

UNIT 4

PLANT PHYSIOLOGY

Chapter 11

Photosynthesis in Higher Plants

Chapter 12

Respiration in Plants

Chapter 13

Plant Growth and Development

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



Melvin Calvin

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

Just after world war II, when the world was under shock after the Hiroshima-Nagasaki bombings, and seeing the ill-effects of radio-activity, Calvin and co-workers put radio-activity to beneficial use. He along with J.A. Bassham studied reactions in green plants forming sugar and other substances from raw materials like carbon dioxide, water and minerals by labelling the carbon dioxide with C¹⁴. Calvin proposed that plants change light energy to chemical energy by transferring an electron in an organised array of pigment molecules and other substances. The mapping of the pathway of carbon assimilation in photosynthesis earned him Nobel Prize in 1961.

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



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CHAPTER 11

PHOTOSYNTHESIS IN HIGHER PLANTS

- 11.1 *What do we Know?*
- 11.2 *Early Experiments*
- 11.3 *Where does Photosynthesis take place?*
- 11.4 *How many Pigments are involved in Photosynthesis?*
- 11.5 *What is Light Reaction?*
- 11.6 *The Electron Transport*
- 11.7 *Where are the ATP and NADPH Used?*
- 11.8 *The C₄ Pathway*
- 11.9 *hotorespiration*
- 11.10 *Factors affecting Photosynthesis*

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

11.1 WHAT DO WE KNOW?

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

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

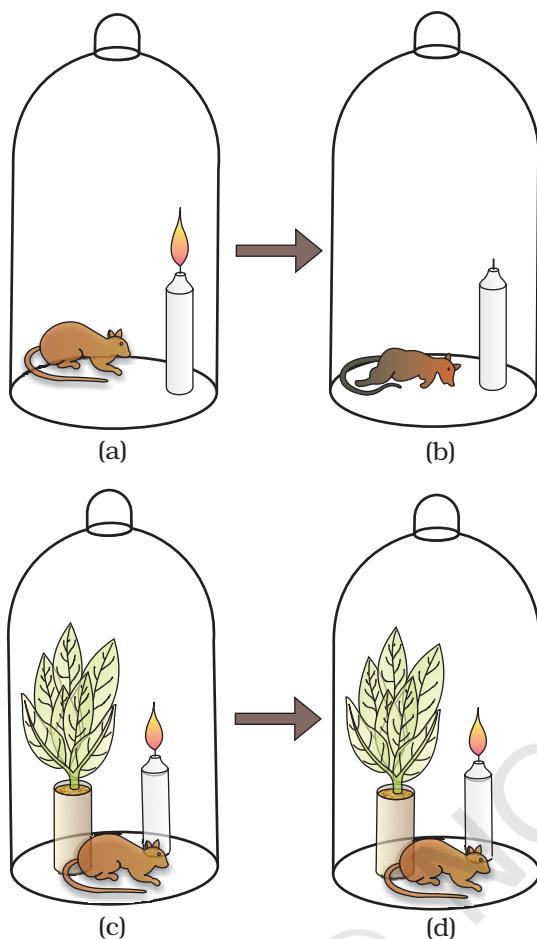


Figure 11.1 Priestley's experiment

Another experiment you may have carried out where a part of a leaf is enclosed in a test tube containing some KOH soaked cotton (which absorbs CO_2), while the other half is exposed to air. The setup is then placed in light for some time. On testing for the presence of starch later in the two parts 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?*

11.2 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 11.1 a, b, c, d). Similarly, a mouse would soon suffocate in a closed space. He concluded that a burning candle or an animal that breathe the air,

both somehow, damage the air. But when he placed a mint plant in the same bell jar, he found that the mouse stayed alive and the candle continued to burn. Priestley hypothesised as follows: Plants restore to the air whatever breathing animals and burning candles remove.

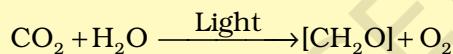
Can you imagine how Priestley would have conducted the experiment using a candle and a plant? Remember, he would need to rekindle the candle to test whether it burns after a few days. *How many different ways can you think of to light the candle without disturbing the set-up?*

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

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

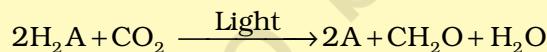
Now consider the interesting experiments done by T.W Engelmann (1843 – 1909). Using a prism he split light into its spectral components and then illuminated a green alga, *Cladophora*, placed in a suspension of aerobic bacteria. The bacteria were used to detect the sites of O₂ evolution. He observed that the bacteria accumulated mainly in the region of blue and red light of the split spectrum. A first action spectrum of photosynthesis was thus described. It resembles roughly the absorption spectra of chlorophyll *a* and *b* (discussed in section 11.4).

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

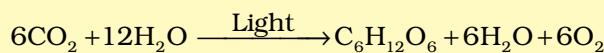


where [CH₂O] represented a carbohydrate (e.g., glucose, a six-carbon sugar).

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



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



where C₆H₁₂O₆ represents glucose. The O₂ released is from water; this was proved using radio isotope techniques. Note that this is not a single

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

11.3 WHERE DOES PHOTOSYNTHESIS TAKE PLACE?

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

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

You have studied the structure of chloroplast in Chapter 8. Within the chloroplast there is membranous system consisting of grana, the stroma lamellae, and the matrix stroma (Figure 11.2). There is a clear division of labour within the chloroplast. The membrane system is responsible for trapping the light energy and also for the synthesis of ATP and NADPH. In stroma, enzymatic reactions synthesise sugar, which in turn forms starch. The former set of reactions, since they are directly light driven are called **light reactions** (photochemical reactions). The latter are not directly light driven but are dependent on the products of light reactions (ATP and NADPH). Hence, to distinguish the latter they are called, by convention, as **dark reactions** (carbon reactions). However, this should not be construed to mean that they occur in darkness or that they are not light-dependent.

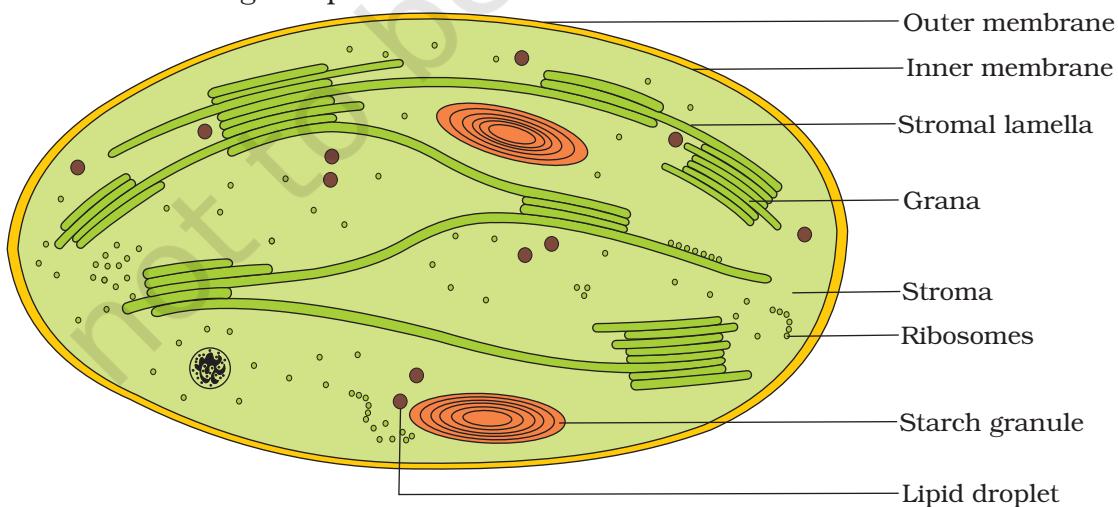


Figure 11.2 Diagrammatic representation of an electron micrograph of a section of chloroplast

11.4 HOW MANY TYPES OF 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 11.3 a). Of course, you are familiar with the wavelength of the visible spectrum of light as well as the VIBGYOR.

From Figure 11.3a 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 11.3b 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 11.3c 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?*

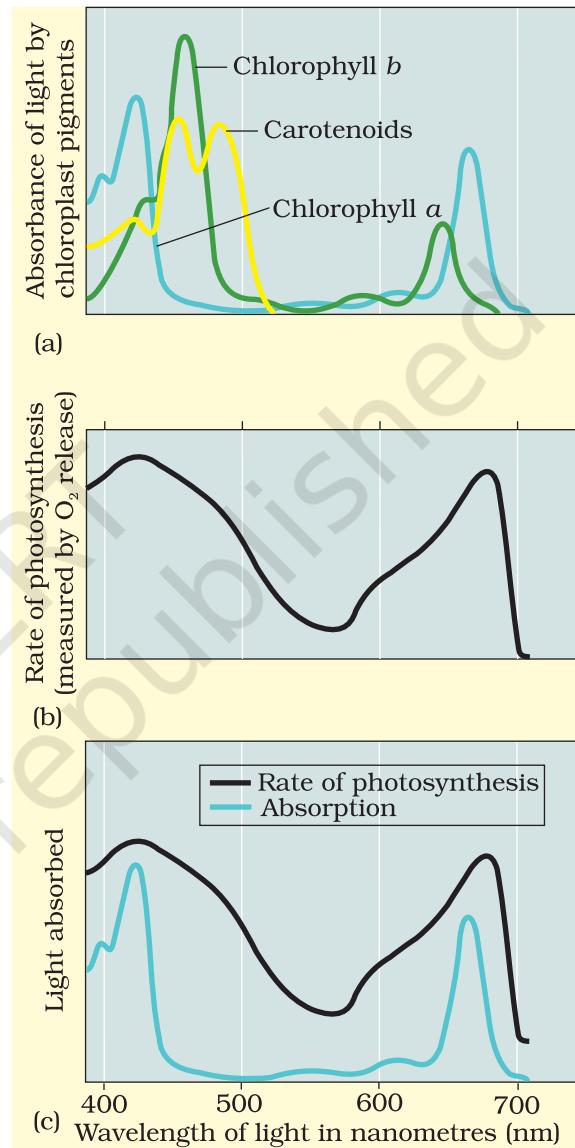


Figure 11.3a Graph showing the absorption spectrum of chlorophyll a, b and the carotenoids

Figure 11.3b Graph showing action spectrum of photosynthesis

Figure 11.3c 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 photosynthesis but also protect chlorophyll *a* from photo-oxidation.

11.5 WHAT IS LIGHT REACTION?

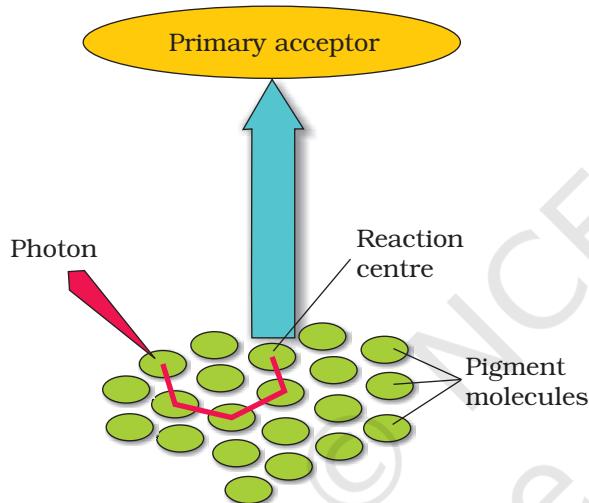


Figure 11.4 The light harvesting complex

Light reactions or the ‘Photochemical’ phase include light absorption, water splitting, oxygen release, and the formation of high-energy chemical intermediates, ATP and NADPH. Several protein complexes are involved in the process. The pigments are organised into two discrete photochemical **light harvesting complexes (LHC)** within the **Photosystem I (PS I)** and **Photosystem II (PS II)**. These are named in the sequence of their discovery, and not in the sequence in which they function during the light reaction. The LHC are made up of hundreds of pigment molecules bound to proteins. Each photosystem has all the pigments (except one molecule of chlorophyll *a*) forming a light harvesting system also called **antennae** (Figure 11.4). 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**.

11.6 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**

system consisting of cytochromes (Figure 11.5). 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 acceptor molecule that has a greater redox potential. These electrons then are moved downhill again, this time to a molecule of energy-rich NADP⁺. The addition of these electrons reduces NADP⁺ to NADPH + H⁺. This whole scheme of transfer of electrons, starting from the PS II, uphill to the acceptor, down the electron transport chain to PS I, excitation of electrons, transfer to another acceptor, and finally down hill to NADP⁺ reducing it to NADPH + H⁺ is called the **Z scheme**, due to its characteristic shape (Figure 11.5). This shape is formed when all the carriers are placed in a sequence on a redox potential scale.

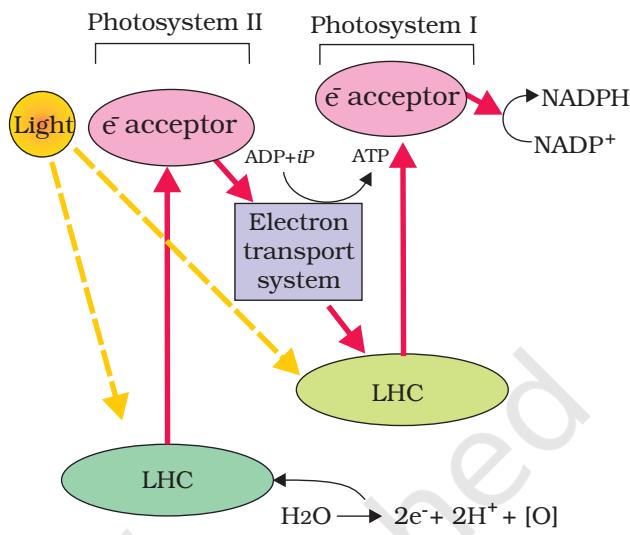
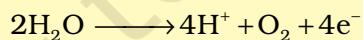


Figure 11.5 Z scheme of light reaction

11.6.1 Splitting of Water

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



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 O₂ formed likely to be released – in the lumen? or on the outer side of the membrane?*

11.6.2 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

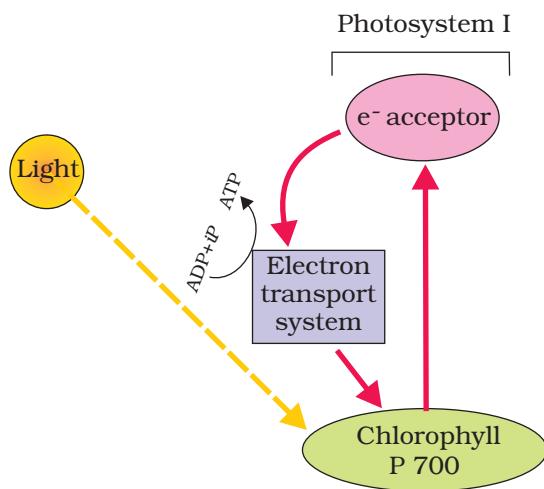


Figure 11.6 Cyclic photophosphorylation

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 PSI, 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 11.5).

When only PS I is functional, the electron is circulated within the photosystem and the phosphorylation occurs due to cyclic flow of electrons (Figure 11.6). 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 11.6). 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.

11.6.3 Chemiosmotic Hypothesis

Let us now try and understand how actually ATP is synthesised in the chloroplast. The chemiosmotic hypothesis has been put forward to explain the mechanism. Like in respiration, in photosynthesis too, ATP synthesis is linked to development of a proton gradient across a membrane. This time these are the membranes of thylakoid. There is one difference though, here the proton accumulation is towards the inside of the membrane, i.e., in the lumen. In respiration, protons accumulate in the intermembrane space of the mitochondria when electrons move through the ETS (Chapter 12).

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 11.7).

- (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.

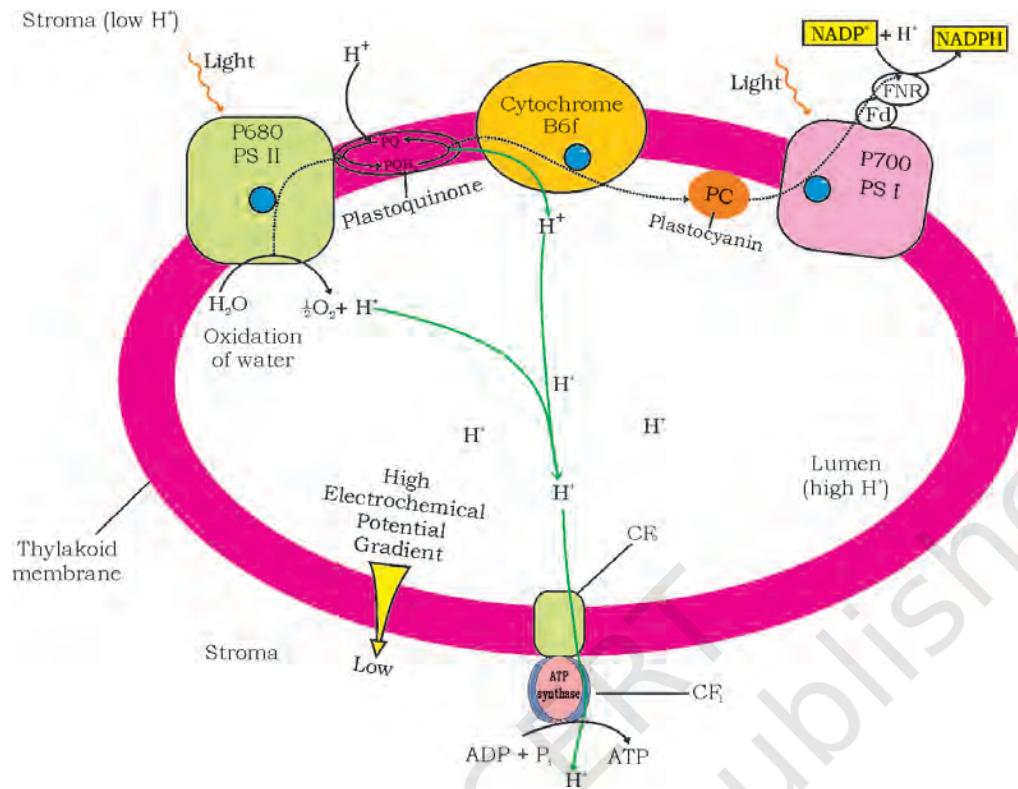


Figure 11.7 ATP synthesis through chemiosmosis

- (b) As electrons move through the photosystems, protons are transported across the membrane. This happens because the primary acceptor 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 $\text{NADPH} + \text{H}^+$. These protons are also removed from the stroma.

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

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

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

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

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

11.7 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 $(\text{CH}_2\text{O})_n$ 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.

11.7.1 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.

11.7.2 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 11.8).

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**.

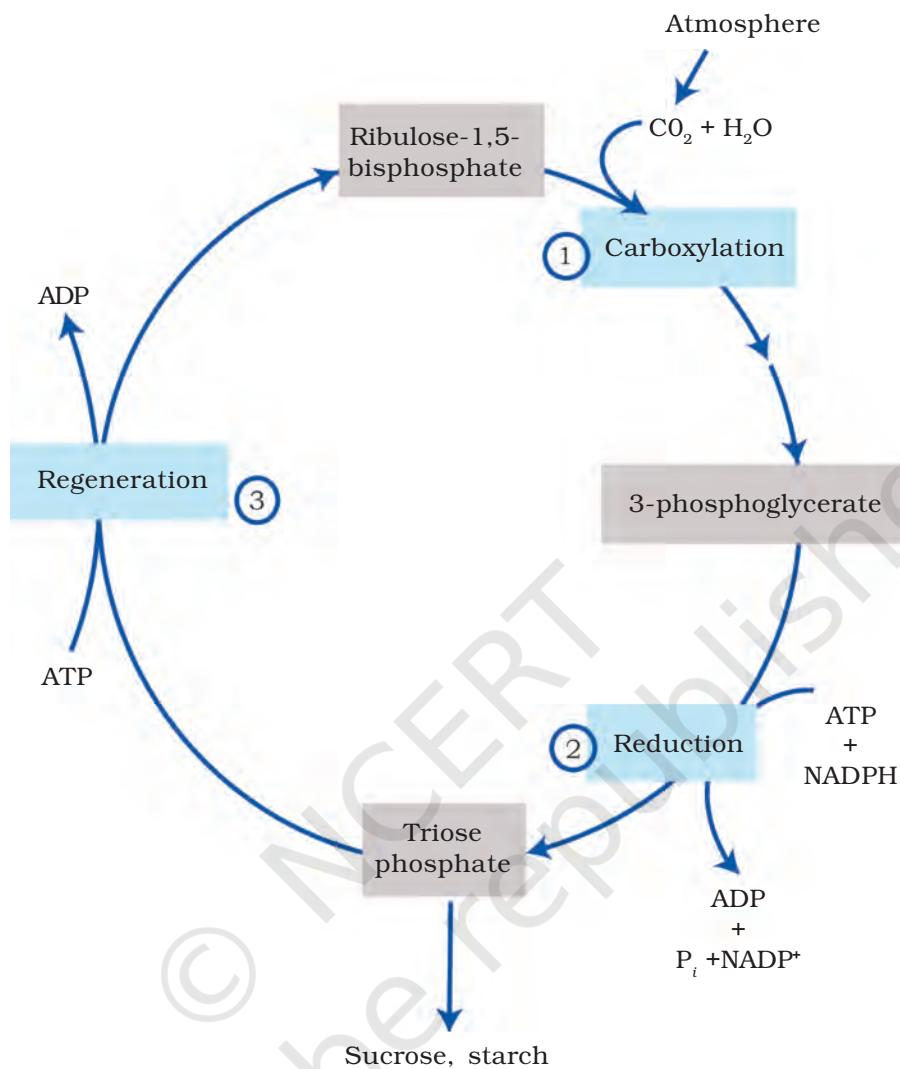


Figure 11.8 The Calvin cycle proceeds in three stages : (1) carboxylation, during which CO₂ combines with ribulose-1,5-bisphosphate; (2) reduction, during which carbohydrate is formed at the expense of the photochemically made ATP and NADPH; and (3) regeneration during which the CO₂ acceptor ribulose-1,5-bisphosphate is formed again so that the cycle continues

- 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 CO₂ molecule fixed. The fixation of six molecules of CO₂ and 6 turns of the cycle are required for the formation of one molecule of glucose from the pathway.
- Regeneration** – Regeneration of the CO₂ 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

11.8 THE C_4 PATHWAY

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

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

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

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

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

Now study the pathway shown in Figure 11.9. 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

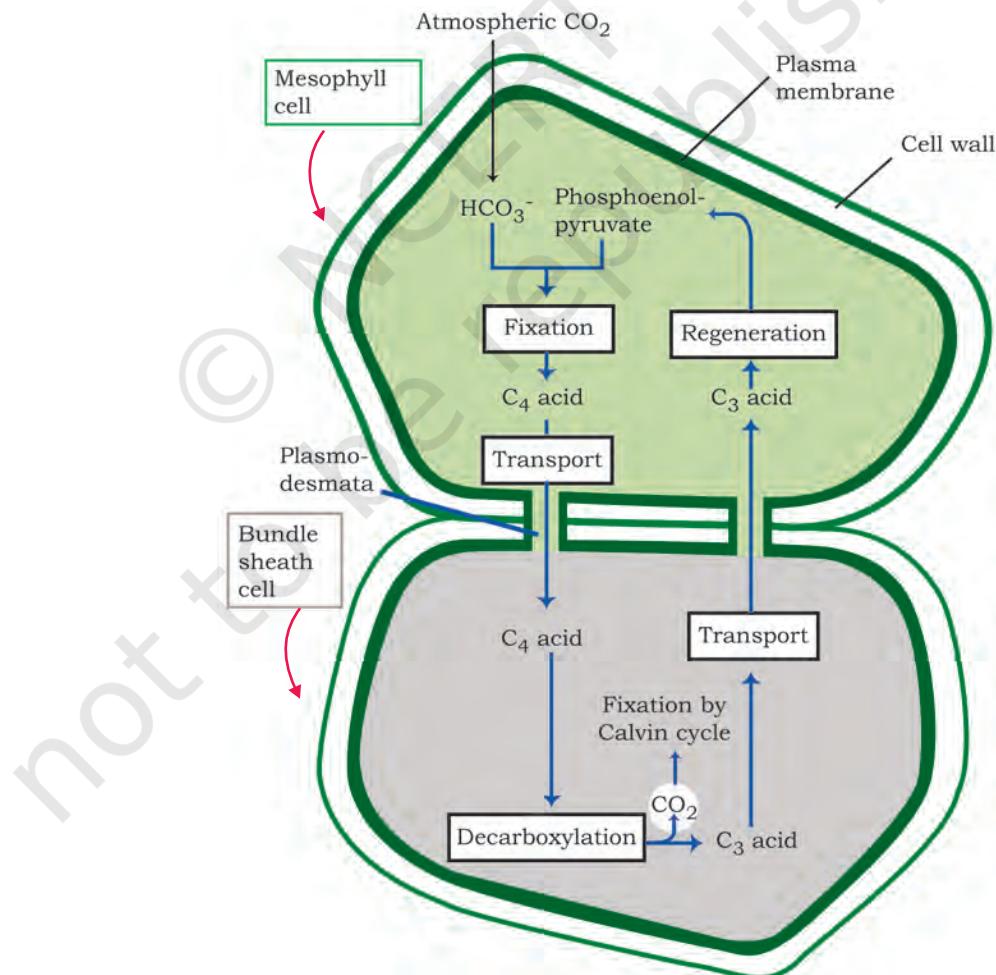


Figure 11.9 Diagrammatic representation of the Hatch and Slack Pathway

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 C₃ and C₄ plants.

Did you note that the Calvin pathway occurs in all the mesophyll cells of the C₃ plants? In the C₄ plants it does not take place in the mesophyll cells but does so only in the bundle sheath cells.

11.9 PHOTORESPIRATION

Let us try and understand one more process that creates an important difference between C₃ and C₄ plants – **Photorespiration**. To understand photorespiration we have to know a little bit more about the first step of the Calvin pathway – the first CO₂ fixation step. This is the reaction where RuBP combines with CO₂ to form 2 molecules of 3PGA, that is catalysed by RuBisCO.



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 CO₂ and O₂ – hence the name. *Can you think how this could be possible?* RuBisCO has a much greater affinity for CO₂ when the CO₂: O₂ is nearly equal. Imagine what would happen if this were not so! This binding is competitive. It is the relative concentration of O₂ and CO₂ that determines which of the two will bind to the enzyme.

In C₃ plants some O₂ does bind to RuBisCO, and hence CO₂ fixation is decreased. Here the RuBP instead of being converted to 2 molecules of PGA binds with O₂ to form one molecule of phosphoglycerate and phosphoglycolate (2 Carbon) in a pathway called photorespiration. In the photorespiratory pathway, there is neither synthesis of sugars, nor of ATP. Rather it results in the release of CO₂ with the utilisation of ATP. In the photorespiratory pathway there is no synthesis of ATP or NADPH. The biological function of photorespiration is not known yet.

In C₄ plants photorespiration does not occur. This is because they have a mechanism that increases the concentration of CO₂ at the enzyme site. This takes place when the C₄ acid from the mesophyll is broken down in the bundle sheath cells to release CO₂ – this results in increasing the intracellular concentration of CO₂. In turn, this ensures that the RuBisCO functions as a carboxylase minimising the oxygenase activity.

Now that you know that the C₄ 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 C₃ and the C₄ pathway? Use the table format given in table 11.1 and fill in the information.

TABLE 11.1 Fill in the Columns 2 and 3 in this table to highlight the differences between C₃ and C₄ 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 One: Mesophyll 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.

11.10 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.

11.10.1 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 11.10). 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

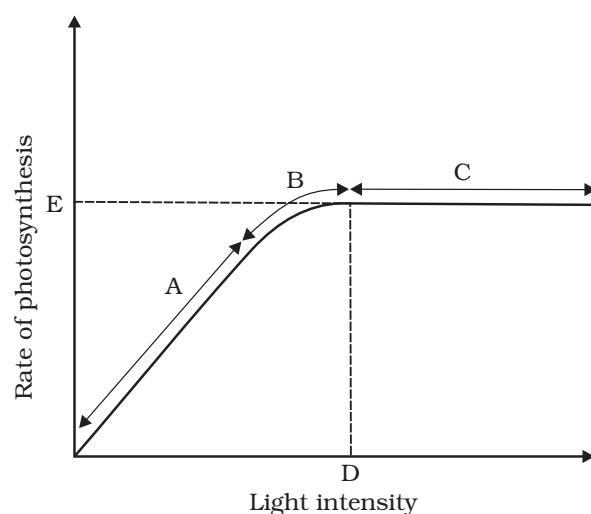


Figure 11.10 Graph of light intensity on the rate of photosynthesis

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

11.10.2 Carbon dioxide Concentration

Carbon dioxide is the major limiting factor for photosynthesis. The concentration of CO₂ 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 CO₂ fixation rates; beyond this the levels can become damaging over longer periods.

The C₃ and C₄ plants respond differently to CO₂ concentrations. At low light conditions neither group responds to high CO₂ conditions. At high light intensities, both C₃ and C₄ plants show increase in the rates of photosynthesis. What is important to note is that the C₄ plants show saturation at about 360 µL⁻¹ while C₃ responds to increased CO₂ concentration and saturation is seen only beyond 450 µL⁻¹. Thus, current availability of CO₂ levels is limiting to the C₃ plants.

The fact that C₃ plants respond to higher CO₂ 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.

11.10.3 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 C₄ plants respond to higher temperatures and show higher rate of photosynthesis while C₃ 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.

11.10.4 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 CO₂ 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 C₄ 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 11.10 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) C₃ and C₄ pathways
 - (b) Cyclic and non-cyclic photophosphorylation
 - (c) Anatomy of leaf in C₃ and C₄ plants



CHAPTER 12

RESPIRATION IN PLANTS

12.1 Do Plants Breathe?

12.2 Glycolysis

12.3 Fermentation

12.4 Aerobic Respiration

12.5 The Respiratory Balance Sheet

12.6 Amphibolic Pathway

12.7 Respiratory Quotient

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.

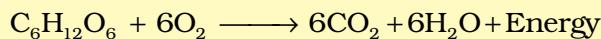
Photosynthesis, of course, takes place within the chloroplasts (in the eukaryotes), whereas the breakdown of complex molecules to yield energy takes place in the cytoplasm and in the mitochondria (also only in eukaryotes). The breaking of the C-C bonds of complex compounds through oxidation within the 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.

12.1 Do PLANTS BREATHE?

Well, the answer to this question is not quite so direct. Yes, plants require O₂ for respiration to occur and they also give out CO₂. Hence, plants have systems in place that ensure the availability of O₂. 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 O₂ is not a problem in these cells since O₂ 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.



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**.

12.2 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

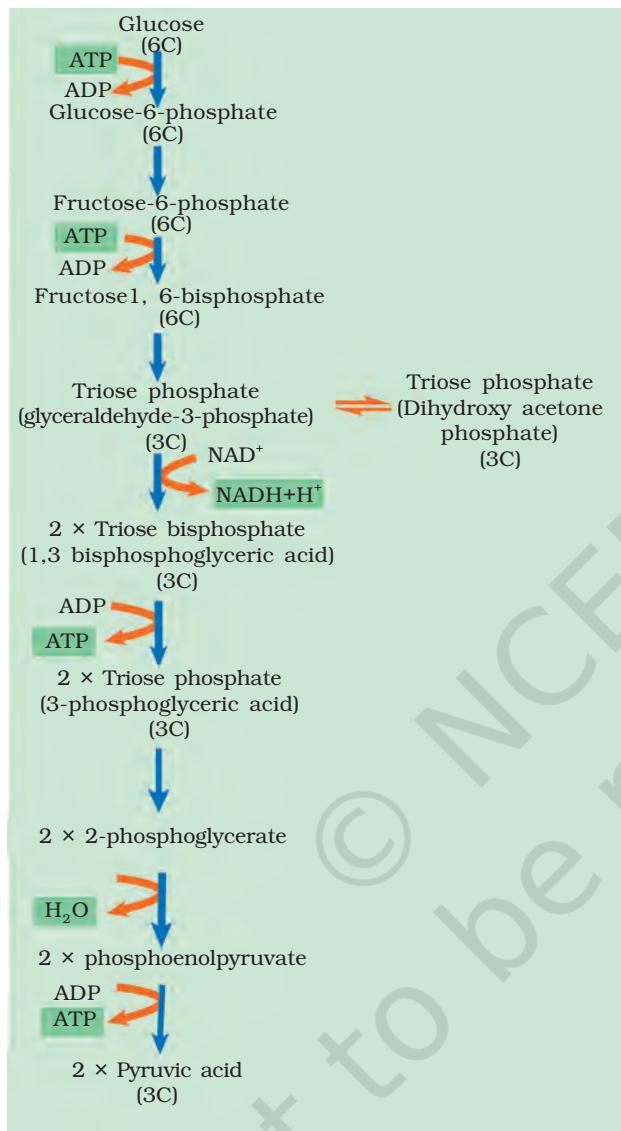


Figure 12.1 Steps of glycolysis

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 12.1. 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.

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.

12.3 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 12.2. 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 $\text{NADH}+\text{H}^+$ which is reoxidised to NAD^+ in both the processes.

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

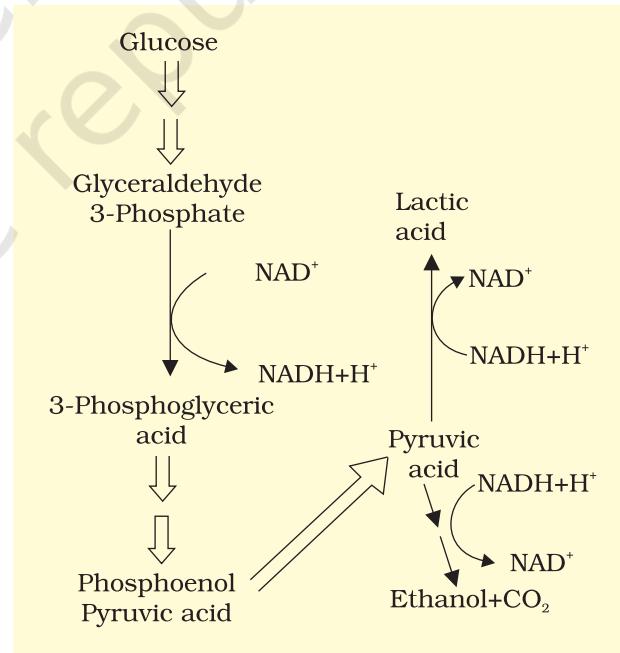


Figure 12.2 Major pathways of anaerobic respiration

synthesise a larger number of ATP molecules needed for cellular metabolism? In eukaryotes these steps take place within the mitochondria and this requires O₂. **Aerobic respiration** is the process that leads to a complete oxidation of organic substances in the presence of oxygen, and releases CO₂, 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.

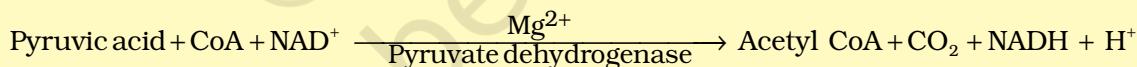
12.4 AEROBIC RESPIRATION

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 CO₂.
- The passing on of the electrons removed as part of the hydrogen atoms to molecular O₂ 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.



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.

12.4.1 Tricarboxylic Acid Cycle

The TCA cycle starts with the condensation of acetyl group with oxaloacetic acid (OAA) and water to yield citric acid (Figure 12.3). 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

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 FADH₂. 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 FADH₂ respectively. The summary equation for this phase of respiration may be written as follows:

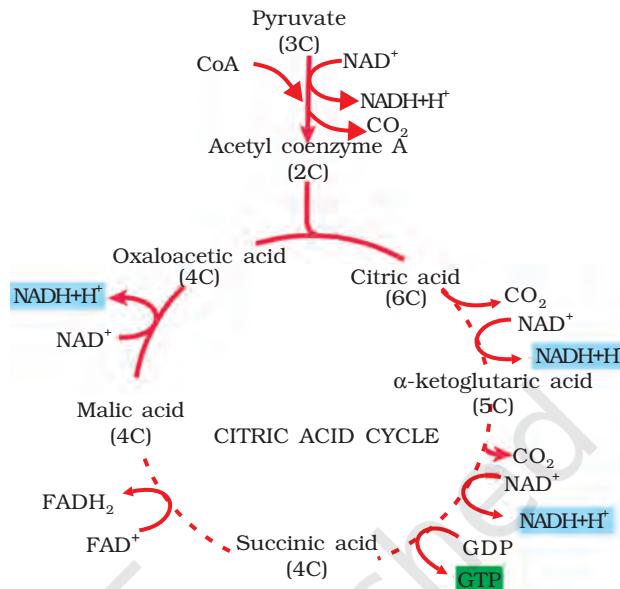
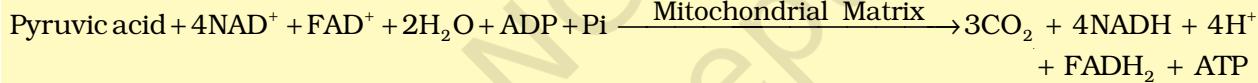


Figure 12.3 The Citric acid cycle



We have till now seen that glucose has been broken down to release CO₂ and eight molecules of NADH + H⁺; two of FADH₂ 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 O₂ 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 FADH₂ that is synthesised? Let us now understand the role of O₂ in respiration and how ATP is synthesised.

12.4.2 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 FADH₂. This is accomplished when they are oxidised through the electron transport system and the electrons are passed on to O₂ resulting in the formation of H₂O. The metabolic pathway through which the electron passes from one carrier to another, is called the **electron transport system** (ETS) (Figure 12.4) and it is present in the inner mitochondrial membrane. Electrons from NADH

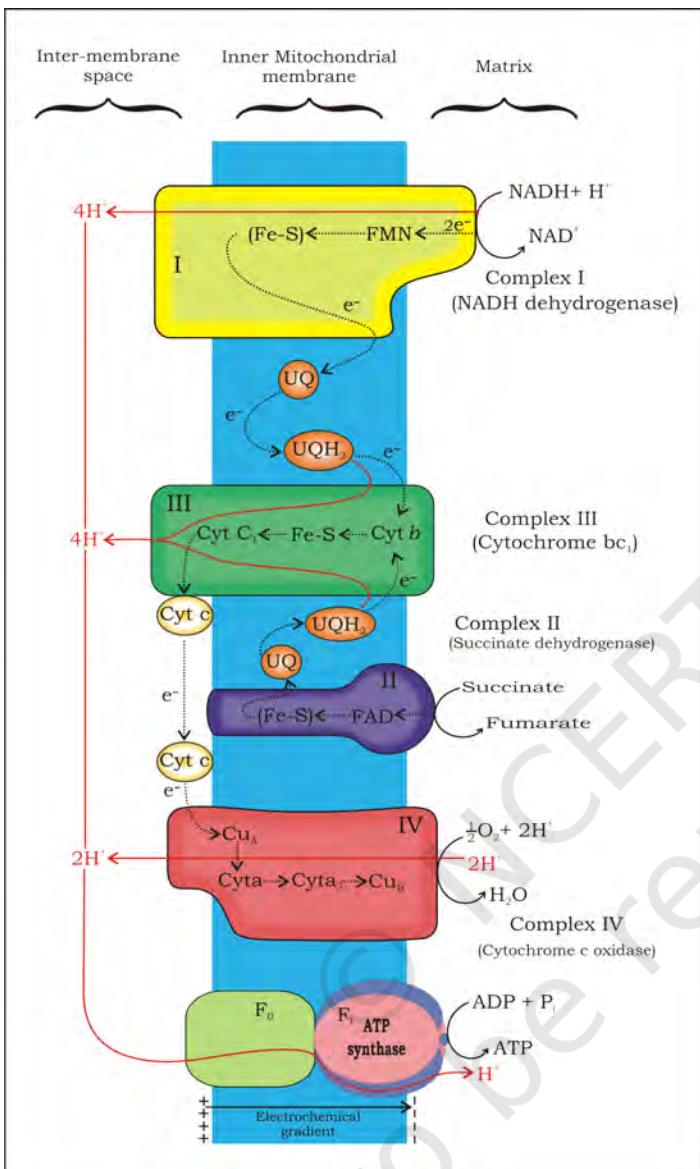


Figure 12.4 Electron Transport System (ETS)

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 FADH₂ (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.

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 FADH₂ 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

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 12.5). 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, 4H⁺ passes through F_0 from the intermembrane space to the matrix down the electrochemical proton gradient.

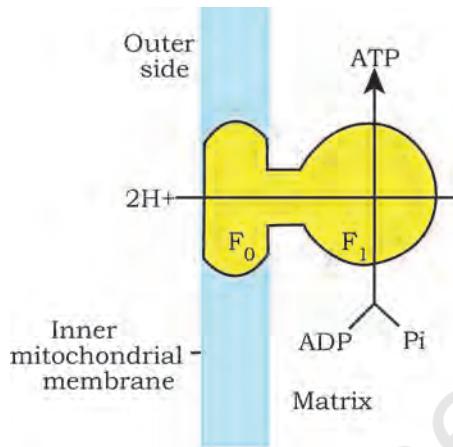


Figure 12.5 Diagrammatic presentation of ATP synthesis in mitochondria

12.5 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 respiration – no other alternative substrates are entering in the pathway at any of the intermediary stages.

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

Now let us compare fermentation and aerobic respiration:

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

12.6 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 12.6 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.

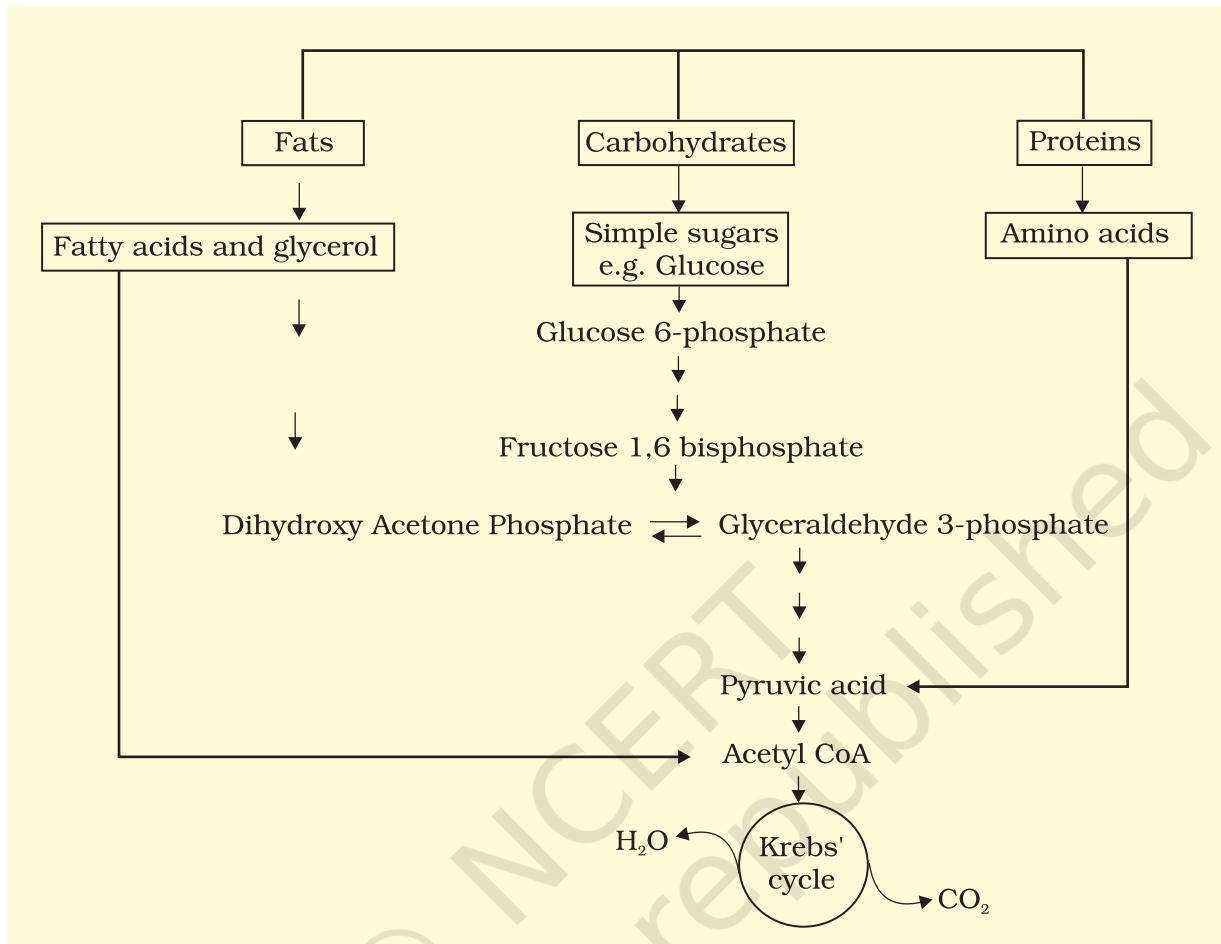


Figure 12.6 Interrelationship among metabolic pathways showing respiration mediated breakdown of different organic molecules to CO_2 and H_2O

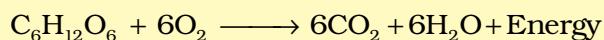
12.7 RESPIRATORY QUOTIENT

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.

$$\text{RQ} = \frac{\text{volume of } \text{CO}_2 \text{ evolved}}{\text{volume of } \text{O}_2 \text{ consumed}}$$

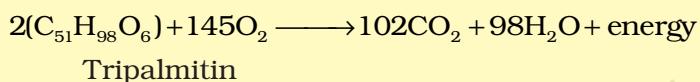
The respiratory quotient depends upon the type of respiratory substrate used during respiration.

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 :



$$\text{RQ} = \frac{6\text{CO}_2}{6\text{O}_2} = 1.0$$

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:



$$\text{RQ} = \frac{102\text{CO}_2}{145\text{O}_2} = 0.7$$

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 CO₂. Acetyl CoA then enters the tricarboxylic acid pathway or Krebs' cycle operating in the matrix of the mitochondria. NADH + H⁺ and FADH₂ 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?



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CHAPTER 13

PLANT GROWTH AND DEVELOPMENT

13.1 Growth

*13.2 Differentiation,
Dedifferentiation
and
Redifferentiation*

13.3 Development

*13.4 Plant Growth
Regulators*

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 descendants 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 13.1). The first step in the process of plant growth is seed germination. The seed germinates when favourable conditions for growth exist in the environment. In absence of such favourable conditions the seeds do not germinate and goes into a period of suspended growth or rest. Once favourable conditions return, the seeds resume metabolic activities and growth takes place.

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

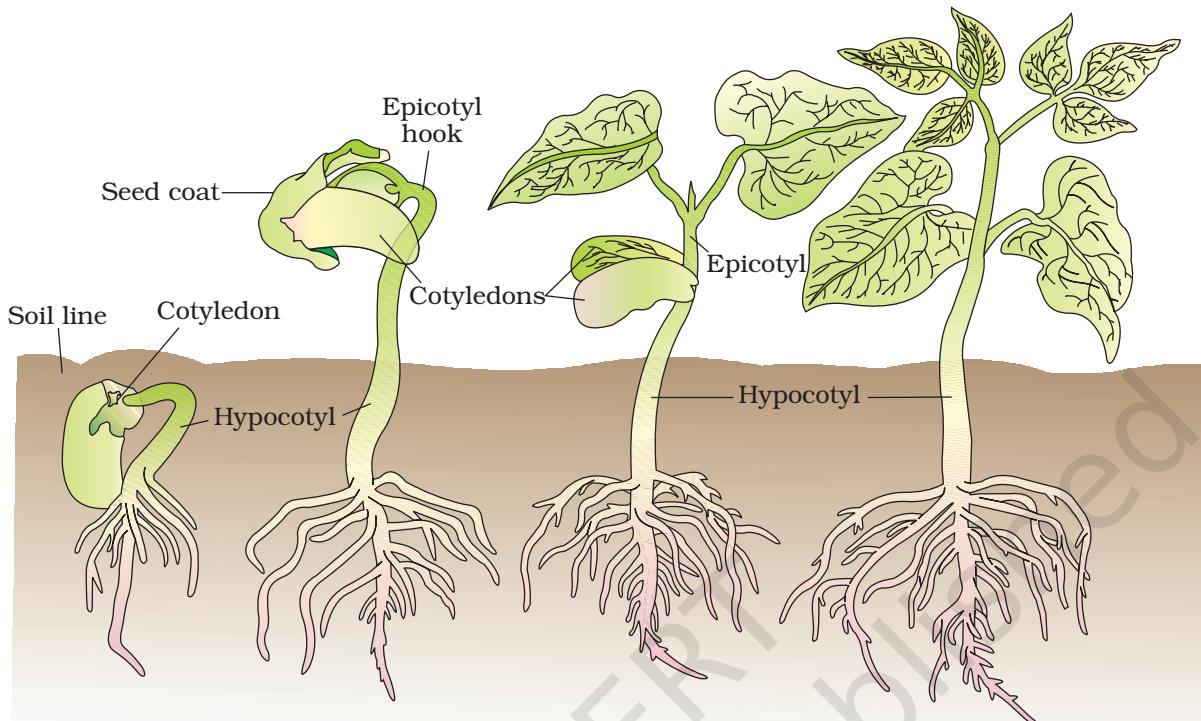


Figure 13.1 Germination and seedling development in bean

13.1 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?

13.1.1 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 earlier classes, you have studied about the root apical meristem and the shoot apical meristem. You know that they are responsible for

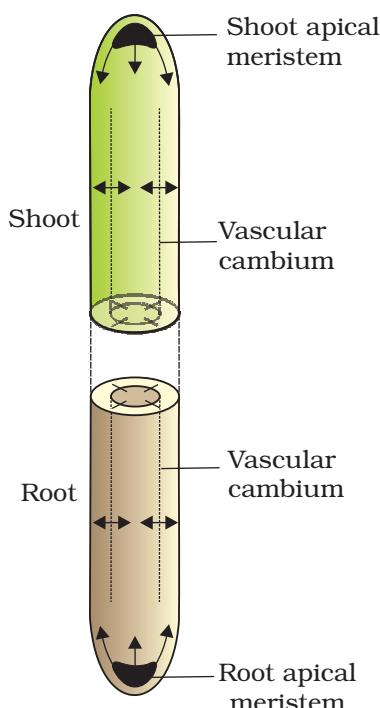


Figure 13.2 Diagrammatic representation of locations of root apical meristem, shoot apical meristem and vascular cambium. Arrows exhibit the direction of growth of cells and organ

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 13.2).

13.1.2 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 meristem 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.

13.1.3 Phases of Growth

The period of growth is generally divided into three phases, namely, meristematic, elongation and maturation (Figure 13.3). 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 cellulose with abundant plasmodesmatal connections. The cells proximal (just next, away from the tip) to the

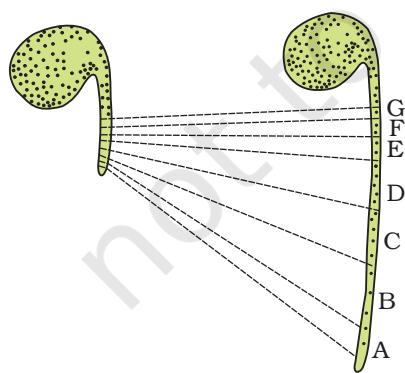


Figure 13.3 Detection of zones of elongation by the parallel line technique. Zones A, B, C, D immediately behind the apex have elongated most.

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 earlier classes represent this phase.

13.1.4 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.

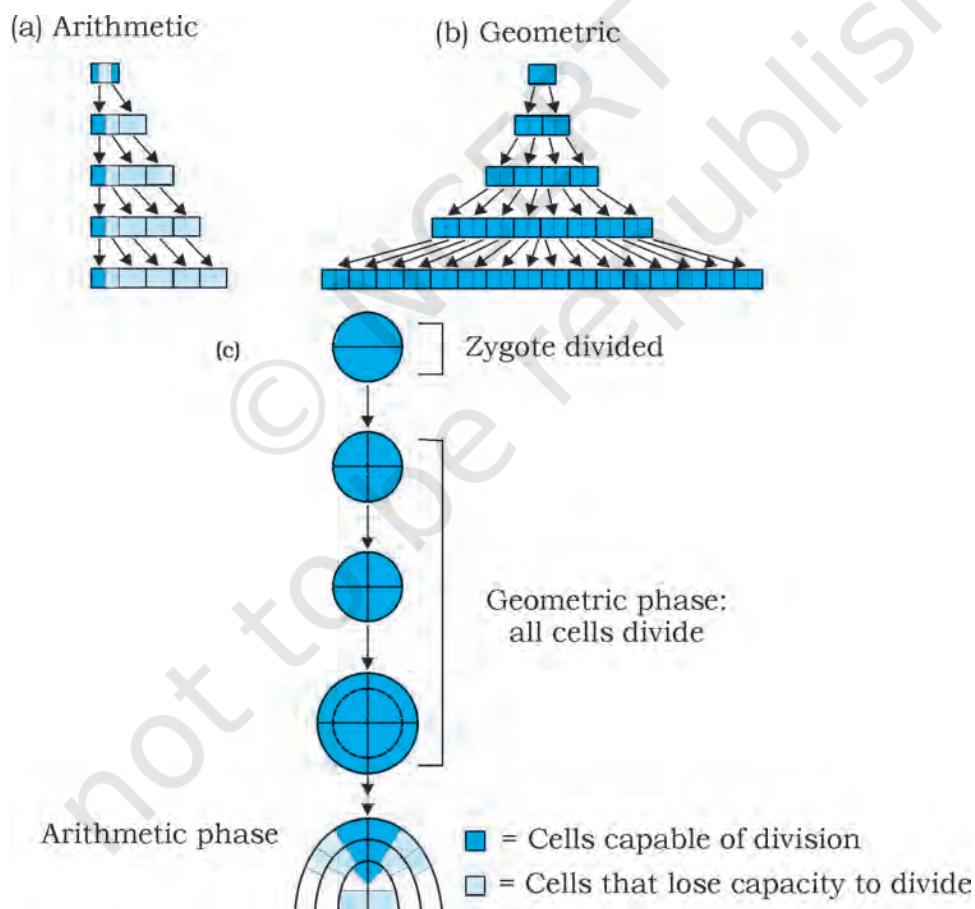


Figure 13.4 Diagrammatic representation of : (a) Arithmetic (b) Geometric growth and (c) Stages during embryo development showing geometric and arithmetic phases

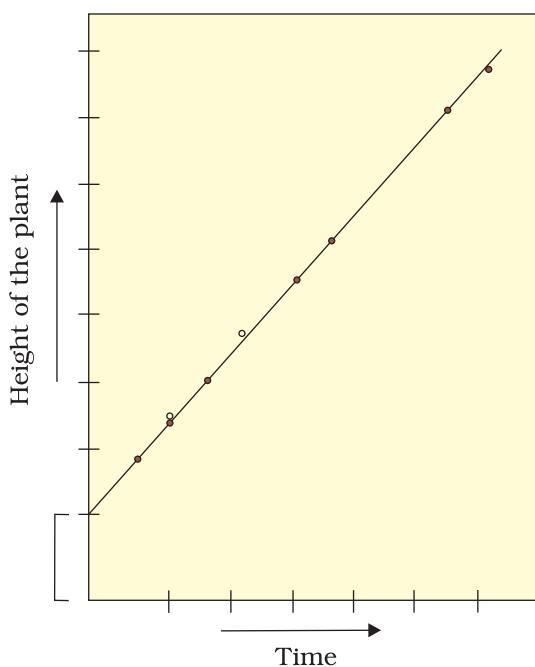


Figure 13.5 Constant linear growth, a plot of length L against time t

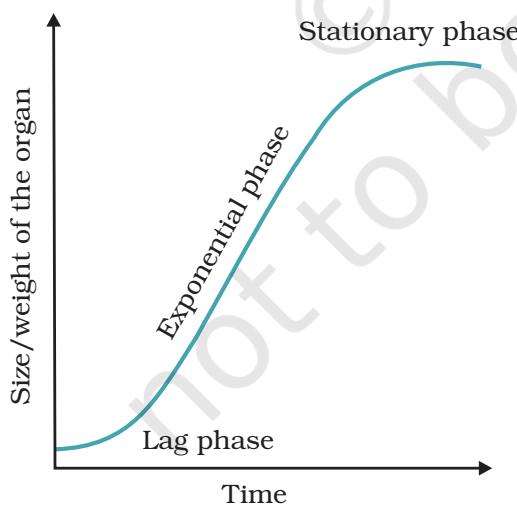


Figure 13.6 An idealised sigmoid growth curve typical of cells in culture, and many higher plants and plant organs

The growth rate shows an increase that may be arithmetic or geometrical (Figure 13.4).

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 13.5. 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 13.6). 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 .

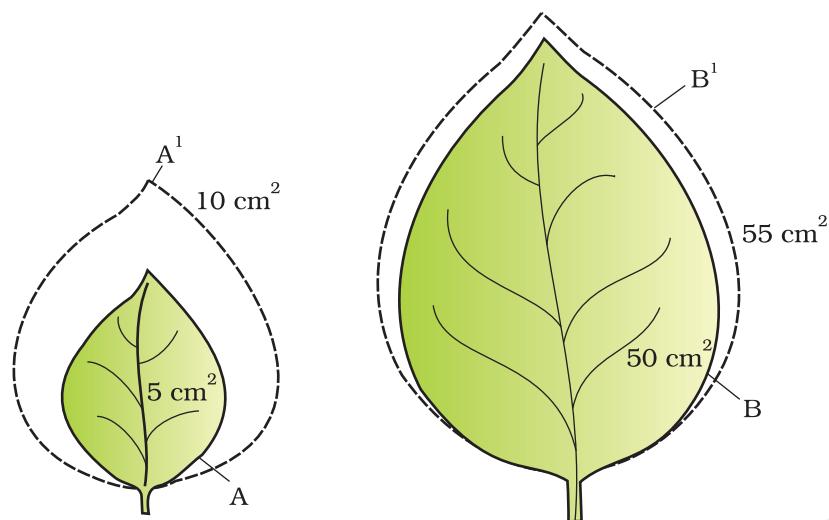


Figure 13.7 Diagrammatic comparison of absolute and relative growth rates. Both leaves A and B have increased their area by 5 cm^2 in a given time to produce A¹, B¹ leaves.

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 13.7 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, A¹ and B¹. However, one of them shows much higher relative growth rate. Which one and why?

13.1.5 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.

13.2 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 13.1.1, 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?

13.3 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 13.8. It is also applicable to tissues/organs.

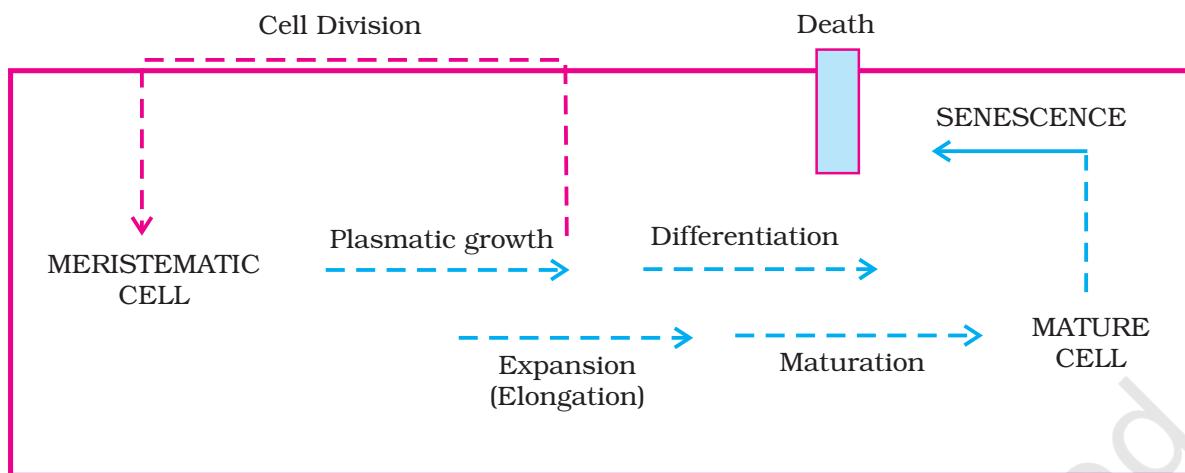


Figure 13.8 Sequence of the developmental process in a plant cell

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., heterophyly 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 13.9). This phenomenon of heterophyly is an example of plasticity.

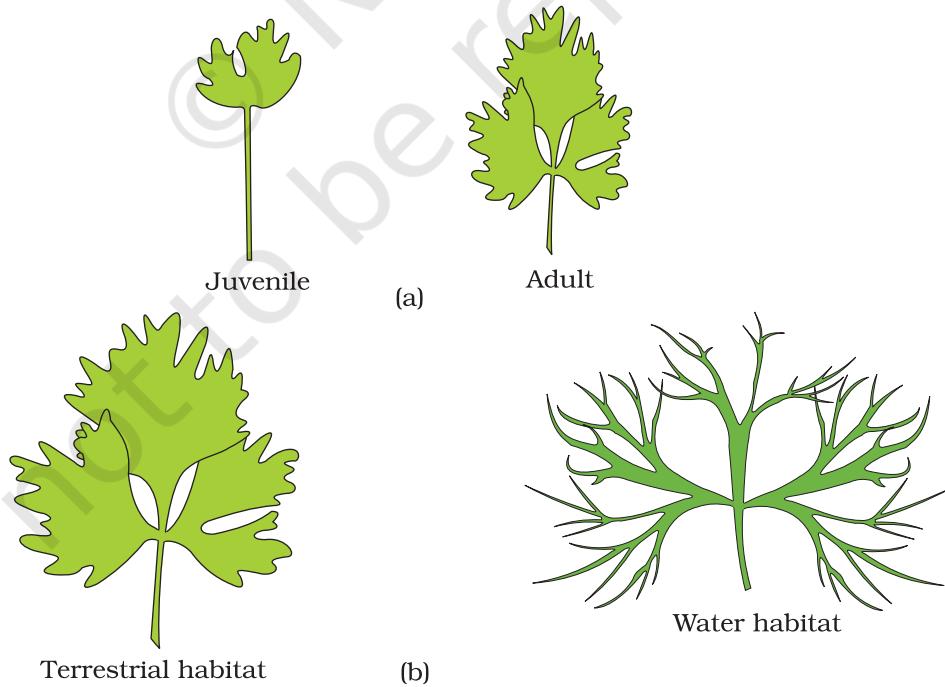


Figure 13.9 Heterophyly in (a) larkspur and (b) buttercup

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.

13.4 PLANT GROWTH REGULATORS

13.4.1 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 -furfurylaminopurine, kinetin), derivatives of carotenoids (abscisic acid, ABA); terpenes (gibberellic acid, GA₃) or gases (ethylene, C₂H₄). 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.

13.4.2 The Discovery of Plant Growth Regulators

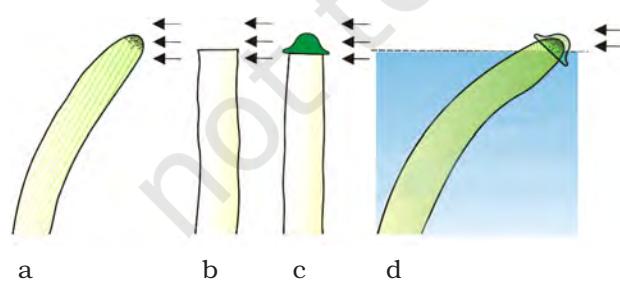


Figure 13.10 Experiment used to demonstrate that tip of the coleoptile is the source of auxin. Arrows indicate direction of light

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 13.10). Auxin was isolated by F.W. Went from tips of coleoptiles of oat seedlings.

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

F. Skoog and his co-workers observed that from the internodal segments of tobacco stems the callus (a mass of undifferentiated cells) proliferated only if, in addition to auxins the nutrients medium was supplemented with one of the following: extracts of vascular tissues, yeast extract, coconut milk or DNA. Miller et al. (1955), 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).

H.H. Cousins (1910) 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.

13.4.3 Physiological Effects of Plant Growth Regulators

13.4.3.1 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 13.11). It is widely applied in tea plantations, hedge-making. Can you explain why?

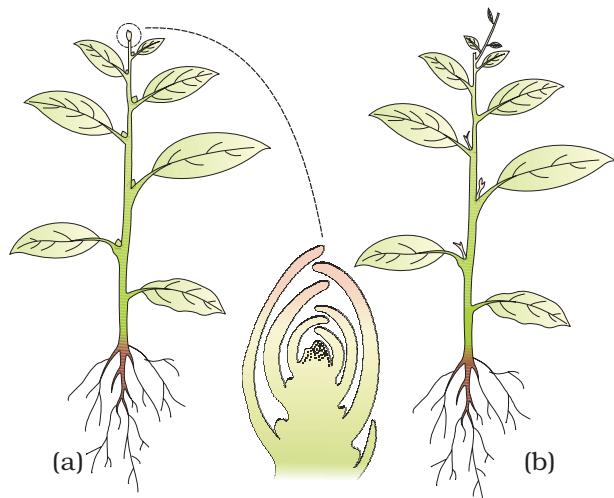


Figure 13.11 Apical dominance in plants :
 (a) A plant with apical bud intact
 (b) A plant with apical bud removed
 Note the growth of lateral buds into branches after decapitation.

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.

13.4.3.2 Gibberellins

Gibberellins are another kind of promotory PGR. There are more than 100 gibberellins reported from widely different organisms such as fungi and higher plants. They are denoted as GA₁, GA₂, GA₃ and so on. However, Gibberellic acid (GA₃) 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. GA₃ 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.

13.4.3.3 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.

13.4.3.4 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. Ethepron in an aqueous solution is readily absorbed and transported within the plant and releases ethylene slowly. Ethepron 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.

13.4.3.5 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 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.

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.

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. Why is abscisic acid also known as stress hormone?
6. 'Both growth and differentiation in higher plants are open'. Comment.
7. 'Both a short day plant and a long day plant can flower simultaneously in a given place'. Explain.
8. 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.
9. Would a defoliated plant respond to photoperiodic cycle? Why?

10. What would be expected to happen if:
- (a) GA₃ 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.

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