sucrose/proton antiport. Sucrose uptake by filial tissues is mediated by sucrose/proton symport (Figure 5.23).

A fascinating aspect of phloem unloading in legume seed pods is how photoassimilate demand by filial tissues is integrated with supply from maternal tissues, itself an integration of photoassimilate efflux and import from phloem. One variable that could regulate rates of photoassimilate transport through seed coat symplasm and efflux into apoplasm of the maternal–filial interface is *P of seed coat cells* ($P_{sc}$): this would sense depletion of apoplasmic sucrose through uptake by cotyledons, producing a signal in the form of a $\Delta P_{sc}$ (Figure 5.24). Specifically, $P_{sc}$ is determined by $\Delta\Pi$ between the seed coat ($\Pi_{sc}$) and seed apoplasm ($\Pi_{apo}$), which fluctuates according to photoassimilate withdrawal by cotyledons.

A pressure difference ($\Delta P$) between the points of photo-assimilate arrival (sieve tubes) and efflux (seed coats) drives bulk flow of photoassimilates through the seed coat symplasm. Turgor pressure of seed coat efflux cells is maintained homeostatically at a set point ($P_{set}$) by *P-dependent efflux* into the seed apoplasm. Changes in apoplasmic assimilate

concentrations and hence Π are sensed immediately as deviations of $P_{sc}$ from $P_{set}$. A rise in $P_{sc}$ produced by photoassimilate depletion around filial tissues elicits an error signal, activating *P*-dependent solute efflux (Figure 5.25b) and thereby raising photoassimilate concentrations in the apoplasm to meet demand by cotyledons (Figure 5.24c). Long-term increases of sucrose influx by cotyledons, for example over hours, are accompanied by adjustments in $P_{set}$ (light to dark arrows in Figure 5.24b) which elicit commensurate increases in phloem import rates (light to dark arrows in Figure 5.24a).

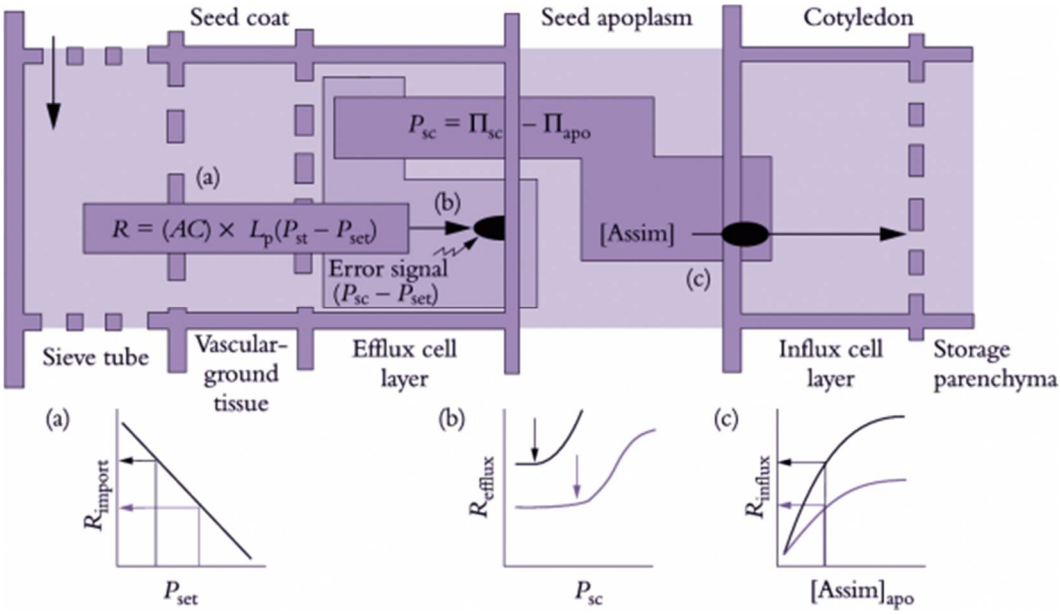



Figure 5.24. A turgor-homeostat model describing the integration of photoassimilate transport to developing legume seeds. Photoassimilate import through phloem (a) and efflux from seed coat to seed apoplasm (b) is mediated by a turgor (*P*)-dependent efflux mechanism and uptake of sugars by cotyledons (c). Metabolic activity in growing seeds influences sucrose concentrations within cotyledon cells, possibly feeding back on activities of symporters located in plasma membranes of the cotyledon dermal cell complex. Graph (c) denotes increased influx (*R*) from light to dark curve as sugar demand increases. The apoplasmic solute pool size is small and turns over in less than one hour (Patrick and Offler 1995). Thus, faster photoassimilate withdrawal from the seed apoplasm by cotyledons will rapidly lower apoplasmic osmotic concentration. Since the osmotic difference between seed apoplasm ($\Pi_{apo}$) and seed coat ($\Pi_{sc}$) is only 0.1-0.2 MPa, a small decrease in osmolality of the apoplasm will elicit a significant increase in seed coat *P* ($P_{sc}$).A shift in $P_{sc}$ above the turgor set point ($P_{set}$) results in an error signal (see model) which in turn induces an immediate compensatory increase in photoassimilate efflux to the apoplasm (light curve in graph b). Increased photoassimilate efflux acts to maintain a constant $\Pi_{apo}$ in spite of enhanced flux through the apoplasm (graph c). Consequently, the increased potential for photoassimilate uptake by cotyledons can be fully realised (dark curve in graph c). In the short term (minutes), the turgor-homeostat ensures that $P_{sc}$ is maintained and hence phloem import, which is driven by the turgor difference between sieve tubes and unloading cells ($P_{st}$- $P_{sc}$) is also maintained. Under conditions where cotyledon demand is sustained, $P_{set}$ in the seed coat adjusts downwards. This results from decreases in $\Pi_{sc}$, while $\Pi_{apo}$ is homeostatically maintained. The decrease in $P_{set}$ of efflux cells serves to enhance the pressure difference between these cells and the importing sieve elements. As a result, the rate of phloem import into seed coats ($R_{import}$) is increased (graph a, light to dark curve). This new rate of import is commensurate with accelerated sucrose efflux from seed coats to the apoplasm (graph b, light to dark curve) and, ultimately, cotyledons.

# 5.4.3 - Sugar metabolism and compartmentation in sinks

The fate of imported photoassimilates depends on sink cell function. In broad terms, imported photoassimilates are primarily used to provide carbon skeletons or signals for *growth* or *storage*. Some photoassimilates provide energy for *maintenance*. Relative flows of photoassimilates to these fates change during cell development and sometimes over shorter time scales depending upon a plant's physiological state.

### (a) Cell maintenance

Irrespective of sink function, a portion of imported sugars is respired to provide energy (ATP) for maintenance of cell function and structure. Most of this energy is required for continual turnover of cellular constituents such as enzymes and mem-branes. Rates of synthesis and degradation of individual macromolecules vary widely, as does the energy invested in different molecular configurations, so sugar demand for maintenance respiration could differ substantially between tissues.

### (b) Cell growth

In growing organs, photoassimilates become substrates for synthesis of new cell material either directly or after biochemical conversions. Other fates for sugars include catabolism in energy-generating pathways which support growth (growth respiration) and storage in vacuolar pools. Stored sugars make an osmotic contribution to growing cells and can act as energy stores in species such as sugar cane. In roots of young barley plants, 40% and 55% of imported sugars are respired and used in structural growth, respectively. Stored sugars turn over each 30 min but account for only 1% of root weight.

### (c) Reserve storage in cells

In mature cells, imported sugars enter physical (e.g. vacuoles) and chemical (e.g. starch) storage pools with lesser amounts diverted to respiration (15–20%) and structural components. In contrast to growth sinks, stored carbohydrates are ultimately retrieved from storage pools and used by other storage sinks (e.g. germinating seeds) or translocated to support growth and storage processes elsewhere in the plant. Carbohydrate storage can be brief (hours, days) or extend over considerable periods (months to years). Short-term storage of carbohydrates in stems and roots buffers phloem sap sugar concentrations against changes in photoassimilate export from photosynthetic leaves.

Sugars can also be stored in soluble forms by compartmentation into vacuoles. In this case, the tonoplast provides a physical barrier to protect stored sugars from molecular interconversion by cytoplasmic sugar-metabolising enzymes. Vacuolar sugars are accumulated as sucrose, hexoses or fructans (short-chain polymers of fructose). Sucrose and hexoses can accumulate to molar concentrations (0.1–1.5M) in storage parenchyma cells of roots, stems and fruits. For instance, tap roots of sugar beet and stems of sugar cane accumulate 1M sucrose thereby providing 90% of the world's sucrose. Hexoses are a common form of sugar storage in fruit, contributing to sweetness of edible fruits such as tomato, grape, orange and cucumber. The wine industry depends upon hexoses accumulating

to high concentrations (1.5M) in grape berries to fuel fermentation of ‘must’ in wine making. Fructans are stored in significant quantities in leaf sheaths and stems of temperate grasses and cereals. In pasture species, they contribute to forage quality, and in cereals constitute an assimilate pool that is mobilised to support grain filling.

Alternatively, imported sugars may be stored as starch along the axial transport pathway (available for remobilisation to buffer phloem sap sugar concentrations) or in more long term storage pools of terminal sink organs such as tubers, fruits and seeds. The proportion of photoassimilates diverted into starch differs widely between species and organs. Starch accounts for some 90% of dry weight of potato tubers and cereal grains.

The chemistry of storage products can change during organ development. For instance, starch is the principal storage carbohydrate in young tomato fruit. Later in fruit development, stored starch is hydrolysed and contributes to hexose accumulation in vacuoles of fruit storage parenchyma cells. In other fruits, significant switches between hexose and sucrose accumulation occur during development. All these changes are brought about by ontogenetic shifts in activities of sugar-metabolising enzymes.

# 5.4.4 - Key transfer events in sugar metabolism and compartmentation

Phloem-imported sucrose can reach the cytoplasm of recipient sink cells chemically unaltered or be hydrolysed en route by extracellular invertase into its hexose moieties. These sugars may then enter a number of metabolic pathways or be compartmented to vacuolar storage (Figure 5.25).

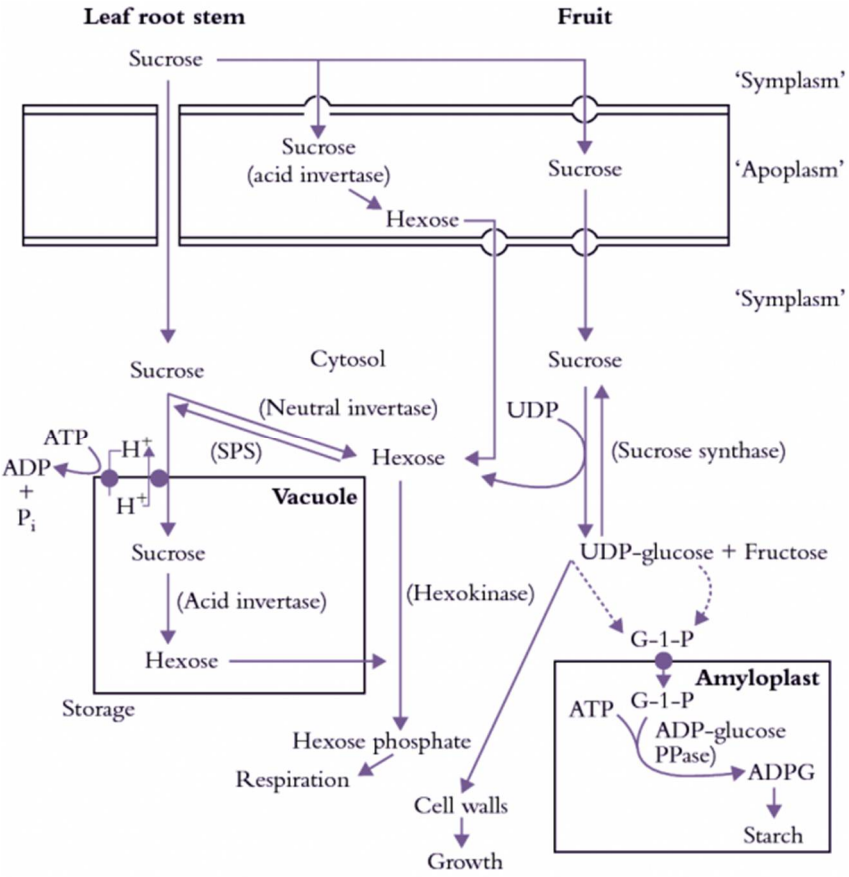



Figure 5.25. Pathways of sugar metabolism and compartmentation within sink cells. Sugars can be delivered to sink cells through either apoplasmic or symplasmic pathways. Within the sink apoplasm, sucrose can be hydrolysed to hexoses by an extracellular invertase. Apoplasmic sugars are transported across plasma membranes of sink cells by proton/ sugar symporters. Alternatively, sucrose enters the sink cytoplasm through a symplasmic path. Within the sink cytoplasm, sucrose can be hydrolysed or compartmented into vacuolar storage. Sucrose hydrolysis provides substrates for energy metabolism or for synthesis of macro-molecules. Invertase activity is important to sustain hexose supply for glycolysis. *Sucrose synthesis* from the hexose pool is catalysed by sucrose phosphate synthase (SPS). *Degradation of sucrose* by sucrose synthase generates fructose and uridine diphosphate glucose (UDP-glucose) which enters various biosynthetic pathways including cellulose and starch synthesis. In the case of starch biosynthesis, UDP-glucose and fructose generate glucose-1-phosphate (G-1-P) which is transported across the amyloplast membrane. Accumulated G-1-P is interconverted into adenine diphosphate glucose (ADP-glucose) by the enzyme adenine diphosphate glucose pyrophosphorylase (ADP-glucose PPase). ADP-glucose is the substrate for starch polymer formation. Sucrose compartmentation into the vacuole is mediated by a sucrose/ proton antiporter located on the tonoplast. Within the vacuole, sucrose can be exposed to invertase hydrolysis with the hexose products accumulating or leaking back to the cytoplasm.

## (a) Sucrose metabolism

Sucrose is metabolically inert and, in order to be metabolised, must be hydrolysed to glucose and fructose. Only two enzymes are capable of metabolising sucrose in green plants. These are invertase and sucrose synthase (Figure 5.25) and they are paramount in sugar metabolism after phloem unloading.

Invertase catalyses irreversible hydrolysis of sucrose to its hexose moieties, glucose and fructose. Both acid and neutral invertases occur in plants, with pH optima of about 5 and 7.5, respectively. The activity of invertases varies with plant species, organ type and stage of development. Acid invertases, located in cell wall or in vacuole, are usually active in rapidly growing leaves, stems and fruits and seeds (Ruan *et al*. 2010), making hexoses available for

regulating gene expression and for respiration and biosynthesis. Reduced acid invertase activity in vacuoles during development of sugar cane stems, and its absence from sucrose-accumulating tomato fruit, is a major factor in sucrose accumulation in vacuoles of these tissues. Suppression of cell wall invertase activity led to shrunken seed in maize and small fruit in tomato and loss of pollen fertility in tomato, wheat and rice, demonstrating its critical roles in these reproductive organs. Less is known about the physiological role of neutral invertases..

Sucrose synthase is mainly located in the cytoplasm but recent research also shows that the enzyme may also be associated with plasma membrane and even present in cell wall matrix. It catalyses sucrose cleavage to fructose and UDP-glucose, a high-energy ester of glucose. UDP-glucose is a substrate for biosynthesis of cellulose and may be converted further for starch synthesis.High activities of sucrose synthase are found in both growing and starch storage tissues. In the cytoplasm of starchy tissues, UDP-glucose is converted by UDP-glucose pyro-phosphorylase to glucose-1-phosphate, which is transported across amyloplast membranes. In amyloplasts, glucose-1-phosphate provides glucose moieties for starch synthesis in a pathway comparable to starch formation in chloroplasts of photosynthetic leaves. The critical role of sucrose synthase in starch synthesis is demonstrated with potatoes transformed with an antisense construct of the gene encoding tuber-specific sucrose synthase. Tuber sucrose synthase activity in transformed plants was depressed significantly while the activities of key starch biosynthetic enzymes were unaltered. Low sucrose synthase activity was directly responsible for a proportional decrease in starch accumulation (Zrenner *et al*. 1995).

Sinks that accumulate soluble sugars have predictably low sucrose synthase activities. Contrastingly high sucrose synthase activities in phloem vessels may be responsible for energy production for phloem loading or unloading and maintaining cellular function of companion cells.

### (b) Hexose metabolism

Hexoses transported to the sink cytoplasm are rapidly phosphorylated to hexose-6-phosphates by glucose- and fructose kinases. In these forms, hexoses can be used as substrates for respiration or for synthesis of new cell constituents. Alternatively, sucrose phosphate synthetase can convert them to sucrose, as in leaves (Chapters 1 and 2). Sucrose synthesized by this reaction can be accumulated in vacuoles (e.g. sugar beet tap roots, sugar cane stems) or be rehydrolysed into hexoses by a vacuolar acid invertase (e.g. grape berries).

# 5.4.5 - Sink control of photoassimilate partitioning

The pressure-flow hypothesis provides a compelling model to explain sink strength in plants. Evidence such as accelerated import of photoassimilate into roots with artificially lowered *P* lends empirical support to the model.

Knowledge of cellular and molecular events in phloem unloading and photoassimilate use begins to reveal the array of control steps which underlie photoassimilate unloading and relative sink strength. Photoassimilate import into sinks by apoplasmic pathways or by diffusion through symplasmic pathways (Figure 5.19) is controlled by *P* in se–cc complexes. In contrast, when phloem unloading is by bulk flow through a symplasmic route (e.g. legume seed coats), *P* in cells responsible for photoassimilate efflux to the apoplasm controls unloading. Unloading into storage tissues is controlled by *P* in sink (storage) cells.

These processes at the cell and tissue level must now be related to a whole-plant perspective of sink control of photoassimilate partitioning, taking into account influences of plant development and environmental factors. How plants use photoassimilates (e.g. switch from growth to storage) is accompanied by alterations in the cellular pathway of import. Phytohormones also play a role in photoassimilate partitioning through their influence on development and intercellular signalling.

Plant development generates new sinks, for example in meristems where cells undergo division or growth zones where enlarging cells import photoassimilates.

### (a) Meristematic sinks

Potential sink size is set largely during the meristematic phase of development through determination of total cell number per organ. Photoassimilate supply has been implicated as a limiting factor in initiation of leaf primordia at the apical dome and subsequent early development by cell division. Substrate supply for developing seeds (endosperm, embryo) and root and floral apices might also be restricted.

Agricultural yields might therefore increase if plants could be modified to enhance the supply of photoassimilates to meristems. Which factors regulate photoassimilate supply to meristematic sinks? Rate equations describing mass flow of phloem sap (Section 5.2.5) predict that photoassimilate supply reaching a sink will be determined by source output (setting photoassimilate concentration in sap and *P* in sieve elements at the source) and modulated by $L_p$ of the transport pathway. Increased photoassimilate output from source leaves increases growth activity of primary meristems. Even during reduced source output, photoassimilate import and meristematic sink strength can be maintained by remobilisation of storage reserves. Manipulating competition for photo-assimilates by more established sinks also suggests that source output influences sink behaviour.

Cultivated plants demonstrate these principles. For example, flushing $CO_2$ into glasshouses increases flower set and hence yield of floral and fruit crops. Similarly, applying growth regulators to induce abscission of some floral apices lessens the number of sinks competing for photoassimilates at fruit set and leads to larger and more uniform fruit at harvest. Alternatively, breeding programs have reduced sink strength of non-harvestable portions of crops and hence the severity of competition. For instance, breeding dwarf varieties of cereals has reduced photoassimilate demand by stems with a consequent increase in floret numbers set and grain size.

These observations imply that increases in net leaf photosynthesis and phloem loading should set higher yield potentials. Yet meristems import only a small proportion of total plant photoassimilate. It may be that phloem conductance limits photoassimilate delivery to

meristems; increases in source output would amplify the driving force for transport and hence bulk flow through a low-conductance pathway.

Given that *mature* phloem pathways have spare transport capacity (Section 5.2.5), any transport limitation imposed by low path conductance might be expected within immature sinks. Photoassimilate import into meristematic sinks involves transport through partially differentiated provascular strands that might extend up to 400µm. Movement through this partially differentiated path is symplasmic (Section 5.2.2(b)). Hence, plasmodesmal numbers and transport properties of plasmodesmata could play a critical role in photoassimilate supplies to sinks and determination of sink size (Equation 5.7).

### (b) Expansion and storage sinks

As cells expand and approach cell maturity, photoassimilates are increasingly diverted into storage products. Towards maturity, fully differentiated phloem pathways with spare transport capacity link expansion/storage sinks with photosynthetic leaves. Photoassimilate import by these sinks depends on duration of the storage phase. This can be short term for sinks located along the axial transport pathway and long term for sinks sited at the ends of transport pathways (e.g. tubers, fruits and seeds).

Storage along the axial pathway occurs mainly when photoassimilate production exceeds photoassimilate demands by terminal sinks. However, storage is not necessarily a passive response to excess photoassimilate supply. Stems of sugar cane store large quantities of photoassimilates (50% of dry weight is sucrose) even during rapid growth of terminal sinks. Photoassimilates might be stored as simple sugars (e.g. sugar cane stems) or as polymers (fructans in stems of temperate grasses; starch in stems and roots of subtropical cereals, herbaceous annuals and woody perennials). Photoassimilates stored along axial pathways buffer against diurnal and more long term fluctuations in photoassimilate supply to terminal sinks. In woody deciduous species, axially stored photoassimilates also provide a long-term seasonal storage pool that is drawn on to support bud growth following budburst. Remobilised photoassimilates can contribute substantially to biomass gain of terminal sinks. For instance, in some mature trees, over half the photoassimilates for new growth come from remobilised reserves; similar proportions of stem-stored fructans contribute to grain growth in cereals when photoassimilate production is reduced (e.g. by drought). Physiological switching between net storage and remobilisation is an intriguing regulatory question.

Growth and development of meristems is determined by phloem unloading events and metabolic interconversion of photoassimilates within recipient sink cells. These transport and transfer processes vary between sinks and can alter during sink development. Techniques now exist to alter expression of membrane porter proteins and possibly enhance photoassimilate import by sinks such as seeds which have an apoplasmic step in the phloem unloading pathway. Prospects of altering plasmodesmal conductivity will improve once plasmodesmal proteins are identified and their encoding genes known.

# 5.4.6 - Follow the flow: unloading of water and its destination

Phloem unloading of nutrients follows water release from vascular system. As discussed before, because of their low transpiration rates, developing sinks typically import water through phloem, not xylem. Water unloaded from phloem is used for cell growth or recycled back to the parental bodies. Water is unloaded from se-cc complexes symplasmically in majority of sinks by bulk flow. For growth sinks such as shoot or root apices, continued symplasmic flow of phloem-imported water can drive cells expansion. In post-phloem unloading pathways interrupted with an apoplasmic step, water must exit cells of the unloading path across cell membranes, facilitated by aquaporins (AQPs). AQPs responsible for water flow across cell membranes are plasma membrane intrinsic proteins (PIPs) and tonoplast intrinsic proteins (TIPs). Strong PIP expression in expanding post-veraison grape berries has been shown to correlate with water flows into, and from, the berry apoplasm. For sinks that stop expansion but continuously accumulate biomass, water transported to storage sink apoplasms is recycled back to the parent plant body through a xylem route. Important roles played by AQPs in water recycling are indicated by their high expression at this stage in developing seed, particularly in the vascular parenchyma cells.

In conclusion, this chapter has shown how growth and development of meristems and other sinks is determined by phloem unloading events, and metabolism of the assimilates within the recipient sink cells. These transport and transfer processes vary between specific sinks, and can alter during development. Molecular techniques that alter expression of membrane transporters can be used to study the pathways and limitations of photoassimilate transport into sinks such as seeds that have an apoplasmic step in the phloem unloading pathway, with the possibility of enhancing the rate of grain growth and crop yield in the future.

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# Theories of Ascent of Sap

**The following points highlight the top four theories of ascent of sap. The theories are:**

**1. Vital Force Theory**
**2. Root Pressure Theory**
**3. Theory of Capillarity**
**4. Cohesion Tension Theory.**

## *1. Vital Force Theory:*

A common vital force theory about the ascent of sap was put forward by J.C. Bose (1923). It is called pulsation theory. The theory believes that the innermost cortical cells of the root absorb water from the outer side and pump the same into xylem channels.

However, living cells do not seem to be involved in the ascent of sap as water continues to rise upward in the plant in which roots have been cut or the living cells of the stem are killed by poison and heat.

## *2. Root Pressure Theory:*

The theory was put forward by Priestley (1916). Root pressure is a positive pressure that develops in the xylem sap of the root of some plants. It is a manifestation of active water absorption. Root pressure is observed in certain seasons which favour optimum metabolic activity and reduce transpiration. It is maximum during rainy season in the tropical countries and during spring in temperate habitats.

The amount of root pressure commonly met in plants is 1-2 bars or atmospheres. Higher values (e.g., 5-10 atm) are also observed occasionally. Root pressure is retarded or becomes absent under conditions of starvation, low temperature, drought and reduced availability of oxygen.

## There are three view points about the mechanism of root pressure development:

### (a) Osmotic:

Tracheary elements of xylem accumulate salts and sugars. High solute concentration causes withdrawal of water from the surrounding cells as well as from the normal pathway of water absorption. As a result a positive pressure develops in the sap of xylem.

### (b) Electro-osmotic:

A bioelectric potential exists between the xylem channels and surrounding cells which favour the passage of water into them,

### (c) Nonosmotic:

Differentiating xylem elements produce hormones that function as metabolic sinks and cause movement of water towards them. The living cells surrounding xylem can actively pump water into them.

## Objections to Root Pressure Theory:

(i) Root pressure has not been found in all plants. No Of little root pressure has been seen in gymnosperms which have some of the tallest trees of the world,

(ii) Root pressure is seen only during the most favourable periods of growth like spring or rainy season. At this time the xylem sap is strongly hypertonic to soil solution and transpiration rate is low. In summer when the water requirements are high, the root pressure is generally absent,

(iii) The normally observed root pressure is generally low which is unable to raise the sap to the top of trees,

(iv) Water continues to rise upwards even in the absence of roots,

(v) The rapidly transpiring plants do not show any root pressure. Instead a negative pressure is observed in most of the plants,

(vi) The amount of exudation due to root pressure is quite low as compared to the rate of passage through the xylem,

(vii) Absorption in de-topped plants is quite low as compared to intact plants,

(viii) Root pressure disappears in un-favourable environmental conditions while ascent of sap continues uninterrupted,

(ix) Root pressure is generally observed at night when evapotranspiration is low. It may be helpful in re-establishing continuous water chains in xylem which often break under enormous tension created by transpiration.

## *3. Theory of Capillarity:*

Water rises in tubes of small diameter, kept in vessel having water, due to force of surface tension or adhesion and cohesion. Water similarly rises up in the walls of xylem channels due to adhesion. Cohesive force present amongst water molecules pulls the water upwards through the xylem channels. The upward movement will continue till the forces of adhesion and cohesion are balanced by the downward pull of gravity.

## Objections to Theory of Capillarity:

(i) The value of capillarity is very small. It can raise water to a height of about 1 metre in vessels of normal diameter (0.03 mm). Therefore, if operational it will be useful to only small sized paints,

(ii) Capillarity occurs only when base of the tube dips in container having water. Xylem vessels are not directly connected with soil water,

(iii) Rise due to capillarity will increase when the lumen of vessels is less. Tall plants should, therefore, have narrow vessels as compared to smaller plants. The truth is, however, reverse,

(iv) Capillarity cannot operate in plants having tracheids due to the presence of end walls.

## *4. Cohesion Tension Theory (Cohesion-Tension and Transpiration Pull Theory):*

The theory was put forward by Dixon and Joly in 1894. It was further improved by Dixon in 1914. Therefore, the theory is also named after him as Dixon's theory of ascent of sap. Today most of the workers believe in this theory.

### The main features of the theory are:

### (a) Continuous Water Column:

There is a continuous column of water from root through the stem and into the leaves. The water column is present in tracheary elements. The latter do operate separately but form a continuous system through their un-thickened areas.

Since there are a large number of tracheary elements running together, the blockage of one or a few of them does not cause any breakage in the continuity of water column. The column of water does not fall down under the impact of gravity because forces of transpiration provide both energy and necessary pull. Cohesion, adhesion and surface tension keep the water in place.

### (b) Cohesion or Tensile Strength:

Water molecules remain attached to one another by a strong mutual force of attraction called cohesion force. The mutual attraction is due to hydrogen bonds formed amongst adjacent water molecules (Fig. 11.26). On account of cohesion force, the water column can bear a tension or pull of up to 100 atm.

Therefore, the cohesion force is also called tensile strength. Its theoretical value is about 15000 atm but the measured value inside the tracheary elements ranges between 45 atm to 207 atm.

Water column does not further break its connection from the tracheary elements (vessels and tracheids) because of another force called adhesion force between their walls and water molecules.

Water molecules are attracted to one another more than the water molecules in the gaseous state. It produces surface tension that accounts for high capillarity through tracheids and vessels.

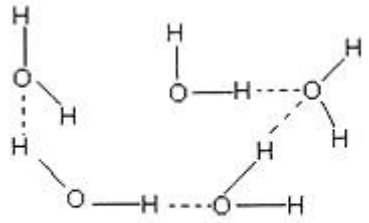


Fig. 11.26. Cohesion force due to hydrogen bonding between water molecules.

## (c) Development of Tension or Transpiration Pull:

Intercellular spaces present amongst mesophyll cells of the leaves are always saturated with water vapours. The latter come from the wet walls of mesophyll cells. The intercellular spaces of mesophyll are connected to the outside air through stomata. Outside air is seldom saturated with water vapours. It has a lower water potential than the moist air present inside the leaf.

Therefore, water vapours diffuse out of the leaves. The mesophyll cells continue to lose water to the intercellular spaces. As a result curvature of meniscus holding water increases resulting in increase in surface tension and decrease in water potential, sometimes to -30 bars. The mesophyll cells withdraw water from the deeper cells as its molecules are held together by hydrogen bonds.

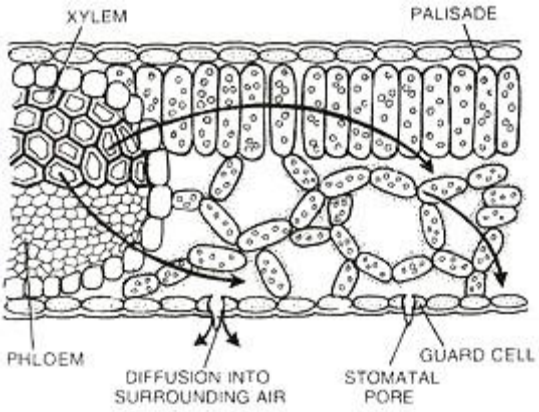



Fig. 11.27. Water movement in leaf and development of pressure gradient between outside air and leaf air spaces, leaf air spaces and mesophyll cells, mesophyll cells and water filled xylem of leaf veins.

The deeper cells in turn obtain water from the tracheary elements. The water in the tracheary elements would, therefore, come under tension. A similar tension is felt in millions of tracheary elements lying adjacent to the transpiring cells.

It causes the whole water column of the plant to come under tension. As the tension develops due to transpiration, it is also called transpiration pull. On account of tension created by transpiration, the water column of the plant is pulled up passively from below to the top of the plant like a rope (Fig. 11.28).

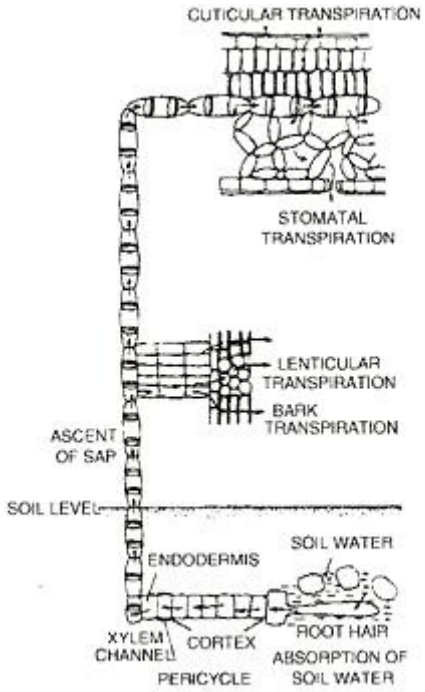



Fig. 11.28. Path of water through the plant.

As a tension of one atmosphere is sufficient to pull water to a height of about 10 metres, a tension of 10-20 atm is sufficient to raise water to the height of the tallest tree over 130 m.

It overcomes, (i) gravitational pull, (ii) resistance of narrow xylem channels and their end walls, (iii) resistance of living cells of the root that lie in the path of water from soil to xylem, (iv) resistance offered by water coming out of narrow capillary pores of the soil.

## Evidences:

(i) The rate of water absorption and hence ascent of sap closely follows the rate of transpiration,

(ii) Evaporation of water from a porous pot or atmometer can produce a tension in the water column present in attached tube. It can even raise a column of mercury to sufficient height (Fig. 11.29).

(iii) Shoot attached to a tube having water and dipping in a beaker having mercury can cause the movement of mercury into the tube showing transpiration pull (Fig. 11.30).

(iv) In a branch cut from a rapidly transpiring plant, water snaps away from the cut end showing that the water column is under tension,

(v) With the help of dendrograph it is found that tree trunks contract during the day time and expand during the night. Contraction is caused by narrowing of tracheary elements when the contained water is under tension,

(vi) The maximum tension observed in water column is 10-20 atm. It is sufficient to pull the water to the top of the tallest trees of even more than 130 metres in height. The tension cannot break the continuity of water column as cohesive force of xylem sap is 45 to 207 atm.

(vii) Gymnosperms are at a disadvantage in the ascent of sap because of the presence of tracheids instead of vessels in angiosperms. However, tracheidal xylem is less prone to gravitation under tension. Therefore, most of the tall trees of the world are redwoods and conifers.

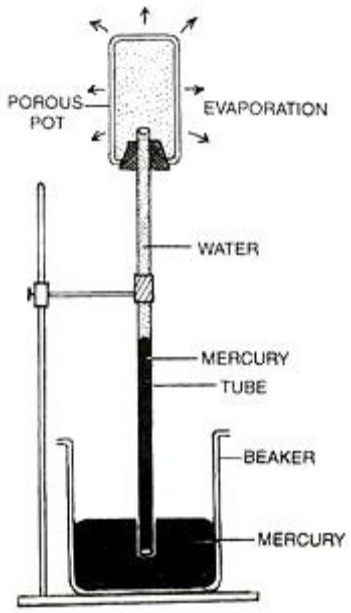



Fig. 11.29. Demonstration of pull due to evaporation.

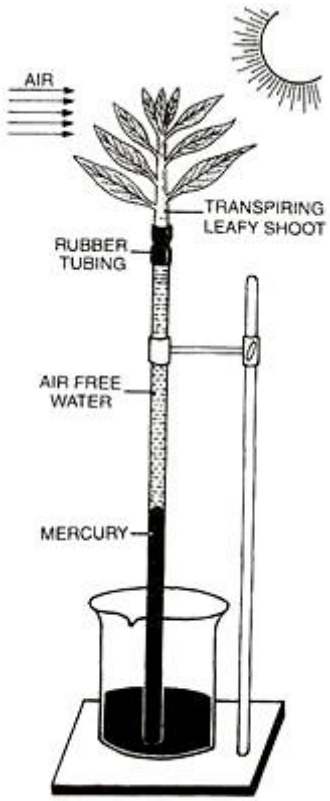



Fig. 11.30. Demonstration of pull due to transpiration.

## Objections:

(i) The gases dissolved in sap shall form air bubbles under tension and high temperature. Air bubbles would break the continuity of water column and stop ascent of sap due to transpiration pull, (ii) A tension of up to 100 atm has been reported in the xylem sap by Mac Dougal (1936) while the cohesive force of sap can be as low as 45 atm.

(iii) Overlapping cuts do not stop ascent of sap though they break the continuity of water column.

# 06. TRANSPIRATION

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

Transpiration is of three types

**1. Stomatal transpiration**

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

**2. Cuticular transpiration**

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

**3. Lenticular transpiration**

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

**Mechanism of stomatal transpiration**

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

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

ii. Opening and closing of stomata (stomatal movement)

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

- Inside the leaf the mesophyll cells are in contact
- With xylem, and on the other hand with intercellular space above the stomata

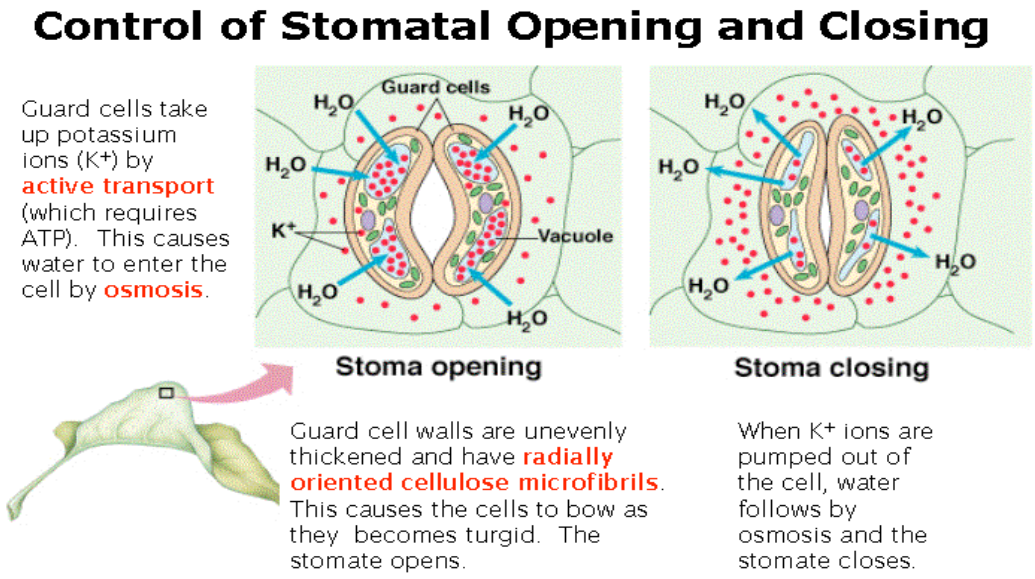
- When mesophyll cells draw water from the xylem they become turgid and their diffusion pressure deficit (DPD) and osmotic pressure (OP) decreases with the result that they release water in the form of vapour in intercellular spaces close to stomata by osmotic diffusion. Now in turn, the O.P and D.P.D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

**Opening and closing of stomata (Stomatal movement)**

The stomata are easily recognized from the surrounding epidermal cells by their peculiar shape. The epidermal cells that immediately surround the stomata may be similar to other epidermal cells or may be different and specialized. In the latter case, they are called as subsidiary cells.

The guard cells differ from other epidermal cells also in containing chloroplasts and peculiar thickening on their adjacent surface (in closed stomata) or on surfaces.

Consequent to an increase in the osmotic pressure (OP) and diffusion pressure deficit (DPD) of the guard cells (which is due to accumulation of osmotically active substances), osmotic diffusion of water from surrounding epidermal cells and mesophyll



cells into guard cells follows. This increase the turgor pressure (TP) of the guard cells and they become turgid. The guard cells swell, increase in length and their adjacent thickened surfaces starch forming a pore and thus the stomata open.

On the other hand, when OP and DPD of guard cells decrease (due to depletion of osmotically active substances) relative to surrounding epidermal and mesophyll cells, water is released back into the latter by osmotic diffusion and the guard cells become flaccid. The thickened surfaces of the guard cells come close to each other, thereby closing the stomatal pore and stomata.

Osmotic diffusion of water into guard cells occur when their osmotic pressure increases and water potential decreases (i.e become more negative) related to those of surrounding epidermal and mesophyll cells. The guard cells become flaccid when their osmotic pressure decreases relative to the surrounding cells (Movement of water takes place from a region of higher water potential to a region of lower water potential.

These may be several different agents or mechanisms which control stomatal movements.

Hydrolysis of starch into sugars in guard cells

Synthesis of sugars or organic acids in them

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

**1. Hydrolysis of starch into sugars in guard cells**

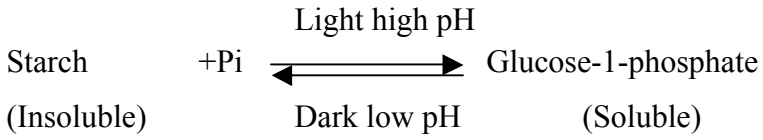
**Starch – sugar Inter conversion theory**

This classical theory is based on the effect of pH on starch phosphorylase enzyme which reversibly catalyses the conversion of starch + inorganic phosphate into glucose -1 phosphate.

During the day, pH is guard cells in high. This favours hydrolysis of starch (which is insoluble into glucose -1- phosphate (which is soluble) so that osmotic pressure is increased in guard cells.

Consequently water enters, into the guard cells by osmotic diffusion from the surrounding epidermal and mesophyll cells. Guard cells become turgid and the stomata open.

During dark, reverse process occurs. Glucose 1- phosphate is converted back into starch in the guard cells thereby decreasing osmotic pressure. The guard cell release water, become flaccid and stomata become closed.

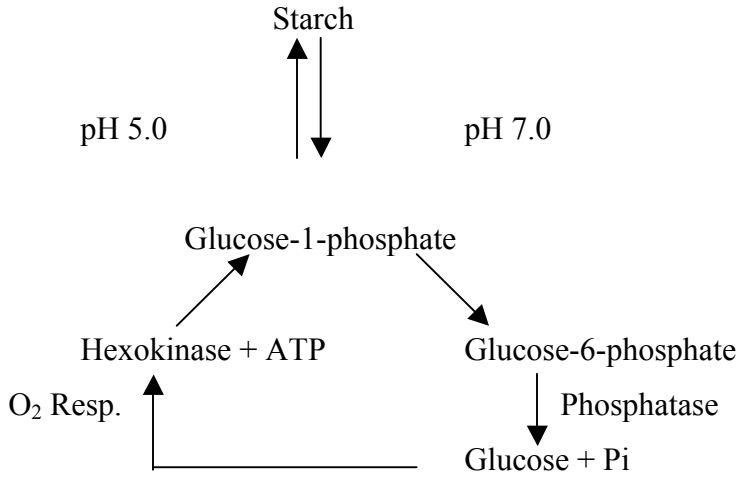



According to Steward 91964), the conversion of starch and inorganic phosphate into glucose-1-phosphate does not cause any appreciable change in the osmotic pressure because the inorganic phosphate and glucose-1-phosphate are equally active osmotically.

In this scheme he has suggested that,

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

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



**2. Synthesis of sugars or organic acids in Guard cells**

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

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

Reverse process would occur in darkness.

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

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

The accumulation of K ion is sufficient enough to significantly decrease the water potential of guard cells during day light. Consequently, water enters into them from the adjacent epidermal and mesophyll cells thereby increasing their turgor pressure and opening the stomatal pore.

Reverse situation prevails during dark when stomata are closed. There is no accumulation of 'K' in g cells in dark.

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

**Significance of Transpiration**

Plants waste much of their energy in absorbing large quantities of water and most of which is ultimately lost through transpiration.

Some people thin that – Transpiration as advantageous to plant.

Others regard it as an unavoidable process which is rather harmful.

**Advances of transpiration**

**1. Role of movement of water**

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

**2. Role in absorption and translocation of mineral salts**

Absorption of water and mineral salts are entirely independent process. Therefore transpiration has nothing to do with the absorption of mineral salts.

However, once mineral salts have been absorbed by the plants, their further translocation and distribution may be facilitated by transpiration through translocation of water in the xylem elements.

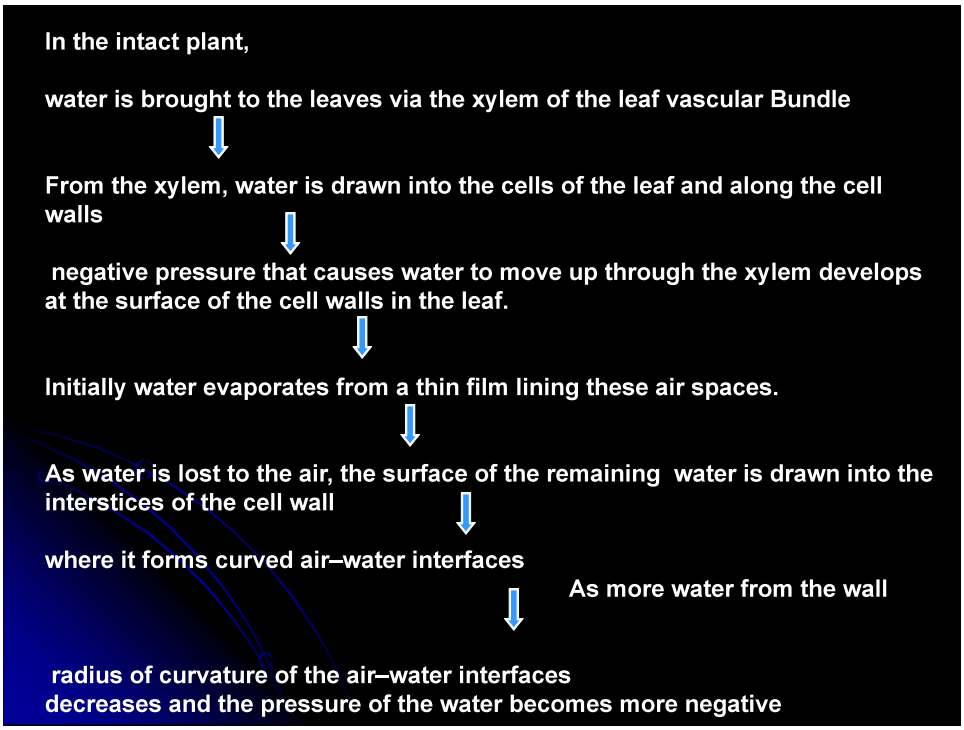
**3. Role of regulation of temperature**

Some light energy absorbed by the leaves is utilized in photosynthesis; rest is converted into heat energy

**Transpiration from the leaf regulates by**

1. **difference in water vapor concentration between the leaf air spaces and the external air**
2. **diffusional resistance (*r*) of this pathway**
3. **leaf stomatal resistance (*r*s)**
4. **leaf boundary layer resistance**
5. **control of stomatal apertures by the guard cells**

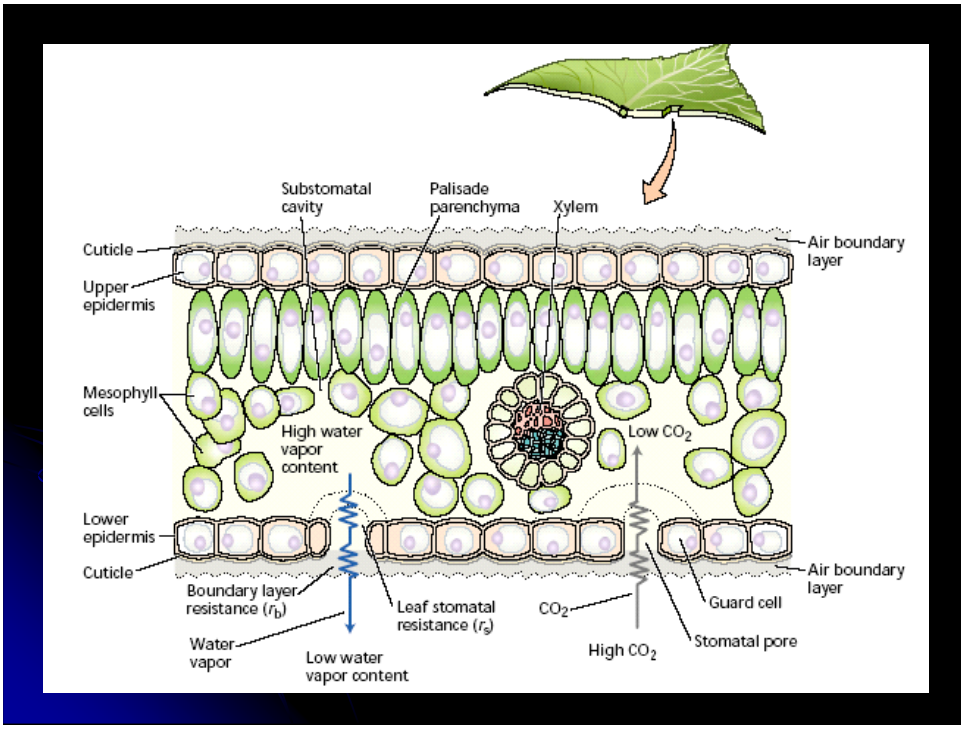
which raises their temperature. Transpiration plays an important role in controlling the temperature of the plants. Rapid evaporation of water from the aerial parts of the plant through transpiration brings down their temperature and thus prevents them from excessive heating.




**Transpiration as a necessary evil**

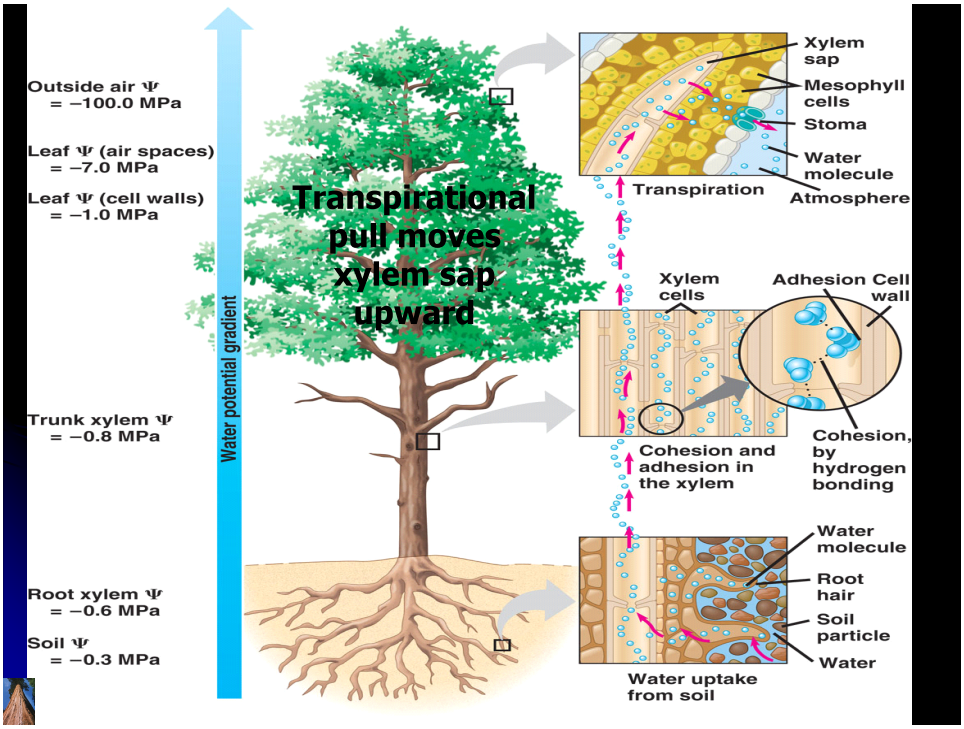
1. When the rate of transpiration is high and soil is deficient in water, an internal water deficit is created in the plants which may affect metabolic processes

2. Many xerophytes have to develop structural modification and adaptation to check transpiration.




3. Deciduous tress has to shed their leaves during autumn to check loss of water.

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




**Factors affecting transpiration rate**

**A. External factors**

***1. Atmospheric humidity***

In humid atmosphere, (when relative humidity) is high), the rate of transpiration decreases. It is because atmosphere is more saturated with moisture and retards the diffusion of water vapour from the intercellular spaces of the leaves to the outer atmosphere through stomata.

In dry atmosphere, the RH is low and the air is not saturated with moisture and hence, the rate of transpiration increases.

***2. Temperature***

An increase in temperature brings about an increase in the rate of transpiration by

1. lowering the relative humidity
2. Opening of stomata widely

***3. Wind***

i. When wind is stagnant (not blowing), the rate of transpiration remains normal

ii. When the wind is blowing gently, the rate of transpiration increases because it removes moisture from the vicinity of the transpiration parts of the plant thus facilitating the diffusion of waster vapour from the intercellular spaces of the leaves to the outer atmosphere though stomata.

iii. When the wind is blowing violently, the rate of transpiration decreased because it creates hindrance in the outward diffusion of water vapours from the transpiring part and it may also close the stomata.

***4. Light***

Light increases the rate of transpiration because,

In light stomata open; It increases the temperature

In dark, due to closure of stomata, the stomatal transpiration is almost stopped.

***5. Available soil water***

Rate of transpiration will decrease if there is not enough water in the soil in such from which can be easily absorbed by the roots.

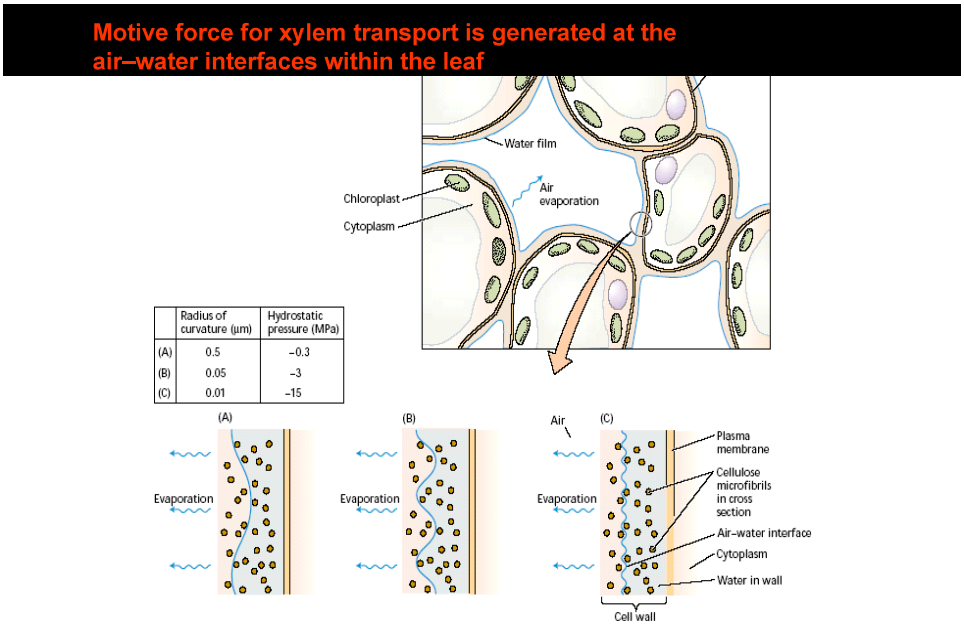
***6. $CO_2$***

An increase in $CO_2$ concentration in the atmosphere (Ova the usual concentration) more so inside the leaf, leads towards stomatal closure and hence it retards transpiration.

**B. Internal factors**

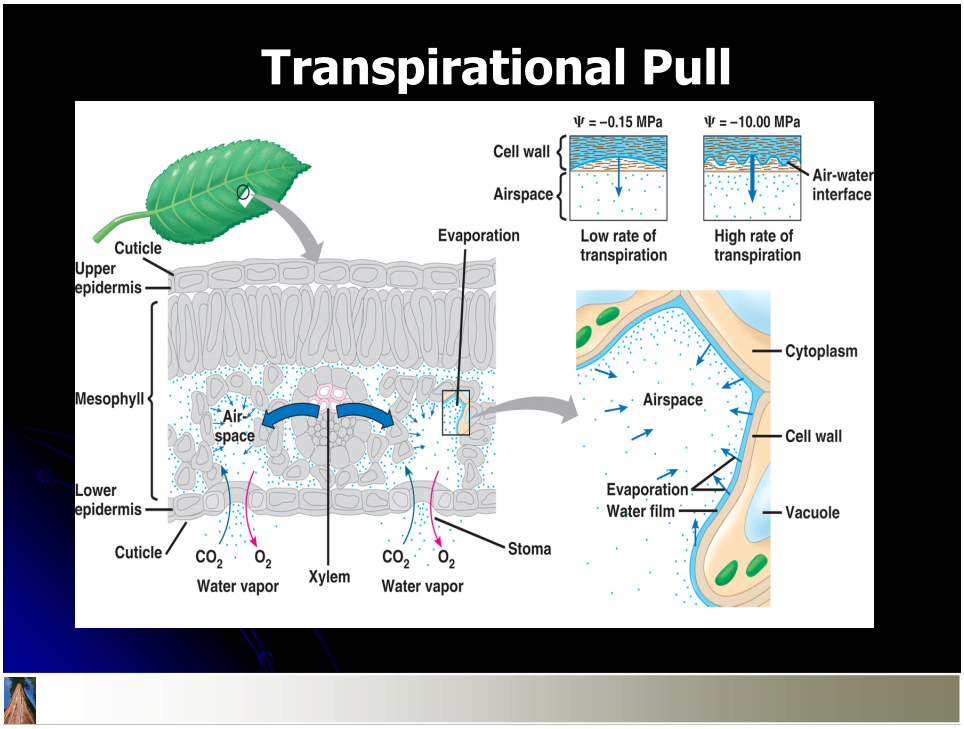
***1. Internal water conditions***

It is very essential for transpiration. Deficiency of water in the plants will result in decrease of transpiration rate. Increase rate of transpiration containing for longer periods often create internal water deficit in plants because absorption of water does not keep pace with it.




## *2. Structural features*

The number, size, position and the movement of stomata affect rate of transpiration. In dark stomata are closed and stomatal transpiration is checked. Sunken stomata help in reducing the rate of stomatal transpiration. In xerophytes the leaves are reduced in size or may even fall to check transpiration. Thick cuticle on presence of wax coating on exposed parts reduces cuticles transpiration.

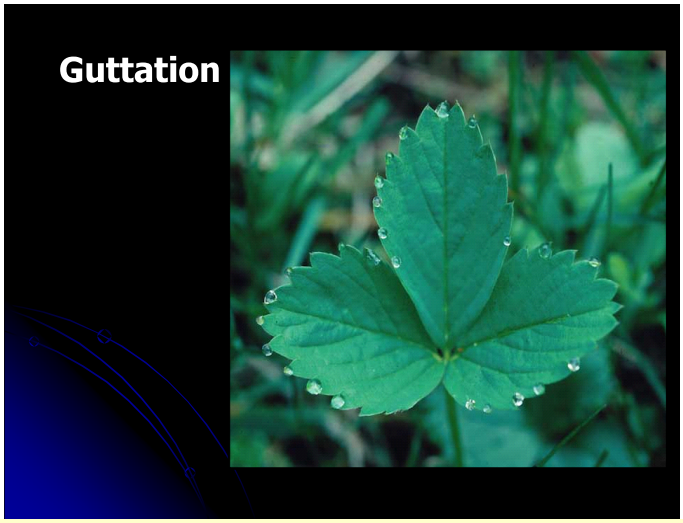


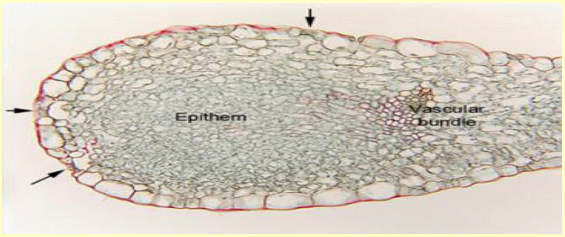

**Antitranspirants**

A number of substances are known which when applied to the plants retard their transpiration. Such substances are called as antitranspirants. Some examples of antitranspirants are colourless plastics, silicone, oils, low viscosity waxes, phenyl mercuric acetate, abscisic acid, $CO_2$, etc. Colourless plastic, silicone oils and low viscosity waxes belong to one group as these are sprayed on the leaves, form after film which is permeable to $O_2$ and $CO_2$ but not to water.

Fungicide phenyl mercuric acetate, when applied in low concentration ($10^{-4}$ m), it exercised a very little toxic effect on leaves and resulted in partial closure of stomatal pores for a period of two weeks. Similarly ABA a plant hormone also induces stomatal closure. $CO_2$ is an effective antitranspirants. A little rise in $CO_2$ concentration from the natural 0.03% to 0.05% induces partial closure of stomata. Its higher concentration cannot be used which results in complete closure of stomata affecting adversely the photosynthesis and respiration.

**GUTTATION**




In some plants such as garden nasturtium, tomato, colocasia etc, water drops ooze out from the uninjured margins of the leaves where a main vein ends. This is called as guttation and takes place usually early in the morning when the rate of absorption and root pressure are high while the transpiration is very low.

The phenomenon of guttation is associated with the presence of special types of stomata at the margins of the leaves which are called as **water stomata or hydathodes**. Each hydathode consists of a water pore which remains permanently open.

Below this there is a small cavity followed by a loose tissue called as epithem. This epithem is in close association with the ends of the vascular elements of veins. Under high root pressure the water is given to the epithem by the xylem of the veins. From epithem

water is released into the cavity. When this cavity is completely filled with watery solution, the later begins to ooze out in the form of watery drops through the water pore.

**Difference between transpiration and Guttation**

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

# CHAPTER 2. SOIL-WATER POTENTIAL: CONCEPTS AND MEASUREMENT

**Contents:**

- Transport mechanisms
- Water properties
- Definition of soil-water potential
- Measurement of soil-water potential
- Soil-water retention curve
- Components of total soil-water potential

**Transport:**

1. Solute molecules, dissolved in gas or liquid, move from high to low concentration
   Driving force for movement is difference in concentration with distance
   Transport is formulated by Fick's law of diffusion

2. Heat moves from high to low temperature
   Driving force for heat transport is difference in temperature with distance
   Heat transport is formulated by Fourier's law of heat conduction

3. Current (movement of electrons) is caused by electrical potential differences
   Electrons move from high to low potential
   Current is computed from Ohm's law

4. Water moves from high to low pressure or potential
   Driving force is difference in its pressure with distance
   In addition water will move by gravitational forces

   In soils, water moves by pressure (or water potential) and gravitational forces

   Water flow is described by DARCY's law

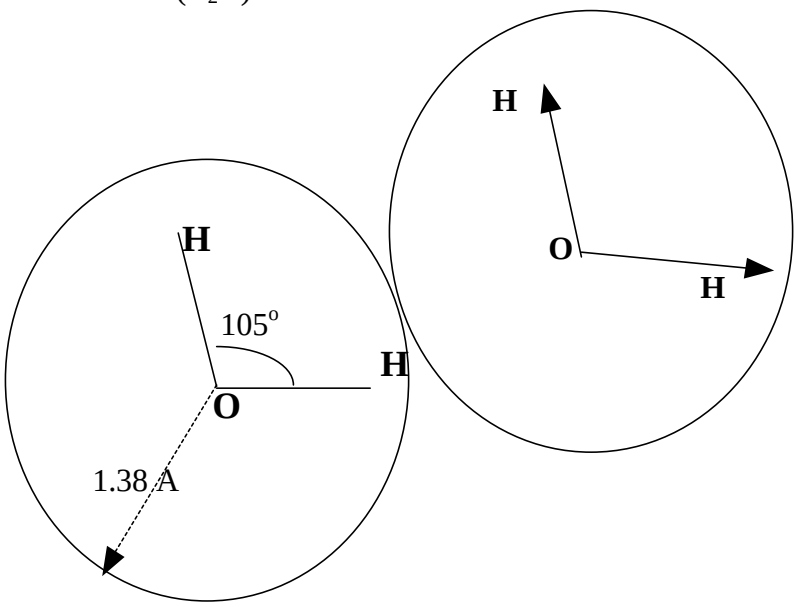
**ALL TRANSPORT FOLLOWS SIMILAR MATHEMATICAL LAWS AS DARCY**

**Water properties:**

Water is an unusual fluid and has much different physical properties than other substances with equal molecular weight

- high boiling and melting point
- low fluid density
- high heat of melting and vaporization
- high specific heat
- large dielectric constant
- good solvent

Molecular structure of water ($H_2O$):




$H_2O$ molecule has no net charge (neutral), but charge distribution is asymmetrical, with the center of negative charge not coinciding with center of positive charge;

Consequently, water molecule is polar;

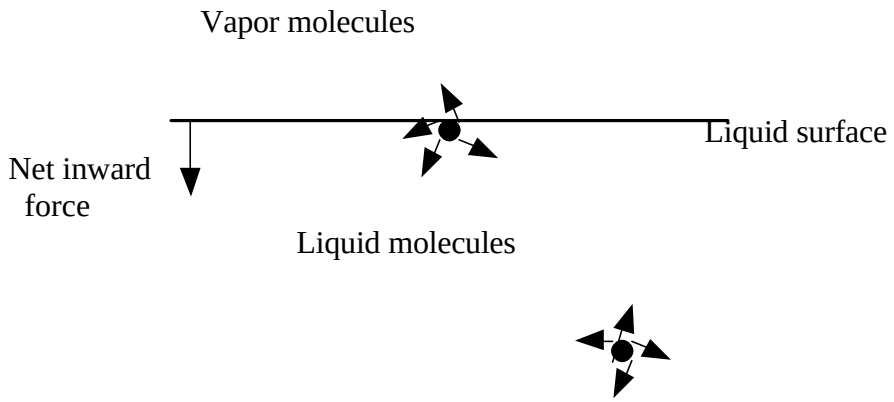
H-atoms of water molecules are hydrogen bonded with O atoms of surrounding molecules, acting as attraction forces between water molecules;

Hydrogen bonding explains the physical properties of water, and its weak crystalline structure;

✪ Why does ice float on water ???

***Surface tension***

Molecules at fluid interface are exposed to different forces than within fluids. For example, at the water-air interface, a net inward force exists because of higher density of water molecules in water than in air. Within the water there is no net attraction in any direction. Therefore, water at interface has membrane-like properties. The extra energy of the water at the interface is called surface tension, $\sigma$, and is defined as energy per unit surface area ($Nm/m^2$) or force per unit length.




Surfactants (organics, detergents) concentrate at liquid-gas interface, because attraction of solute molecules to water is less than cohesive attraction forces (by hydrogen bonding) between water molecules.

Net effect is reduction of surface tension by surfactants

Surface tension of air-water = 72 dynes/cm = 0.072 N/m

✪ How does surface tension depend on temperature ?

*Viscosity*

Resistance of fluid flow is caused by attraction forces between fluid molecules, thereby resisting motion from one laminar fluid layer to another.

Pressure forces causing fluid flow is counteracted by these drag forces.

Viscosity is defined by relation between drag force, $\tau$, ($N/m^2$) and velocity gradient perpendicular to direction of flow:

$$\tau = \nu \, dv/dy \quad ,$$

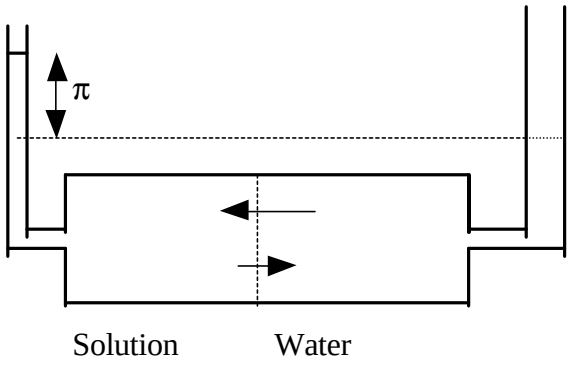
where $\nu$ is dynamic viscosity ($Ns/m^2$)

✪ What has higher viscosity: air or water; which fluid will experience the larger drag force, and how is viscosity affected by temperature ?

***Osmotic pressure**:*

Occurs when solutions of different concentrations are separated by a semi-permeable membrane. Random motion of water and solute molecules create a net movement of water to the compartment with higher concentration, until equilibrium is reached.

This net movement by concentration differences is called diffusion




At equilibrium, net water movement is eliminated by increase in water pressure, $\Pi$,

$$\Pi = CRT$$

where $\Pi$ is defined as osmotic pressure ($N/m^2$), C is solute concentration ($mol/m^3$), T is temperature (K), and R is universal gas constant (8.314 J/mol K).

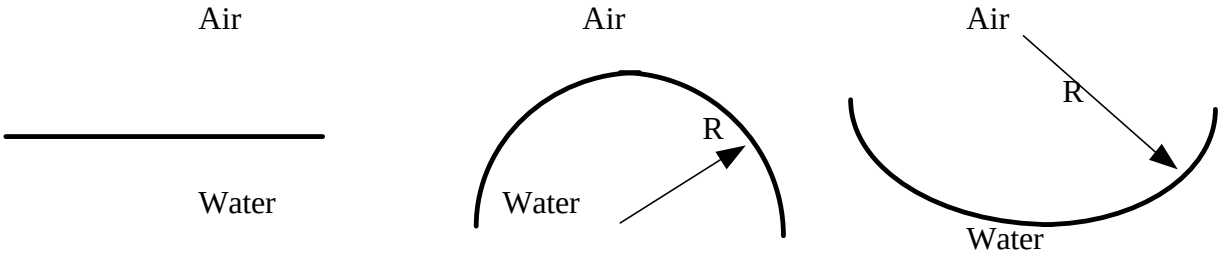
✪ Salt water can be purified to drinking water by reverse osmosis. Explain.

***Capillary Rise**:*

For a curved interface, the pressure is greater on the concave side of the air-water interface. For a hemispherical interface (with radius R), this pressure difference, $\Delta P$, can be derived from a force balance, and is equal to

$$\Delta P = 2\sigma/R = P_{air} - P_{water}$$

That is, the surface tension force is balanced by a pressure drop across the air-water interface:




$P_{air} = P_{water}$ $P_{air} < P_{water}$ $P_{air} > P_{water}$

Hence, the fluid pressure is always greater on the concave side of the interface, with the difference determined by the radius of the curvature and the surface tension.

Contact angle ($\gamma$) is the angle measured from the liquid-solid interface to the liquid-air interface. For a small angle ($<90^o$), liquid is preferentially attracted to the solid surface (by adhesion forces), and wets the solid. If cohesion forces of liquid are stronger than the attractive force to the solid, the liquid repels from the solid surface and $\gamma$ is large (mercury on glass).


Small contact angle, $< 90^o$
Liquid wets the solid
**Hydrophilic surface**

Large contact angle, $> 90^o$
Liquid repelled by solid
**Hydrophobic surface**

The contact angle adjusts to attain equilibrium between various surface tension forces.
If a capillary tube is inserted in water, the water will form a concave meniscus because of net surface tension forces pulling it along the capillary wall. If diameter is small enough, the air-water interface will be curved, causing a pressure difference across the air-water

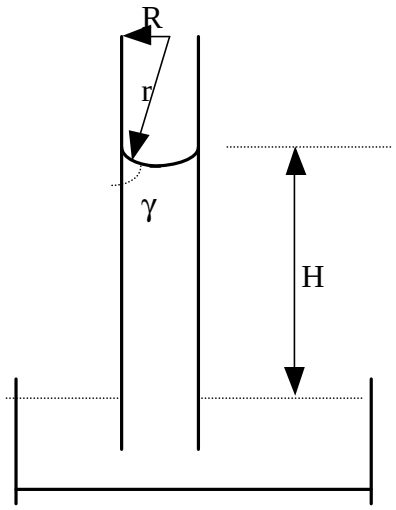
interface.

Consequently, the water in the capillary will rise. At equilibrium, the upward surface tension force is equal to the downward force by the weight of the water in the capillary.

The resulting capillary rise equation for a tube with capillary radius R is:

$$\rho_w g H = 2\sigma/r = 2\sigma \cos\gamma/R,$$

where H is the height of capillary rise and $\rho_w$ is the water density. This is also called the La Place equation ( r is radius of curvature) .




✪ Where would the level of the interface be if the capillary was filled with mercury, which is repelled by the glass capillary?

**Energy state of soil water:**

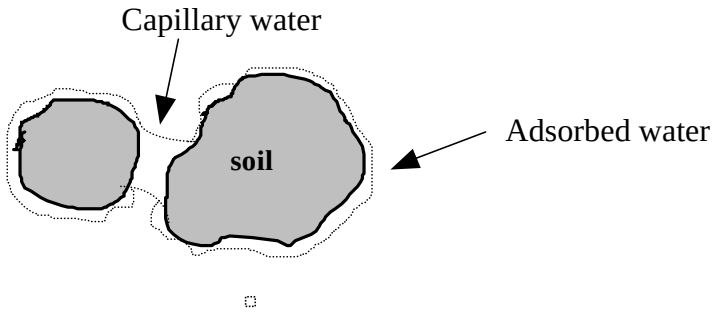
The total energy state of soil water is defined by its equivalent potential energy, as determined by the various forces acting on the water per unit quantity.

In general, flow rates of water in soils is too small to consider kinetic energy. Therefore, the energy state of soil water is defined by its equivalent potential energy, that is by virtue of its position in a force field.

Forces acting on soil water (in the vadose zone) are:

- Capillary forces
- Adsorptive forces (adhesion of water to solid soil surfaces)

Capillary and adsorptive forces together result in soil matric potential




- Gravitational forces
- Drag or shear forces (at soil surface-water interface)

Water in the soil flows from points with high soil-water potential energy to points of lower potential energy:

Driving force for flow is the change in potential energy with distance (soil-water potential gradient)

These driving forces determine:

- Direction and magnitude of water flow
- Plant water extraction rate
- Drainage volumes
- Upward water movement (or capillary rise)
- Soil temperature changes
- Solute (contaminant) transport rates

**Definitions of soil-water potential:**

To quantify potential energy state of soil water, a reference state is needed.

It is defined as the potential energy of pure water, with no external forces acting on it, at a reference pressure (atmospheric), reference temperature, and reference elevation. Soil-water potential is then determined as potential energy per unit quantity of water, relative to the reference potential of zero.

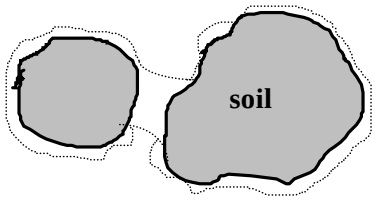
Soil water potential is measured as potential energy per unit quantity of water, relative to this reference soil water potential.

✪ The soil below the groundwater table is saturated with water. Is the soil water potential below the groundwater larger or smaller than this reference potential ?

***Formal definition:***

***Total soil water potential is defined as the amount of work per unit quantity of pure water that must be done by external forces to transfer reversibly and isothermally an infinitesimal amount of water from the standard state to the soil at the point under consideration.***

**Since water in soil has various forces acting upon it, potential energy usually differs from point to point, and hence its potential energy is variable as well.**




✪ Do we need to exert work to move water from the reference state (at atmospheric pressure) to the capillary water with the curved interface of the soil above, and hence is the soil water potential there positive or negative ?

**REMEMBER: Potential = Force x Distance = mgl =** $\rho_w$**Vgl (Nm)**

**Soil water potenial can be expressed in three different units:**

Potential per unit mass ($\mu$) : $\mu$ = potential/mass = gl (Nm/kg)

Potential per unit volume ($\psi$) : $\psi$ = potential/volume = $\rho_w Vgl / V = \rho_w gl$ ($N/m^2$, water pressure units)

Potential per unit weight (h) : h = potential/weight = mgl / mg = l (m, head unit) = equivalent height of water

**Consequently, we do not need to compute the soil-water potential directly by computing the amount of work needed, but measure the soil-water potential indirectly from pressure or water height measurements !!!!**

## Total soil water potential , $\Psi_T$

$\Psi_T = \Psi_p + \Psi_z + \Psi_s + \Psi_a$ ($N/m^2$)

where $\Psi_p$ , $\Psi_z$ , $\Psi_s$ , and $\Psi_a$ are pressure, gravitational, solute (osmotic) and air pressure potentials, respectively.

Most of our discussion will only include $\Psi_p$ and $\Psi_z$

so $\psi_T = \psi_p + \psi_z$

***or when potential expressed per unit weight: H = h + z***

### $Y_p$ - pressure potential

Energy per unit volume of water required to transfer an infinitesimal quantity of water from a reference pool of water at the elevation of the soil to the point of interest in the soil at reference air pressure and temperature.

Pressure potential can be both negative and positive.

If soil is saturated, $Y_p$ is positive, and also denoted by hydrostatic pressure potential.

If the soil is unsaturated, $Y_p$ is negative, and also denoted by matric potential ($\Psi_m$ in book).

[However, if only water column: $Y = Y_p$ (can be both < 0 and > 0), as opposed to water in soil matrix.]

When expressed in energy per unit weight (m) than we use $h_p$ or simply h.

✪ How is pressure potential of water ($\psi_p$) in soil related to $\mu_p$?

$$\mathbf{P = \frac{F}{A}} \quad \textbf{makes} \quad \mathbf{F = PA}$$

$$\mu_p = \frac{Fl}{m} = \frac{PAl}{m} = \frac{PV}{m} = \frac{P}{\rho_w} = \frac{\psi_p}{\rho_w}$$

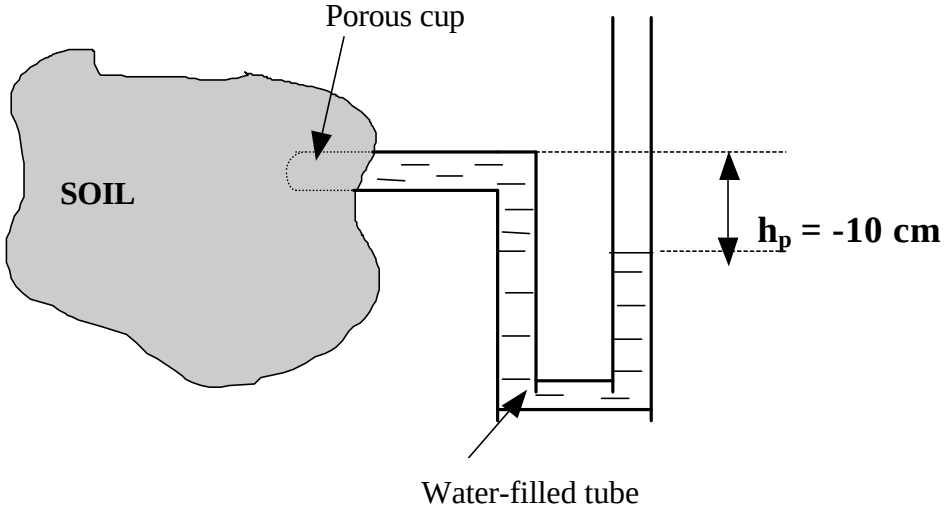
$$\textbf{thus} \quad \psi_p = \mu_p \rho_w$$

Also, soil water pressure head is

$$h_p = h = \Psi_p / \rho_w g$$

Unless otherwise specified, we will express potential on a weight basis.

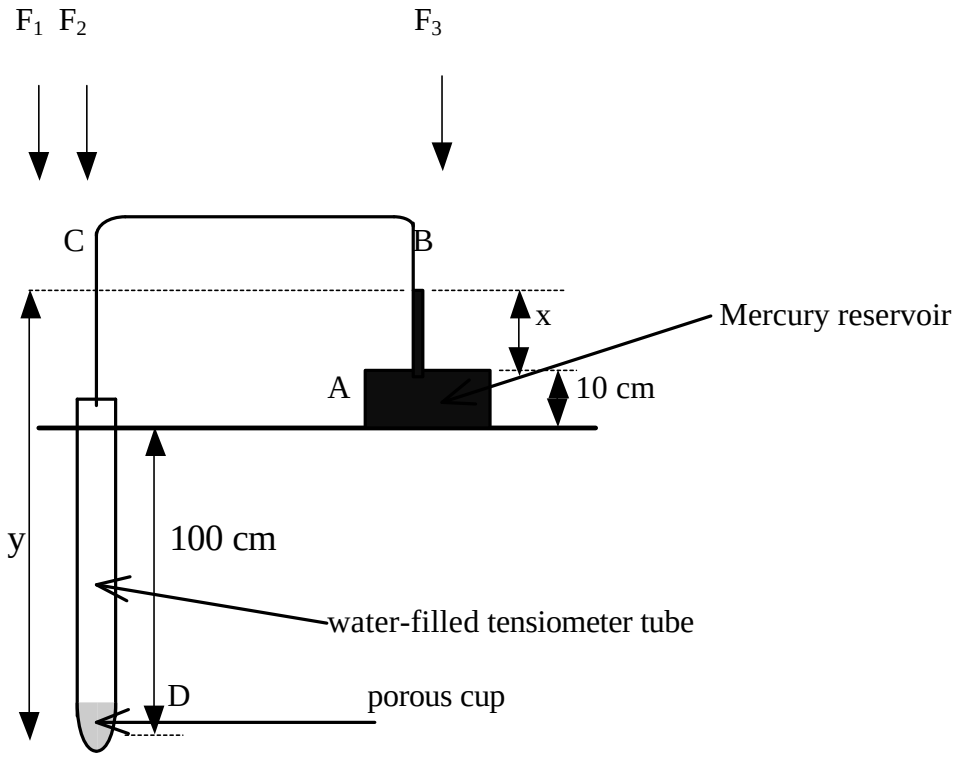
## How to measure pressure potential in soil ?




$\psi_p = \rho_w g h_p = \rho_w g h$ Then if $h = h_p = -10$ cm : $\psi_p = \rho_w g h = 1{,}000$ (kg/m$^3$) x 9.8 (m/s$^2$) x -0.10 (m) = - 980 N/m$^2$ = -980 Pa = -0.98 kPa (1Pa=1 N/m$^2$)

And $\mu_p = \psi_p / \rho_w$ = -980/1,000 = -0.98 Nm/kg

**<u>How to measure $h_p$ in a field soil? (tensiometer)</u>**




At equilibrium, all forces must balance.

The 3 forces in a tensiometer with a Hg manometer are as follows;

That is, $F_1 + F_2 = F_3$

$F_1$ - force due to soil trying to pull $H_2O$ from tensiometer
$F_2$ - weight of $H_2O$ in the tensiometer and tubing up to the level of the Hg meniscus
$F_3$ - weight of mercury in tubing of height X

*Note the $H_2O$ in the tubing above the dashed line BC (see figure) is not considered because the downward forces due to $H_2O$ in both halves of the "U-tube" are equal.

$F_1 + F_2 = F_3$
The sign (+ or -) of $F_1$ is unknown at this stage. However, since $F_2$ and $F_3$ are acting downward, both forces are exerting a negative pull on the water located at the broken line. Thus, they are assigned negative values.

Thus: $M_1g - M_2g = -M_3g$

$\rho_w V_1 g - \rho_w V_2 g = -\rho_{Hg} V_3 g$

If we express this on a per unit area basis (NOTE: $\psi_p = \rho_w g h$)

$\rho_w g h - \rho_w g y = -13.5 \rho_w g X$

Divide by $\rho_w g$ and rearrange to solve for h

$$\mathbf{h = y - 13.5\ X}$$

## Example from tensiometer figure on previous page:

Tensiometer cup is 100 cm below soil surface

$h = 100 + 10 + X - 13.5\ X$

If X = 20 cm,

$h = 100 + 10 + 20 - 13.5\ (20) = -140$ cm

a negative soil-water pressure potential (matric potential head)

If X = 5 cm,

$h = 100 + 10 + 5 - 13.5\ (5) = +47.5$ cm

I.e., a positive pressure potential (hydrostatic pressure head)

Thus, tensiometers can measure both + and – soil-water pressure potentials, i.e., hydrostatic pressure and matric potentials.

Alternatively, instead of force balance, one can state that at hydraulic equilibrium the total water potential at B is equal to total water potential at point D.

Then: At B: $z = 10 + X$ (soil surface is reference level)
$h = -\rho_{Hg}/\rho_{H2O}\, X = -\rho_{Hg}\, X$

At D: $z = -100$
$h = h_D$

Total water potential at B = total water potential at D, or

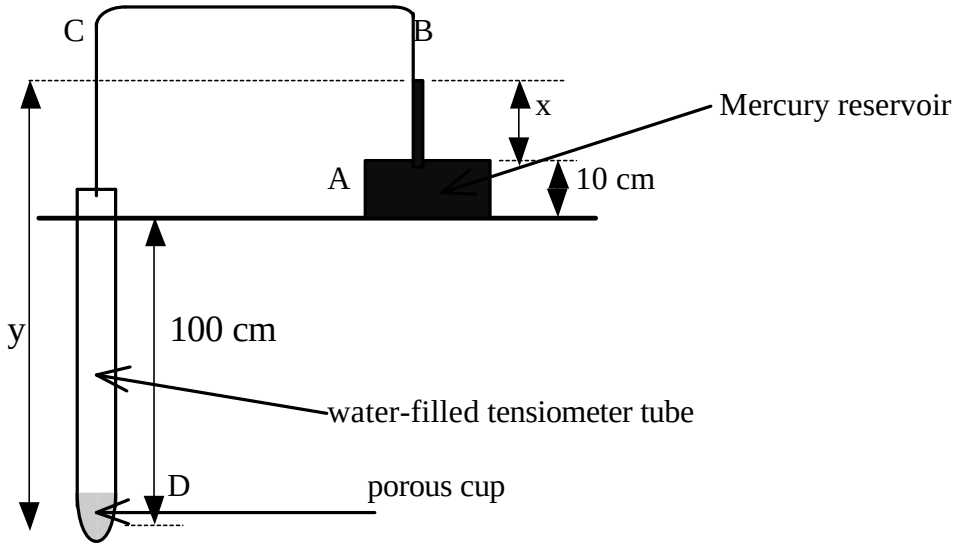
$10 + X - \rho_{Hg}X = -100 + h_D$ or $h_D = 110+X - 13.5X = Y - 13.5X$

✪ Would the rise of fluid in the reservoir (X) increase or decrease if its density would be less than mercury, for the same soil water potential at point D ?

Hence, why would one use mercury instead of a lighter fluid ?

✪ What is the soil-water pressure at location D, if $x = 0$ ?

Let us use yet another method:




- Assume that at the time of measurement, the fluids in the tensiometer are at hydraulic equilibrium, i.e., fluid is static;

- For a static fluid (not in motion), change in pressure occurs only with a change in elevation. That is, along the horizontal plane, the pressure will be constant;

- Also, the fluid pressure changes inversely with elevation. If one travels upward in the fluid (positive z), the pressure decreases; and if one goes downward (negative z), the pressure increases; Therefore, in diagram: pressure at C = pressure at B or $\psi_C = \psi_B$

- $\psi_B = -\rho_{Hg} gX$ (hanging column of mercury)

  $\psi_C = -\rho_w gY + \rho_w gh_{p,D}$ ( $= -\rho_w gY + \psi_D$) , where $Y = 100 + 10 + X$ and $\psi_D$ is the pressure potential of soil water at point D, expressed by potential per unit volume (pressure units);

- Since $\rho_{Hg} = 13.5\rho_w$

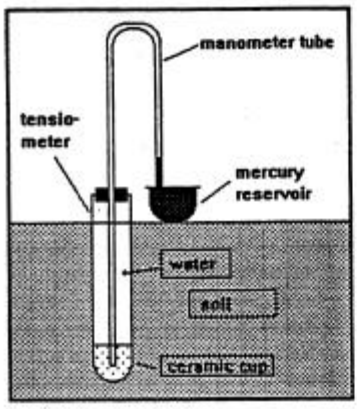
  $-13.5\rho_w gX = -\rho_w gY + \rho_w gh_{p,D}$ or $\underline{h_D = Y - 13.5X}$

**Methods for measuring $h_p$ (soil water pressure potential):**

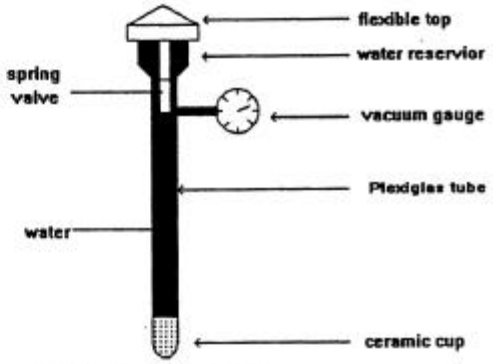
Tensiometer 0 to -80 kPa (= -0.8 bars = approximately -800 cm)

- measures water pressure relative to atmospheric pressure
- water boils if $h_p$ in tensiometer < vapor pressure of water
- measurement range limited by air entry value of porous cup

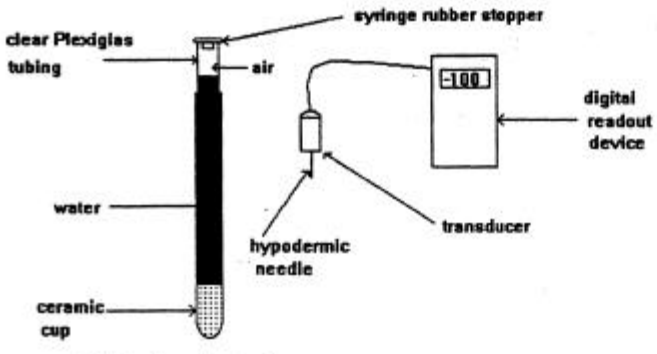
mercury types gauge types transducer types




Schematic illustration of a mercury-water manometer type tensiometer system.




Jet fill type tensiometer system.

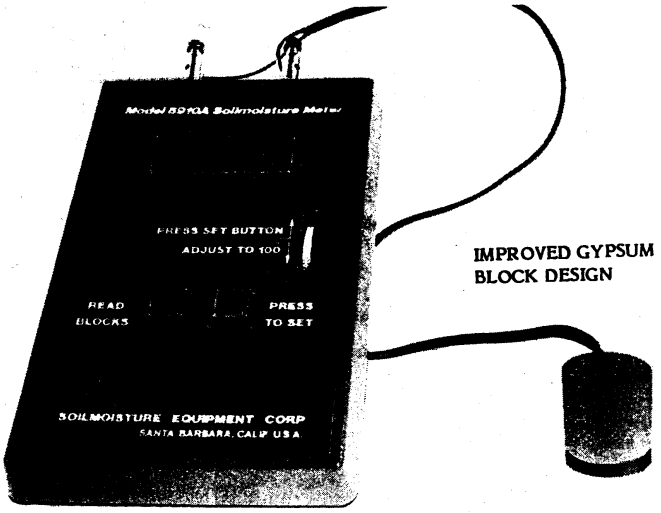


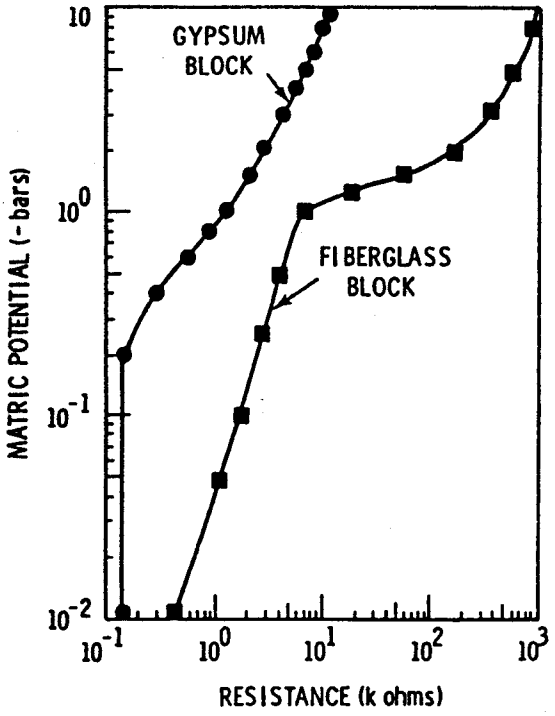

Transducer type tensiometer system.

Electrical Resistance Blocks 0 to -980 or -1,500 kPa

(0 to -10 or -15 bars)

Resistance(R) depends upon amount of $H_2O$ in block. Must be calibrated. Requires good soil contact, and R depends on ionic concentration in block. Accurate only in dry range.




**Typical calibration curves for gypsum and fiberglass block soil moisture sensors.**

Thermocouple Psychrometer -98 to –3,000 kPa

(-1 to -30 bars)

-Measures contribution of matric + osmotic potential to soil-water vapor pressure

-Measures vapor pressure of the thermocouple chamber and assumes equilibrium with the soil in the chamber

-Uses wet and dry junctions to establish vapor pressure deficit

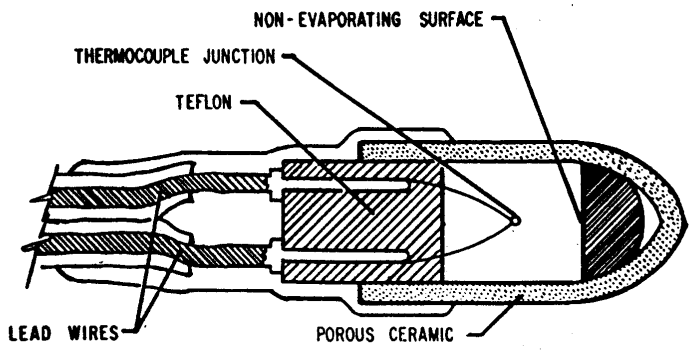



Fig. 24–7. Soil psychrometer modified to reduce error due to thermal gradients by using an additional nonevaporating surface in the chamber (Campbell, 1972).

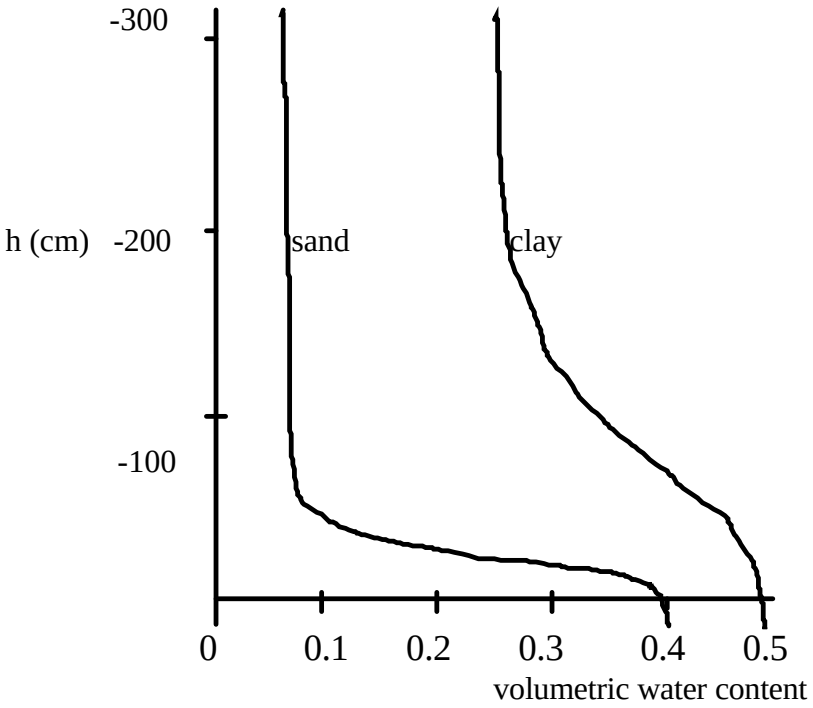
Heat Dissipation Sensor -9.8 to -100,000 kPa)
(-0.1 to –1,000 bars)

-Measures rate at which heat is conducted away from a heat source, using temperature response close to heat source (using thermocouple or thermister).

-The more water in porous matrix, the faster the heat is conducted away.

## Soil-water characteristic curve (soil-water retention curve):

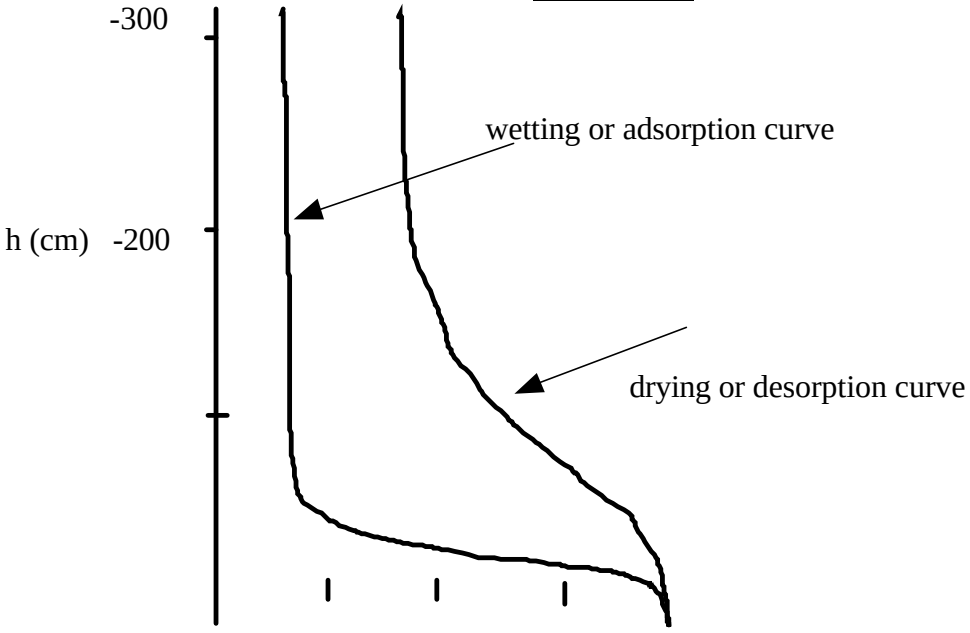
- Describes relationship between soil-water pressure potential and volumetric water content;
- Is determined by simultaneous measurement of water content and pressure potential;
- As soil drains, the largest soil pores empty first since the capillary forces are smallest in these pores. As the soil drains further, the maximum diameter of the water-filled pores further decreases, corresponding with pores that have decreasing values for the pressure potential (water is held by larger capillary forces);
- Soil-water retention is unique, and is a function of pore size distribution;
- At a pressure potential of zero, the soil's volumetric water content is defined as the saturated water content;
- The maximum pressure potential at which soil begins to desaturate (starting at saturation) is defined as the air entry value of the soil, and is determined by the largest pores in the soil;

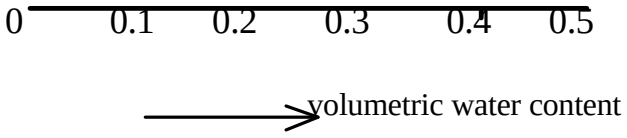



- Soil available water is defined as the water content difference between field capacity (h=-330 cm) and permanent wilting point (h = -15,000 cm); Will be discussed later !!!

✪ Would you expect the soil-water retention curve to be different if it was determined by desorption or adsorption ?

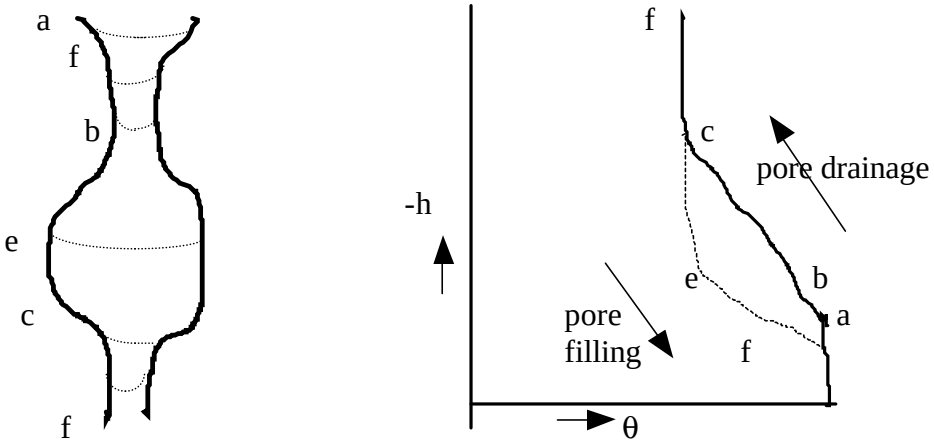
**Hysteresis**





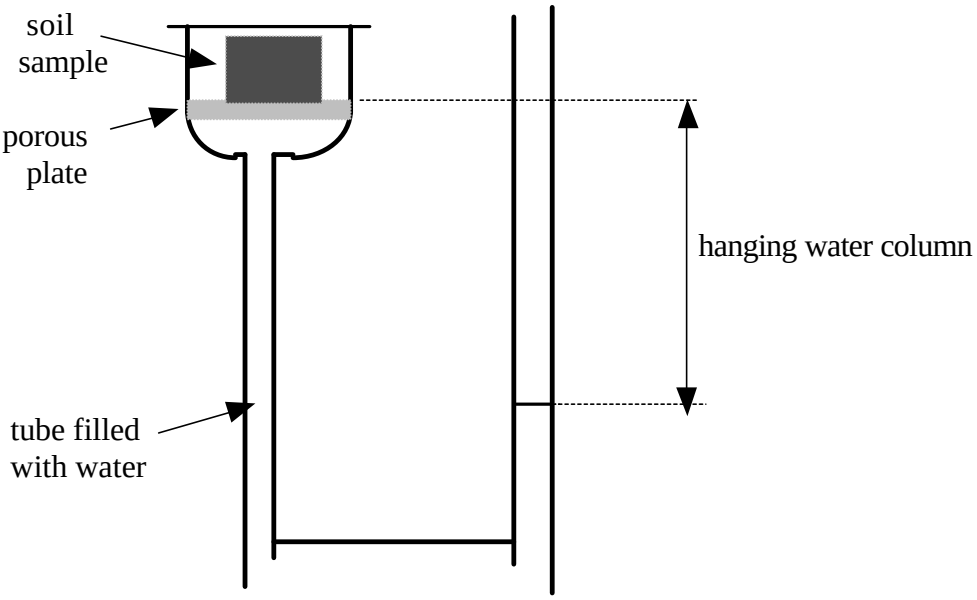

The cause of hysteresis is attributed to:

- Contact angle is larger for wetting than drying of the soil;
- Entrapment of air during soil wetting;
- "Ink bottle" effect. Pore necks (throats prevent complete drainage of soil pores)




**How to measure the soil-water retention curve:**

1. **Buchner funnels** can be used up to about -300 cm



✪ If the length of the hanging water column is X and the system is in hydraulic equilibrium, what is the pressure potential in the soil sample ?

2. **Pressure cell (Tempe Cell)**

- good to -98 kPa (-1 bar)

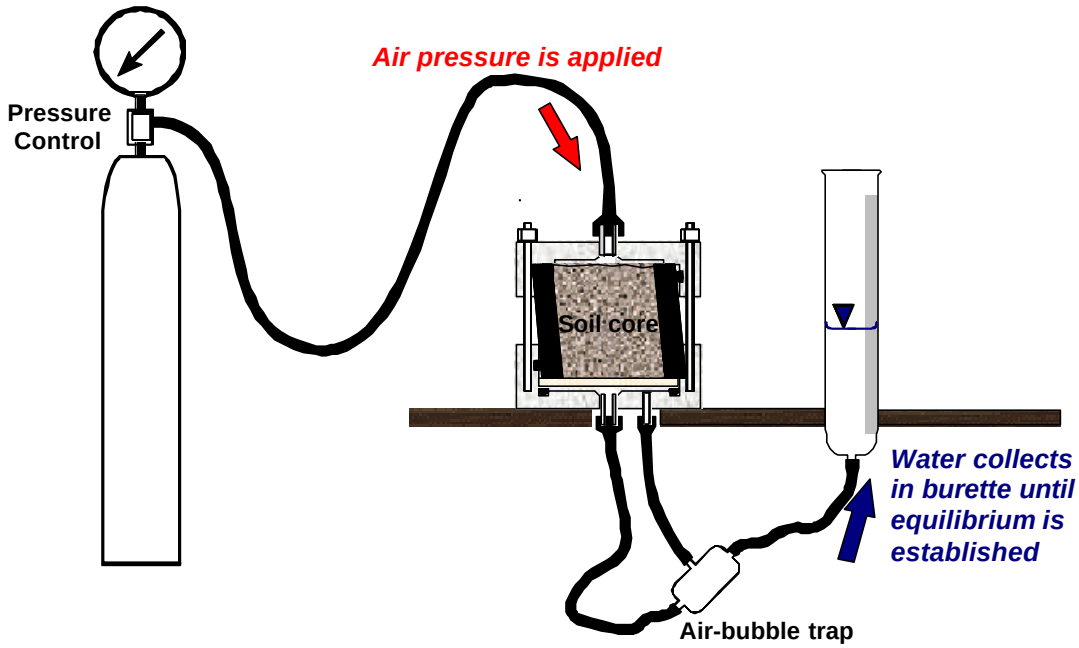
- takes a long time to reach equilibrium in an undisturbed, full- size core

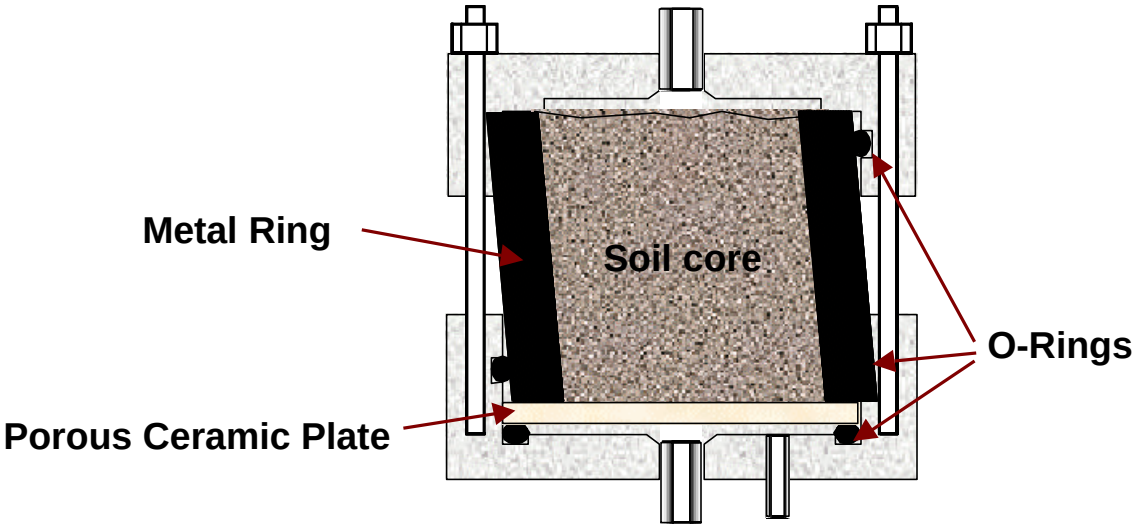
- is used by applying positive air pressure to soil core

3. **Pressure plate**

- good to - 1,500 kPa (-15 bar)
- not convenient for large, undisturbed cores
- soil can be disturbed because the water present in the drier range occurs in soil micropores instead of the normal pore structure
- conductivity of plate too small for wet range determination

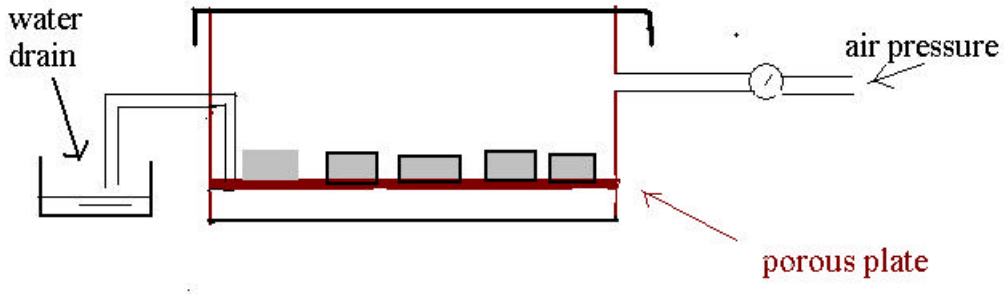
Components of the Tempe cell method






Vertical cross section of the cell

**Pressure plate apparatus**




✪ If 1 bar gauge pressure is applied to the pressure plate apparatus, and water drainage has stopped, then what is the pressure potential of the soil water in the cores ?

**Components of Total Soil-Water Potential:**

**$Y_p$ – soil-water pressure potential**

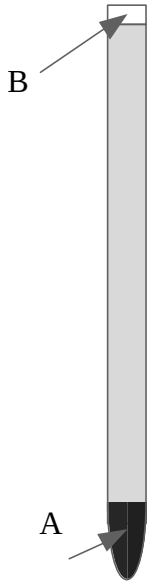
Remember:
Energy per unit volume of water required to transfer an infinitesimal quantity of water from a reference pool of water at the elevation of the soil to the point of interest in the soil at reference air pressure and temperature.

When expressed in energy per unit weight (m), then we simply use h (pressure head)

If the soil is unsaturated: ⟶ soil water matric potential ($h < 0$)

If the soil is saturated: ⟶ hydrostatic pressure potential ($h = p > 0$)

Use tensiometer to measure soil water pressure potential:




Total soil water potential at A
=
Total water potential at B

The pressure in B is measured with a pressure transducer

As we shall see later:
From the length of the tensiometer, the pressure Potential in soil at point A can be determined (either matric or hydrostatic pressure potential).

<u>If soil at A is saturated, then:</u>

Pressure potential or hydrostatic water pressure is equal to the water pressure exerted by overlying unsupported saturated water at the point of interest. This is strictly positive, and is equal to the pressure equivalent of the height of water above the point of interest (h = hydrostatic pressure head, when expressed in potential per unit weight, Nm/m = m, sometimes also denoted by p).

<u>If soil at A is unsaturated, then:</u>

h = matric pressure head given by: $h = \frac{y_p}{r_w g}$ (units: m)

In short notation, we sometimes refer to h = pressure head, independent of its sign (saturated or unsaturated)

✪ If the tensiometer cup at point A is located at the water table (pressure is atmospheric pressure), what is the pressure potential at point A ?

$Y_z$ **- gravitational potential (can be + or -)**

Energy per unit volume of water required to move an infinitesimal amount of pure, free water from the reference elevation ($z_o$) to the soil water elevation ($z_{soil}$).

$$\text{Potential} = \text{Work} = \text{Force X distance} = F(z_{soil} - z_o) = m\,g\,(z_{soil} - z_o)$$

If potential is expressed per unit volume, then gravitational potential = $(z_{soil} - z_o)$, or

$$\Psi_z = m\,g\,(z_{soil} - z_o)/V = \rho_w g(z_{soil} - z_o)$$

where $z_{soil}$ is the height above or below an arbritary reference level

$z_{soil}$ = + , if above ref. level

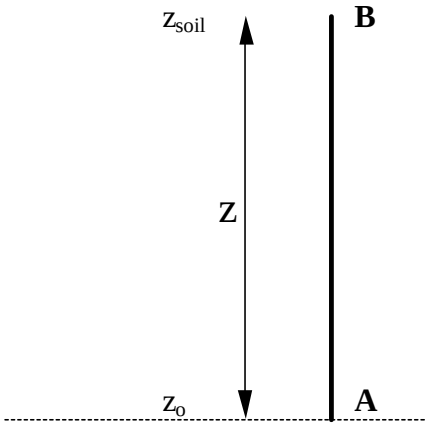
= - , if below ref. level

If potential is expressed per unit weight, then gravitational potential

$$z = \Psi_z / \rho_w g = (z_{soil} - z_o)$$

If one sets $z_o$ to 0, then gravitational potential :

$$z = (z_{soil} - z_o) = z_{soil} = z$$




Thus, z is positive, if point in consideration is above reference level ($z_o$),
and z is negative, if point in consideration is below reference level.

One can chose $z_o$ anywhere, as long as one is consistent in its choice.

✪ Now with this information, compute the soil water pressure potential at point A in the previous diagram with the tensiometer of length L.

### $Y_s$ - solute potential

- always negative since defined relative to pure $H_2O$
- due to amount of salt in soil solution
- only important if have semi-permeable membrane (plant roots) or at air-water interface (controls humidy of air phase)

s = osmotic pressure head given by: $s = \frac{y_s}{r_w g}$ (units: m)

### $Y_a$ - air pressure potential

Accounts for changes in air pressure, different than the reference pressure (atmospheric pressure). Important in pressure cells (Tempe cell) and pressure plate apparatus.

Positive, if air pressure is larger than reference pressure.
Negative, if air pressure is smaller than reference pressure

a = air pressure head given by: $a = \frac{y_a}{r_w g}$ (units: m)

## **Total water potential** , $\Psi_T$

$\Psi_T = \Psi_p + \Psi_z + \Psi_s + \Psi_a + .....$

(Or in head units: H = h + z + s + a + . . . )

Much of our discussion will only include $\Psi_p$ (<0 or >0) and $\Psi_z$

so $\psi_T = \psi_p + \psi_z$ or Total Head H = h + z

(Book Jury: sometimes uses h = p, if h > 0)

**Other terminology found in the literature**:

Suction = absolute value of h, if h < 0)
Hydraulic potential = $\psi_p + \psi_z$

Submergence potential = $\psi_p$ (+ only)

Pneumatic potential - due to air only