Indira Gandhi
National Open University
School of Sciences

BBYCT-137
PLANT PHYSIOLOGY AND METABOLISM

Block

4

NITROGEN METABOLISM AND PLANT GROWTH REGULATORS

Course Design Committee

Prof. G.C. Srivastava (Retd.)
Former Head,
Department of Physiology,
IARI, Pusa,
New Delhi-110012

Prof. Vijay Paul
Principal Scientist,
Division of Plant Physiology
IARI, Pusa,
New Delhi-110012

School of Sciences, IGNOU

Prof. M.S. Nathawat, Director, (Ex.)

Prof. Amrita Nigam

Prof. Jaswant Sokhi (Retd.)

Prof. Bano Saidullah (Retd.)

Prof. Neera Kapoor

Dr. Eklavya Chauhan (Sr. Consultant)

Block Preparation Team

Prof. Amrita Nigam
SOS, IGNOU, New Delhi-110068

Dr. Eklavya Chauhan
Sr. Consultant,
SOS, IGNOU, New Delhi-110068

Editor

Prof. G.C. Srivastava (Retd.)
Former Head,
Department of Physiology,
IARI, Pusa,
New Delhi-110012

Course Coordinator: Prof. Amrita Nigam

Production

Mr. Hemant Kumar
SO(P), MPDD, IGNOU

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BLOCK 4 : NITROGEN METABOLISM AND PLANT GROWTH REGULATORS

In the preceding Blocks 1-3 you have read about the plant water relations, occurrence, uptake and transport of mineral nutrients; energy conservations by plants through the fascinating process of photosynthesis; transformation of photoassimilates to various parts of the plant body; biocatalysts, their structure, properties and mechanism of action; finer details of cellular respiration involing glycolysis, Krebs Cycle, Oxidative phosphorylation, electron transport chain and the modern aspects of ATP generation.

In the final fourth block you will study in **Unit 12** the sources of nitrogen in the atmosphere and soil along with the details of its absorption involving nitrification, along with emphasis on the biochemistry of nitrate and ammonium assimilation. You will read more about the biological nitrogen fixation carried out by the "gifted" species of nitrogen fixers in nature in **Unit 13**. This unit describes the nodule-formation and the ***Rhizobium*** - root associations along with a detailed description of the ***nitrogenase*** enzyme complex.

Unit 14 is devoted exclusively to a study of the chemical messengers of a plant – the hormones. A detailed description of the structure and mode of action of hormones follows their discovery. Synthetic growth regulations along with some recent discoveries in hormonal signaling has also been discussed in details.

Plant responses to light and temperature is the theme of **Unit 15** where you will study the phenomenon of photoperiodism, its variations and the role of phytochrome in flowering. In addition, recent information on the flowering hormones has also been discussed. The process of vernalization has been described along with a discussion on cryptochromes and the movements in plants.

The last of the units, **Unit 16** deals with a detailed description of abiotic and biotic stress conditions and the response of plants. Various ways in which the plants adapt to stress have been described. Examples of specific stress conditions have been highlighted so as to emphasize the changes in plant morphology and metabolism.

Objectives

After studying this block, you will be able to:

- describe the steps involved in the assimilatory reduction of nitrates and the activity of enzyme *nitrate reductase* and *nitrite reductase*;
- evaluate the structural and functional aspects of the *nitrogenase* enzyme;
- describe the process of biological nitrogen fixation with special emphasis on the ***Rhizobium*** legume symbiosis;
- discuss the history of naturally occurring hormones and their usefulness in agriculture and horticulture;
- appreciate the mode of action of phytohormones;
- differentiate between the long-day and short-day plants;
- describe the role of florigens and hormones in flowering;
- distinguish between abiotic and biotic stress; and
- appreciate plant response to osmotic, temperature, wounding and pollution stress.

UNIT 12

NITROGEN METABOLISM

Structure

12.1 INTRODUCTION

You have studied about the role of nitrogen as a macronutrient in Unit 3. Nitrogen is considered to be the fourth most abundant nutrient element after carbon, hydrogen and oxygen in plants. This element occurs in both inorganic and organic forms. Nitrogen is an essential constituent of amino acids, proteins, enzymes, hormones, nucleic acids, alkaloids, chlorophyll, vitamins, glycosides and other important primary and secondary plant constituents. Needless to say protoplasm, the physical basis of life consists of a major portion of proteins.

Although molecular nitrogen (N_2 or dinitrogen) constitutes 78% by volume of the atmosphere, this odorless, colorless gas cannot readily diffuse into the plant and get utilized directly. This is partly due to the exceptionally stable $N \equiv N$ bond. No such enzyme is present in higher plants that can reduce this triple covalent bond. Thus, these plants need to be supplied with nitrogen in a *usable* or *combined* form for the biosynthesis of the above-mentioned substances.

Objectives

After studying this unit, you should be able to:

- describe the steps involved in assimilatory reduction of nitrate and the requirements of the process;
- explain the significance of organelle specific or tissue specific distribution of *nitrate reductase* and *nitrite reductase*;
- explain the significance of induction, repression and control of activity of *nitrate reductase*;
- list the requirements of assimilation of ammonia into organic form; and
- discuss the metabolic interrelation of nitrogen, carbon and sulfur.

12.2 NITRATE ASSIMILATION

The higher green plants can utilize nitrogen from soil as nitrates (NO_3^-), nitrites (NO_2^-), ammonium salts (NH_4^+) and organic nitrogenous compounds.

The higher plants depend on prokaryotic organisms in soil for conversion of dinitrogen into this usable forms. Only certain microbes are 'gifted' to convert the atmospheric nitrogen into utilizable form. You will read about the biological nitrogen fixation in the next Unit 13.

Atmospheric nitrogen fixation by some microbes (discussed in Unit 13) accounts for nearly half of nitrate assimilation by green plants, which is estimated to be about 2×10^4 Mt/year.

Interestingly, nitrogen is often a limiting nutrient for plants, placed after water, since the plants and soil microorganisms compete with each other for this element which is limited in the soil pool.

12.2.1 Sources of Nitrogen

Atmosphere and soil are the main sources of nitrogen. The molecular form of nitrogen (dinitrogen) is prevalent in the atmosphere while both inorganic and organic forms of nitrogen are present in soil. Inorganic forms of nitrogen in the soil are, **nitrite nitrogen**, **nitrate nitrogen** and **ammonical nitrogen**. The organic forms of nitrogen in soil are in the form of amides, amino acids and urea.

Remains of dead animals and plants, excretions and nitrogenous wastes in soil are first acted upon by saprophytic microorganismswhich hydrolyze the proteins into amino acids. The later are then deaminated to release ammonia. This process is called **ammonification**. The liberated ammonia immediately combines with H^+ of water to form NH_4^+ ion.

$$\text{Proteins} + \text{Water} \xrightarrow[\text{hydrolysis}]{\textit{Bacillus} \text{ sp}, \textit{Actinomyces} \text{ sp.}} \text{n RH. NH}_2\text{. COOH}_{\text{amino acids}}$$

$$\underset{\text{Amino acid}}{RH.NH_2.COOH} + Water \xrightarrow{\text{deamination}} \underset{\text{Organic acid}}{R\,HOH.COOH} + NH_3$$

$$NH_3 + H^+ \rightarrow \underset{\text{Ammonium ion}}{NH_4^+}$$

Some of these ammonium ions can be absorbed by roots of some plants. Remaining ammonium ions get converted into nitrate nitrogen by the microbial activity. The process is called **nitrification** which occurs in two steps.

i) **Nitrite Formation** : The nitrite microorganisms like *Nitrosomonas*, *Nitrosococcus* and *Aspergillus* oxidize ammonical nitrogen into nitrites.

$$NH_4^+ + 2O_2 \xrightarrow{\text{nitrite microbes}} NO_2^- + 2H_2O + \text{Energy}$$

The energy is utilized by chemosynthetic bacteria for their carbon assimilation.

ii) **Nitrate Formation** : Both biological as well as non-biological oxidation of nitrite is possible. Biological nitrate formation is carried out by *Aspergillus*, *Nitrobacter*,*Nitrocystis* and *Penicillium*.

Energy released in the process is used for carbon assimilation by the chemosynthetic nitrifying bacteria.

$$2\,NO_2^- + O_2 \xrightarrow{\text{nitrate microbes}} 2\,NO_3^- + \text{Energy}$$

The nitrates are now available to plants for absorption and assimilation.

Most of the plants absorb nitrogen in the form of nitrates. The mechanism of absorption of nitrate ions is essentially similar to that of other ions. A small amount of nitrites can also be absorbed. Interestingly, plants complete with the denitrifiers in soil like *Thiobacillus denitrificans* which readily reduce nitrate into dinitrogen, returning it into the atmosphere. Denitrification itself accounts for a loss of 93-190 million metric tonnes of nitrogen per year! Thus, in order to maintain a steady state of the supply, demand, turnover, recycling and growth of plants, there must be an element of a “nitrogen economy” in the biosphere which can balance the nitrogen recycling. A relationship between inorganic and organic nitrogen metabolism is illustrated in Fig. 12.1.

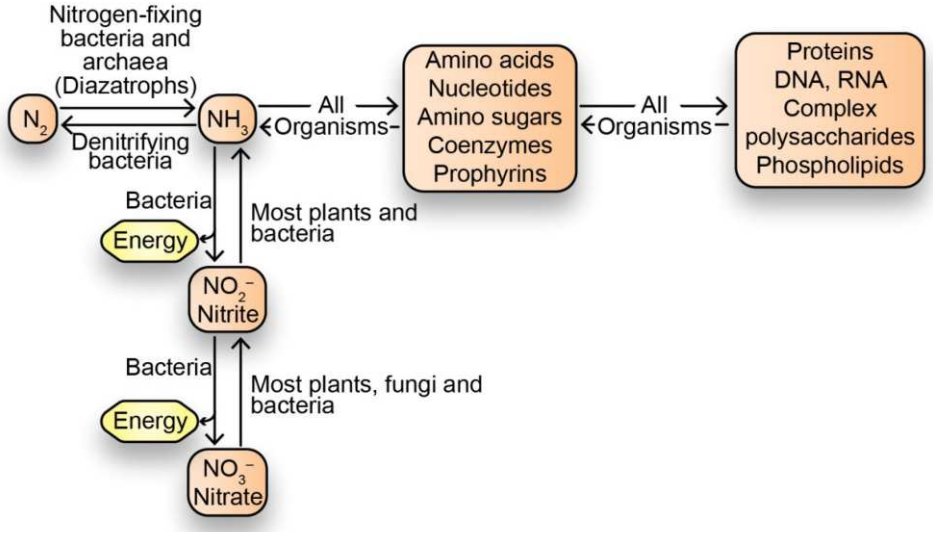

Fig. 12.1: Diagram depicting relationships between inorganic and organic nitrogen metabolism (From Appling et al).

12.2.2 Biochemistry of Nitrate Assimilation

As you have studied in subsection 12.2.1, nitrate is the most readily available and preferred source of nitrogen for plant growth. Once inside the roots and after being transported to leaves through the transpiration stream, the nitrates are converted into ammonia. This assimilatory reduction of NO_3^- to NH_3 occurs in **two steps** as shown below :

i) $NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$

Nitrate Nitrite

ii) $NO_2^- + 7H^+ + 6e^- \longrightarrow NH_3^- + H_2O$

Nitrite Ammonia

Recent evidence suggests that this conversion may involve many more steps as indicated below:

$$NO_3^- \longrightarrow NO_2^- \longrightarrow HNO^- \longrightarrow NH_2OH \longrightarrow NH_3$$

Nitrate Nitrite Nitroxyl Hydroxylamine Ammonia

The first step is catalyzed by ***nitrate reductase*** **(NR)** in the cytosol which reduces NO_3^- to NO_2^- at the expense of two electrons. The second step is catalysed by ***nitrite reductase*** which converts NO_2^- into NH_3 at the expense of six electrons. *Nitrate reductase* is a Mo-enzyme like *dinitrogenase* and *nitrite reductase* is a Fe-protein.

The physiological source of reductant for the two reductive processes could be reduced ferredoxin –$Fd_{(red)}$ or reduced pyridine nucleotides (NADH or NADPH) depending upon the system. It is important to point out here that NO_3^- is also known to undergo dissimilation process in which it is reduced to N_2 gas. Such a process of nitrate metabolism is called **nitrate respiration** or **denitrification** and occurs exclusively in certain bacterial forms under anaerobic conditions. The enzymes of denitrification of nitrate are called *dissimilatory nitrate reductases*.

Assimilatory *Nitrate Reductase* and *Nitrite Reductase*

There are two kinds of *nitrate reductases* depending upon their specificity to reductant. *Nitrate reductase* of cyanobacterial system requires reduced ferredoxin (Fd-dependent) to catalyse the reaction while *nitrate reductase* in plants and fungi requires reduced pyridine nucleotide (NADH or NADPH – dependent) to carry out the reaction.

In general, most pyridine nucleotide *nitrate reductases* are capable of using both NADH and NADPH as source of reductant. The enzyme from photosynthetic organisms like higher plants and algae show preference for NADH but those from fungi show preference for NADPH. This suggests inherent difference between cyanobacterial *nitrate reductase* and eukaryotic *nitrate reductase* in respect to reductant requirement. The two types of reductant dependent nitrate reduction reactions are show below (Fig. 12.2).

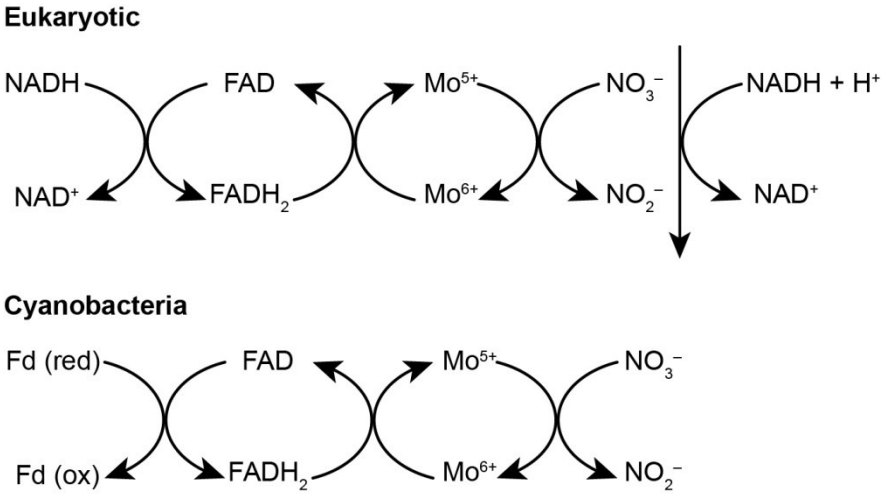

Fig. 12.2: Two major types of reductant dependent nitrate reduction reactions in eukaryotes and cyanobacteria.

The overall reaction of eukaryotic *nitrate reductase* is summarized as:

$$NO_3^- + NAD(P)H + H^+ \longrightarrow NO_2^- + NAD(P)^+ + H_2O$$

In comparison to the Fd-dependent enzyme which contains only molybdenum as prosthetic group, the eukaryotic *nitrate reductase* is made up of two identical sub-units (dimer), each containing one molecule of FAD heme, and a Mo atom as prosthetic group complexed with an organic molecule called **Pterin**. Functionally, while the Fd-dependent enzyme catalyses only reduction of nitrate to nitrite, the NAD (P)H-dependent enzyme catalyses two independent activities, one called ***diaphorase*** activity in which NAD(P)H is oxidized and one electron acceptor like cytochrome $_C$ or flavin mono-nucleotide (FMN) is reduced, and the other called ***terminal nitrate reductase*** activity which is NAD(P)H independent and in which nitrate is reduced at the expense of reduced flavins ($FMNH_2$) or viologens. *In vivo* both activities participate jointly and sequentially in the transfer of electrons from reduced pyridine nucleotide to nitrate as shown in reaction (Fig. 12.3). *Nitrate reductase* in cyanobacteria lacks *diaphorase* activity and contains only Mo as prosthetic group.

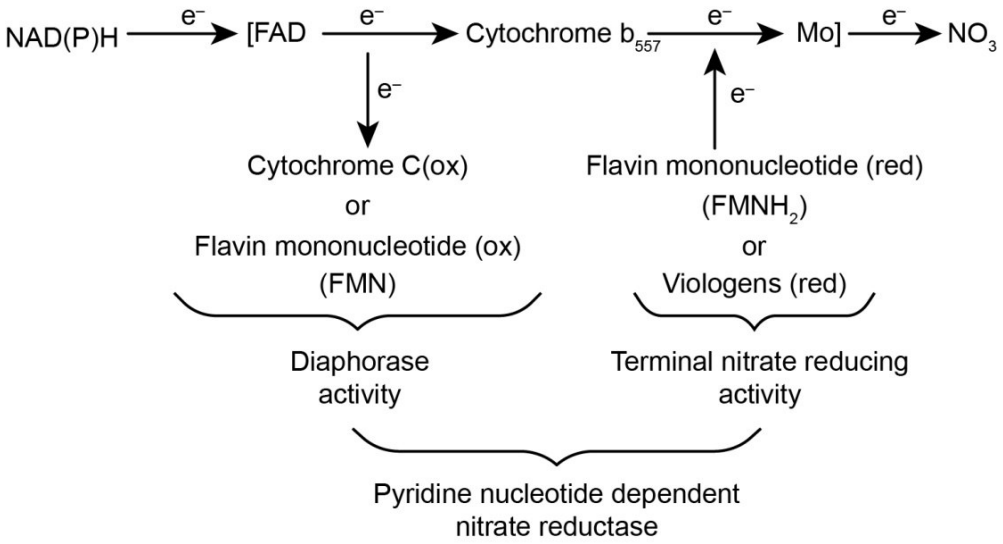

Fig. 12.3: Activities of NAD$^+$(P)-dependent *nitrate reductase* i) *diaphorase* activity; ii) terminal *nitrate reductase* activity.

Reduction of nitrite to ammonia occurs in chloroplasts. The enzyme *nitrite reductase* in cyanobacteria or chloroplasts requires reduced ferredoxin for reduction. *Nitrite reductase* of fungi requires NADPH to carry out the reductive function. The two reactions are shown below (Fig. 12.4).

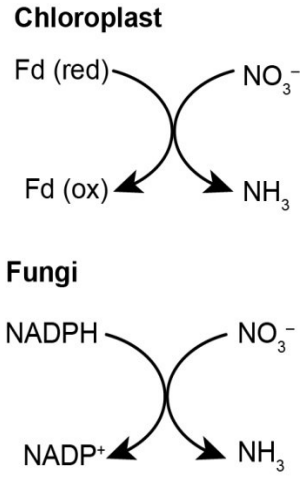

Fig. 12.4: *Nirtite reductase* activity in the chloroplast and in fungi.

Since NO_2^- is highly reactive and does not accumulate in plant tissue, it is immediately transported from the cytosol to the chloroplast in leaves and plastids in roots for reduction. Nitrite is reduced to ammonia by the enzyme *nitrite reductase*. This involves the transfer of six electrons:

$$NO_2^- + 6Fd_{red} + 8H^+ \longrightarrow NH_4^+ + 6Fd_{ox} + 2H_2O$$

Ferredoxins involved in this reaction are obtained from the electron transport system of chloroplasts (see Unit 5).

Nitrate Uptake

Cells accumulate NO_3^- against concentration gradient and this accumulation is a result of the presence of active NO_3^- transport system in the cell membrane. Nitrate uptake and nitrate reduction are independent processes because organisms genetically deficient in *nitrate reductase* activity contain normal transport activity.

12.2.3 Regulation of Nitrate Assimilation

There are two levels of regulation of nitrate assimilation. One is long-term and another is short-term. The long-term regulation operates at the level of enzyme synthesis (**transcription level**) and the short-term regulation operates at the level of enzyme activity (**translation level**).

Enzyme Synthesis

Nitrate assimilating systems in general are known to show an increase in nitrate uptake system and *nitrate reductase* in the presence of nitrate. In other words, nitrate assimilatory system is induced by the presence of nitrate. Similarly, cells or organisms assimilating NH_3 as nitrogen source show lack of nitrate assimilatory system. Such control of nitrate is called **repression control**. Thus, nitrate is an inducer while ammonia is repressor of nitrate assimilatory system.

Red light is known to enhance synthesis of nitrate assimilatory system which is mediated by well-known photomorphogenic pigment called **phytochrome** about which you will learn in detail in Unit 15 of this course.

Enzyme-activity Control

Availability of the substrate, NADH and nitrate would be an important determinant of the rate of nitrate assimilation. In addition, there are number of substances which are known to cause reversible inactivation of *nitrate reductase* under reducing conditions (Fig. 12.5).

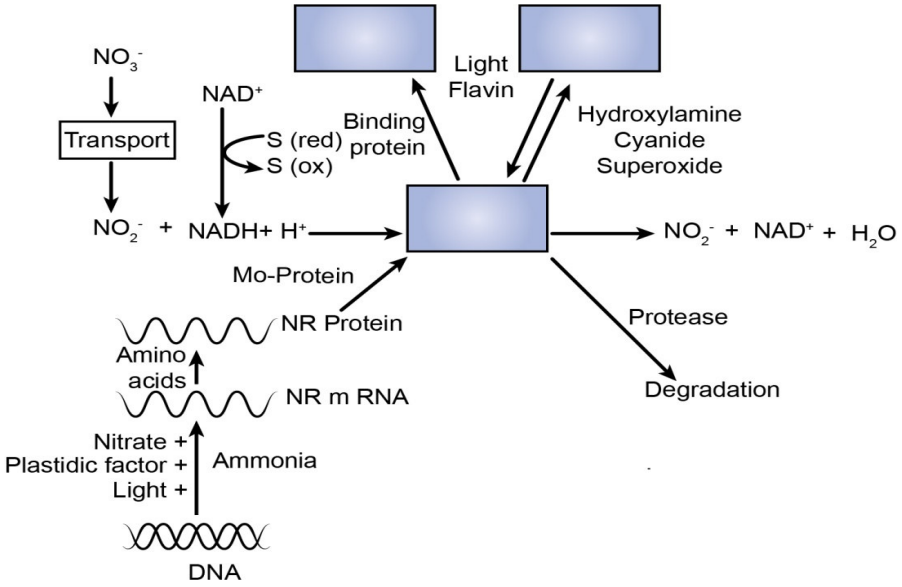

Fig. 12.5: Regulation of assimilatory *nitrate reductase* (NR). Reversible inactivation of NR by combination with hydroxylamine, cyanide, or superoxide and reversal of this inactivation by blue light in the presence of flavin; inactivation by combination with specific binding proteins or by limited proteolysis. Enzyme synthesis is regulated by positive effector-nitrate, plastidic factor, light and negative effectors derived from ammonia.

Cyanide: Plants generate cyanide from cyanogenic glycides and histidines. Ethylene biosynthesis is also accompanied by small amount of cyanide production. *Nitrate reductase* can occur in reduced form following its interaction with NADH in the absence of nitrate. The reduced form of the enzyme has the ability to combine with cyanide forming enzyme-CN complex which is enzymatically inactive. Nitrate, oxygen or blue light oxidise the enzyme-CN complex releasing cyanide and making cyanide - free enzyme active.

Hydroxyl amine or superoxide inactivates the enzyme which on exposure to blue light gets converted to active form. It is observed that plants grown in blue light are higher in protein contents.

Inhibitor proteins: One kind of inhibitor protein found in higher plants is an *endopeptidase* which degrades *nitrate reductase* thus causing irreversible loss of the enzyme. The other kind includes binding proteins that specifically bind to *nitrate reductase* leading to permanent inactivation of enzyme. Such inactivator proteins have been isolated from rice seedings and spinach leaves. These reactions are explained in Fig.12.6. You will see that the transcription of enzyme is mediated by light. It is a unique enzyme in this respect. On the other hand, darkness and Mg^{2+} stimulate a protein *kinase* that phosphorylates

serine residues which interact with an inhibitor to inactivate *nitrite reductase*. This type of regulation is shown to be a much quicker one.

12.2.4 Interaction of Nitrogen and Carbon Assimilation

Application of nitrogenous fertilizers brings about dramatic effects on thegrowth and performance of the plant. One of the most important consequences is the effect on utilisation of carbohydrates in plants. It is known that the level of carbohydrates in the plants goes down with increase in the level of N_2 supplied to them.The form in which the fertilizers are supplied are NO_3^-, and NH_3.In the plant NO_3^- is reduced to NH_3 and assimilation of NH_3 requires carbon-skeleton like α-ketoglutaric acid, an intermediate of TCA cycle for its incorporation into organic form. Naturally, the rate of NH_3 assimilation into organic form would depend on the rate at which TCA cycle supplies the carbon skeleton. The C-skeleton removed during the assimilation of NH_3 must be replenished through the catabolism of carbohydrates. Consequently, the carbohydrate content of the plant decreases in proportion to the amount of organic N_2 produced.

The other places of nitrate assimilation in the plant as you have already seen in photosynthetic tissue and the reductants are reduced ferredoxin and pyridine nucleotide. Since such reductants are also required for carbon assimilation, theproduction of carbohydrates in photosynthesis is bound to go down in proportion tothe increase in rate of reductive assimilation of NO_3^-. In other words; the level of NO_3^- utilisation is inversely related to the level of carbohydrate in the plant.

We will describe how the practical applications of this knowledge is used in raising crops with desired protein and carbohydrate content. Take the example of celery eaten as salad; the stalks of this plant are most edible when soft and this softness is the result of lower availability of carbohydrates. Now, to raise a celery crop with soft stalks one uses nitrate fertilizers which divert a major part of carbohydrate in the synthesis of amino acids and proteins. Another example is sugarcane, a crop of tropical region. It requires a period of ten months from the time of planting to the period of harvest. Here, the growers apply nitrogen fertilizers in the beginning and not near the time of harvest.Similar practices have resulted in production of beet roots highly rich in sugar concentration. The reason for rise in sugar in the beet root is again due to withholding of N_2-fertilizer near the harvest time, so that the C-skeleton is not utilized for producing amino acids and proteins.

SAQ 1

a) Match the characteristics listed below of Column I with enzymes given in Column II.

Column I	Column II
i) Nitrate reductase	a) Non-heme Fe-protein
ii) *Nitrite reductase*	b) Present in chloroplast
	c) Present in cytoplasm
	d) Molybdo-flavo protein
	e) *Diaphorase* activity

b) Choose the alternate correct word given in parenthesis in the following statements:

i) Nitrate assimilation in higher plants occurs mainly in (roots/leaves).

ii) Number of electrons used when NO_3^- is reduced to NO_2^- (1/2).

iii) Number of electrons used when NO_3^- is reduced to ammonia (2/6).

iv) In cyanobacteria *nitrate reductase* is located in the (photosynthetic membranes/plasma membrane).

v) In higher plants *nitrate reductase* is in (cytoplasm/chloroplast) and *nitrite reductase* is in (cytoplasm/chloroplast).

vi) In C_4 plants nitrate assimilation occurs in (bundle sheath/mesophyll cells) and CO_2 assimilation occurs in (bundle sheath/mesophyll cells).

vii) *Nitrate reductase* activity is induced in the presence of (NO_3^- / NH_4^+).

12.3 AMMONIUM ASSIMILATION AND ITS REGULATION

Atmospheric nitrogen (N_2) and NO_3 are the most common available source of inorganic nitrogen. Both are enzymatically reduced to ammonia because it is only ammonia that is incorporated into organic form. The major product of ammonia assimilation is usually considered to be amino nitrogen. Ammonium generated during nitrite assimilation (as discussed in Unit 12.2.2) and during photorespiration (Unit 7) has to be rapidly converted as its accumulation is toxic to a plant. Molecular nitrogen fixation has been discussed in Unit 13.2.4.

12.3.1 Biochemistry of Ammonium Assimilation

Ammonia resulting from N_2- fixation or nitrate reduction or supplied exogenously is assimilated in plants by the following two primary pathways, i) **Reductive amination**/***glutamate dehydrogenase*** **(GDH) pathway**, and ii) ***glutamine synthetase(GS)-glutamate synthase*** also called (***Glutamine oxoglutarate aminotransferase-GOGAT*****) pathway.**

i) **Reductive Amination (High cellular ammonia Pathway):**

$$NH_3 + \alpha - \text{Ketoglutarate} + NADPH + H^+ \xrightarrow{GDH} \text{Glutamate} + NADP^+ + H_2O$$

ii) **GS-GOGAT (Low cellular ammonia Pathway): (**NADH-GOGAT occurs in diatoms, fungi and in the nucleus of vascular plants). Fd-GOGAT is prevalent in photosynthetic eukaryotes and cyanobacteria. NADPH-GOGAT occurs in mitochondria, chloroplasts, archaea and non-photosynthetic bacteria.

$$\text{Glutamate} + NH_3 + ATP \xrightarrow{\textit{Glutamate Synthetase}} \text{Glutamine} + ADP + Pi$$

$$\text{Glutamine} + \alpha - \text{Ketoglutarate} + NADPH + H + (\text{or}Fd_{(red)}) \xrightarrow[\text{synthase}]{\text{Glutamate}}$$

$$2\text{Glutamate} + NADP^+ + H_2O \quad (\text{or } Fd_{(ox)})$$

As you may note, both the pathways generate the same amino acid, **glutamate** as end product of the reaction. The two pathways may operate simultaneously as in higher plants and eukaryotic algae or alternatively as in certain heterotrophic enterobacteria.In cyanobacteria ***glutamine synthetase-glutamate synthetase* (*GS*)** is the main primary pathway of ammonia assimilation.

The two enzymes involved in ammonia assimilation differ significantly in their affinity for ammonia which is very high for *glutamine synthetase* and significantly low for*glutamate dehydrogenase*. In other words, *glutamate dehydrogenase* - mediated pathway functions under conditions of high cellular ammonia and *glutamine synthetase*-mediated pathway under conditions of low cellular ammonia.

The *GS-glutamate synthase* pathway is characteristically cyclic in nature in which glutamate acts as both the acceptor and product of ammonia assimilation. This pathway of ammonia assimilation is called ***glutamate synthase* cycle** (Fig. 12.6)**.**

12.3.2 *Glutamate Synthase* (GOGAT) Route

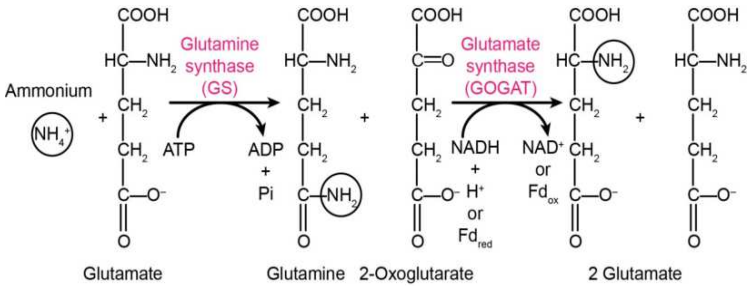

Fig.12.6 : *Glutamine Synthase* (GS) Route.

Two classes of GS exist in plants:

1. Cytosolic GS expresses itself in vascular bundles of roots and shoots to produce glutamine for intracellular nitrogen transport.

2. GS present in root plastids or shoot chloroplasts generate amides and catalyze reassimilation of photorespiratory NH_4^+ respectively.

When there is an enhanced concentration of glutamine in the plastids, the enzyme *glutamate synthase* (also called *glutamine: 2-oxoglutarate aminotransferase, or GOGAT*) getsactivated. The amide group of glutamine is transferred to 2-oxoglutarate, finally yielding two molecules of glutamate (Fig. 12.6).

There are two types of GOGAT. One accepts electrons from NADH and the other (Fd).

i) $\text{Glutamine} + 2-\text{oxoglutarate} + \text{NADH} + \text{H}^+ \rightarrow 2\ \text{glutamate} + 2\text{NAD}^+$

ii) $\text{Glutamine} + 2-\text{oxoglutarate} + \text{Fd}_{\text{red}} \rightarrow 2\ \text{glutamate} + \text{Fd}_{\text{ox}}$

The NADH type of enzyme (***NADH-GOGAT***) is

- Located in plastids of non-photosynthetic tissues e.g., roots and vascular bundles of developing leaves.
- NADH-GOGAT helps in the assimilation of NH_4^+ absorbed from the rhizosphere and also assimilates glutamine which has been translocated from the senescing leaves or roots.

The Fd-dependent type (***Fd-GOGAT)*** is

- Located in the chloroplasts and help in photorespiratory nitrogen metabolism.
- Helps to incorporate glutamine generated during nitrate assimilation.

12.3.3 *Glutamate Dehydrogenase* Pathway

An alternate pathway involving ***Glutamate dehydrogenase***(***GDH***) can also assimilate ammonium ions in rice and under anaerobic conditions (Fig. 12.7), particularly when ammonia concentration is high. This NADH-dependent enzyme is present in mitochondria whereas its NADPH-dependent enzyme is present in the chloroplasts of photosynthetic parts. This mechanism of ammonia assimilation is less expensive than the GS-GOGAT Pathway.

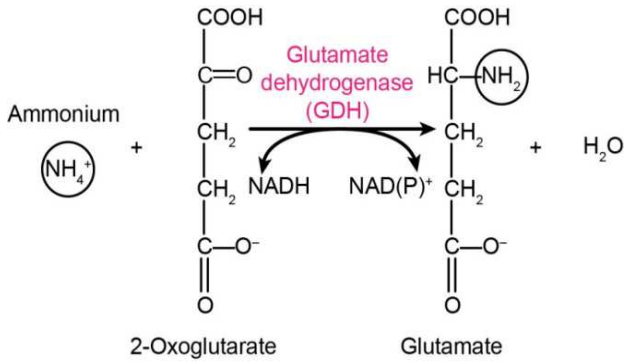

Fig. 12.7 : *Glutamate Dehydrogenase* Route.

Nutrient assimilation consumes a considerable amount of energy. The reduction of nitrate to nitrite and subsequently to ammonium involves the transfer of about 10 electrons. This amounts to nearly 25% of the total energy expenditure of both roots and shoots. Interestingly, nitrogen accounts for only 2% of the total dry weight of the plant, yet requires nearly one fourth of energy for its assimilation.

As most of these assimilation reactions occur in the chloroplast stroma, and utilize the reducing agents of the photosynthetic electron transport chain, the coupling of the two processes viz., nutrient assimilation and photosynthetic electron transport chain, is called **Photoassimilation**. The processes involved in the assimilation of mineral nitrogen and the energy expenditure is depicted in Fig. 12.8.

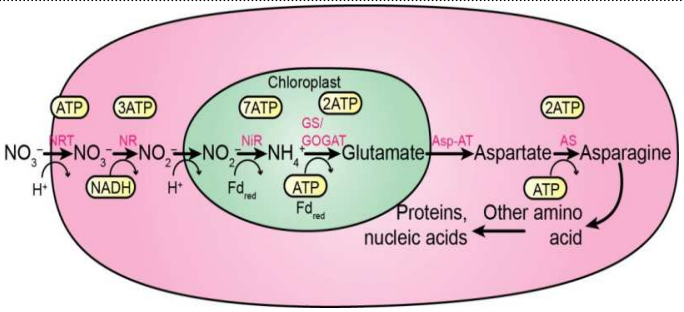

Fig. 12.8: A generalized diagram depicting the processes involved in the assimilation of mineral nitrogen in the leaf (From Taiz & Zeiger).

Since enhanced CO_2 levels inhibit nitrate inhibition in both C_3 and bundle sheath of C_4 plants, this phenomenon is bound to affect the plant nutrient reactions significantly by the end of this century, when the atmospheric CO_2 levels are expected to rise further.

12.3.4 Uptake of Ammonia

Ammonia (NH_3) diffuses freely across biological membranes according to its concentration gradient. However, ammonium (NH_4^+) ion requires a specific transport system to cross the biological membrane.

12.3.5 Ammonia Assimilation and its Regulation

Heterotrophic bacteria like *Escherichia coli* and *Klebsiella aerogenes* induce theoperation of *GS-glutamate synthase* pathway of ammonia assimilation underconditions of low ammonia and of *GDH*-pathway under conditions of high ammonia.Thus the two pathways are mutually exclusive and the level of cellular ammonia determines which pathway of ammonia assimilation is likely to operate.Cyanobacteria like systems have only GS-*glutamate synthase* pathway functioning under low or high cellular ammonia. Information about the role of ammonia in metabolic regulation of its two assimilatory pathways in higher plants is not clearly understood.

12.3.6 Metabolic Interrelation of Nitrogen, Carbon and Sulphur

Plants grow and develop into characteristic individuals as a result of a series of well programmed and regulated biochemical and morphological events. The main elements that enter into the composition of an individual plant are carbon, hydrogen, oxygen, nitrogen and sulphur. The three elements are taken by plants in the oxidized form CO_2, NO_3^-, SO_4^{2-}, and thus need to be reduced for assimilation. Assimilation of nitrate and sulphate, like carbon dioxide, requires ATP and reductants. It seems that plants must have evolved mechanisms to integrate the three assimilatory reductions with light harvesting photosynthetic reactions. Eukaryotic algae and higher plants have achieved this integration by localizing most of these assimilatory reactions within the chloroplast.

Photosynthetically produced ATP and reductant (ferredoxin or NADPH) together constitute what is called assimilatory power of the plants. Light reactions of photosynthesis produce ATP and reductants and dark reactions of photosynthesis are related to the reductive assimilation of carbon dioxide, nitrate and sulphate.

In plants, since carbon, nitrogen and sulphur also occur together in sulphur containing amino acids there must be regulatory mechanisms controlling integrated assimilation of carbon dioxide, nitrate and sulphate into organic forms. The results of scientific studies also support this view. Inhibition of photosynthetic carbon dioxide assimilation is known to simultaneously inhibit nitrate assimilation. This is partly because the carbon skeleton for compounds like α-ketoglutarate is ultimately produced from photosynthetically generated organic carbon from carbon dioxide. Assimilation of N_2 by nodulated legumes also depends upon the extent and amount of photosynthate like organic carbon reaching them. There is evidence which suggests that sulphur also controls nitrogen metabolism. Protein synthesis is known to decline under sulphate limitation and comes to a halt after sulphate exhaustion. During sulphur starvation, nitrate or ammonia is assimilated mainly into free amino acids and not in proteins. Because, in the absence of sulphur, the sulphur containing amino acids (cysteine and methionine) cannot be synthesized and the disulphide bridges cannot be formed in proteins. Some of the amino acids preferentially synthesized under these conditions are arginine, glutamine and alanine.

The major regulatory mechanisms controlling interdependence of inorganic carbon, nitrogen and sulphur metabolism are to be studied and understood in detail as they hold the key to the desired production of major plant products at commercial scale.

SAQ 2

a) i) Name the two pathways of ammonia assimilation in plants.

ii) Which of the two pathways operates under high concentration of cellular ammonia?

iii) Which of the two pathways is costly in respect of energy and material needs?

b) i) Which of the source among NH_4^+, NO_3^- and N_2 is preferred by plants?

ii) What signal in plant causes repression of both NO_3^- assimilation and N_2-fixation?

12.4 SUMMARY

- Nitrate assimilation occurs in two enzymatic steps. Nitrate reduction to nitrite is catalysed by *nitrate reductase* and nitrite reduction to NH_3 is catalysed by *nitrite reductase.* NADH is the reductant for *nitrate reductase* in plants, NADPH is the reductant in fungi and reduced ferredoxin in cyanobacteria. Reductant for *nitrite reductase* in fungi is NADPH and in plants and cyanobacteria is reduced ferredoxin.
- *Nitrate reductase* is predominantly localized in cytoplasm while *nitrite reductase* is localized exclusively in chloroplast.

- Nitrate assimilation is regulated by inducer and repressor control at synthetic level and activity control involving reversible inhibition of enzyme activity by cyanide, hydroxyl amine, superoxide radical and reactivation by blue light.
- In C_4 plants nitrate assimilatory pathway remains localized within mesophyll cell and CO_2 assimilation in bundle sheath cells.
- Plants prefer ammonia as a source of nitrogen over NO_3^- or N_2 and this is achieved by repression control of nitrate assimilation and N_2 fixation.
- *Glutamate dehydrogenase* and *glutamine synthatase/synthase* are the two primary enzymes of ammonia assimilation.
- Sulphate like NO_3^- is also assimilated reductively. Unlike NO_3^-, it is required to undergo ATP dependent activation before entering into pathway of assimilatory reduction.
- A clear understanding of metabolic interrelationship of N,C and S nutrition are a must from agricultural point of view.

12.5 TERMINAL QUESTIONS

1. Trace the steps of nitrification.
2. Distinguish between the eukaryotic and bacterial *nitrate reductase* enzyme.
3. What are the three basic modes of NO_3^- reduction?
4. Where are the *nitrate reductase* and *nitrite reductase* enzymes present in higher plants (C_3)?
5. Explain the control of *nitrate reductase* by various regulatory factors.
6. Explain the two major pathways for ammonium assimilation.
7. How expensive is the nitrogen uptake?
8. How are the sulphates, phosphates and cations assimilated?

12.6 ANSWERS

Self-Assessment Questions

1. a) i) c, d and e;
   ii) a and b

   b) i) leaves
   ii) 2
   iii) 6
   iv) photosynthetic membranes

v) cytoplasm, chloroplast

vi) mesophyll cells, bundle sheath cells

vii) NO_3^-

2. a) i) *Glutamate dehydrogenase* pathway and *glutamine synthetase-glutamate synthetase* pathway;

ii) *Glutamate dehydrogenase* pathway

iii) *GS-glutamate synthase*.

b) i) NH_4^+;

ii) high ratio of glutamine to α-ketoglutarate

Terminal Questions

1. Refer to Subsection 12.2.1.
2. Refer to Subsection 12.2.2.
3. Refer to Subsection 12.2.3.
4. Cytoplasm and chloroplast respectively.
5. Refer to Subsection 12.2.4.
6. Refer to Subsection 12.3.1.
7. Refer to Subsection 12.3.3.
8. Refer to Subsection 12.3.7.

UNIT 13

BIOLOGICAL NITROGEN FIXATION

Structure

13.1 INTRODUCTION

You have learnt in the preceding Unit 12 how nitrogen gets metabolized in the plant. No doubt, all plants have the capability of converting NH_3 to organic nitrogen compounds –(with C-N) bonds). But can all organisms synthesize ammonia from inorganic nitrogen – dinitrogen gas N_2, which is the most abundant component of earth's atmosphere? Although many plants and microorganisms do have the ability to reduce NO_3^- to NH_3, there is no plant known so far which can reduce N_2 to NH_3. This ability is confined only to some prokaryotes, and these nitrogen-fixing microorganisms are termed **diazotrophs**. These nitrogen-fixers or the **'gifted'** prokaryotes, are both free-living as well as in symbiotic association with plants.

Objectives

After studying this unit, you should be able to:

- list and classify the nitrogen fixing organisms based on mode of living;
- write the conditions necessary for nitrogen fixation and the essential requirements of the process;

- describe the metabolic modifications occurring during bacteroid formation in nodules;
- explain the basis of specificity of *Rhizobium*-legume symbiosis; and
- list the components of *nitrogenase* enzyme, describe the reactions carried out by each component, and explain the significance of H_2 evolution during the process.

13.2 BIOLOGICAL NITROGEN-FIXATION

The process by which molecular nitrogen (N_2) is reduced to ammonia (NH_3) is called nitrogen-fixation (N_2-fixation).

This is the most important section of this unit. Here we first introduce you to the species capable of N_2-fixation and describe the formation of nodules during symbiotic association. Then we go through the reactions and enzymes involved in the conversion of molecular nitrogen to NH_3. Brief section on the genetics of N_2-fixation will introduce you to the possibility of transferring N_2-fixing capability to non N_2-fixers.

13.2.1 Nitrogen Fixers in Nature - The 'Gifted' Species

Biological nitrogen fixation remains mainly confined to a few distinct nutritional types of prokaryotes, some of which are free-living while others live in symbiotic association with eukaryotic partners. This process contributes annually about 60 %(nearly 150-190 million metric tons annually) to the earth's newly fixed nitrogen budget. Tables 13.1 and 13.2 show the ranges and types of biological nitrogen fixers. They have been variously classified as free living (*Azotobacter*, *Nostoc*, *Anabaena* (Fig. 13.1a) or symbiotic (*Rhizobium*-legume association Fig. 13.1 b).

Free-living nitrogen fixers can further be classified into phototrophic (*Nostoc*), chemotrophic (*Klebsiella*), aerobic (*Azotobacter*) or anaerobic (*Chromatium*) depending upon the state of existence and mode of nutrition. Agronomically the most important nitrogen fixing systems are *Rhizobium*-legume association (Fig 13. 1 b), *Frankia* (actinorhizal)-woody plant association, *Anabaena-Azolla* and Cyanobacteria-rice system. *Rhizobium*-legume association invariably occurs in the form of root nodules. This group of nitrogen fixers has been divided into three categories: i) *Rhizobium* which includes fast growing species, ii) *Bradyrhizobium* which include slow growing strains and iii) *Azorhizobium* which is a combination of traits from *Rhizobium* and *Bradyrhizobium*.

Rhizobium is highly specific with respect to its host and that is why the nomenclature is based on the specific host which it infects. For example, the *Rhizobium* infecting clovers and peas are called *trifolii* and *leguminosarum,* respectively. The genus *Bradyrhizobium* is not so rigid regarding the host partners. All the strains of *Rhizobium* and *Bradyrhizobium* produce nodules on the host, but *Azorhizobium* produces nodules on the stem of *Sesbania rostrata* and *Aeschynomene afraspera*. *Parasponia* is the only non-legume genus where the bacterial partner is *Rhizobium*. This observation has raised the

hope of creating nitrogen fixing associations between *Rhizobium* and important crops like cereals. Nitrogen fixing nodules on the roots of woody plants like *Alnus*, *Casuarina*, *Myrica*are formed by symbiotic association with *Frankia,* a member of actinomycetes.*Anabaena-Azolla* association is experimentally shown to be a strong source of nitrogen fertilizer in wetland rice agriculture. Free-living heterocyst- possessing cyanobacteria are also known to contribute significant amount of nitrogen to the wetland rice ecosystem. Lichens are associations between fungi and N_2-fixing cyanobacteria. They are very important source of N_2 in barren harsh habitats. N_2-fixing root nodules are also known to occur in tropical plants like *Psychotria* with *Klebsiella* as the N_2-fixing partner. Several nitrogen-fixing bacteria get associated with C_4 plants like grasses *Miscanthus* and Sugarcane.

(a) **(b)**

Fig. 13.1: The gifted N_2-fixers (photographs). a) *Anabaena* (arrow-heterocyst); b) Symbiotic nitrogen fixation by legume-*Rhizobium* association(Root nodules on Soybean)(from Taiz e*t al*)

Table 13.1: Some free-living diazotrophs

Archaebacteria	*Methanococcus voltae* *Halobacterium halobium*
Eubacteria ***Cyanobacteria***	
Unicellular	*Gloeothece*
Filamentous forms	*Oscillatoria*, *Plectonema*
Heterocystous forms	*Nostoc*, *Anabaena*, *Calothrix*
Other bacteria	
Aerobes	*Azotobacter*, *Derxia*, *Beijerinckia,* *Azospirillum lipoferum**
Facultative anaerobes	*Klebsiella pneumoniae*, *Enterobacter, Bacillus*
Microaerobes	*Aquaspirillum*, *Arthrobacter*
Anaerobic	*Clostridium*
Photosynthetic	*Rhodospirillum rubrum*, *Rhodopseudomonas*, *Chromatium*
Chemotrophic	*Thiobacillus ferroxidans*

Cyanobacterial Associations	**Co symbionts**
Cyanobacteria	*Gunnera* (Angiosperm) *Agathis*, *Cycas*, *Microzamia* (Gymnosperms) *Azolla* (Pteridophyte) *Blasia*, *Anthoceros*, *Caricula* (Bryophytes) *Collema*, *Lichina*, *Peltigera* (Lichens)
Cyanobacterial Animal Association	*Siphonochalina* (Coral reef sponge)

*Some*Azospirillum* spp are often found in association with roots of grasses, and fix nitrogen microaerobically.

Table 13.2: Some Symbiotic Diazotrophs

a) Legumes	**N_2-fixing Symbiont**
Bradyrhizobium japonicum (slow growing)	Soybean (*Glycine max*) Peanut (*Arachis hypogaea*)
Sinorhizobium fredii (Fast growing)	Soybean
Sinorhizobium meliloti	Alfalfa (*Medicago sativa*) Sweet clover (*Melilotus*)
Rhizobium leguminosarum bv. *phaseoli* *Rhizobium tropicii*; *Rhizobium etli*	Bean (*Phaseolus*)
Rhizobium leguminosarum bv *viciae*	Pea (*Pisum sativum*), Sweet pea (*Lathyrus*)
Photorhizobium (Photosynthetically active rhizobia form stem nodules. Associated with adventitious roots)	*Aescheomene* (aquatic)
b) Non-legumes	***Rhizobia***
Bradyrhizobium spp. *Rhizobium loti*	*Parasponia* (Ulmaceae) Lotus (*Nelumbo sp.*) (Nelumbonaceae)
c) Non-legume (**Actinorhizal Association**) *Frankia* (Actinomycetales)	*Shepherdia* (Eleagnaceae),*Hippophae* (Elaeagnaceae) *Alnus* (Betulaceae),*Casuarina* (Casuarinaceae), *Elaeagnus* (Elaeagnaceae), *Myrica* (Myricaceae), *Ceanothus* (Rhamnaceae), *Datisca* (Datiscaceae), *Coriarea* (Coriariaceae).
d) Endophytic bacteria	
Acetobacter diazotrophicus	Sugarcane
Azoarcus spp.	Kallar grass
Herbaspirillum spp.	Rice, maize, sorghum, sugar cane

13.2.2 Conditions for Nitrogen Fixation

The following are the essential requirement for N_2-fixation.

i) The enzyme catalyzing the conversion of molecular N_2 to NH_3 is called ***nitrogenase***. It is produced by a group of genes called the ***nif*** genes. Expression of the *nif* genes leading to the formation of functional *nitrogenase* is thus one of the essential requirements.

ii) The activity of *nitrogenase* is extremely sensitive to oxygen. The aerobic N_2-fixing organisms, therefore, must have a cellular mechanism to protect *nitrorgenase* activity from oxygen inhibition or creating anaerobic environment (discussed later in the Unit).

iii) The reduction of N_2 to NH_3 is a high-energy requiring process consuming 16 to 24 ATP molecules per N_2 reduced. Therefore, the organism must have provision for an abundant supply of ATP.

iv) Mo and Fe are integral constituents of *nitrogenase* enzyme and therefore, they must be available to the plant to ensure the formation of this enzyme complex.

v) *Nitrogenase* enzyme is produced by bacteria and not produced in the cells that have access to fixed nitrogen source like nitrate or ammonia. Hence, the process of N_2-fixation occurs in situations devoid of such fixed nitrogen sources.

13.3 SYMBIOTIC NITROGEN FIXATION – NODULE FORMATION-LEGHAEMOGLOBIN

As you read in earlier subsection 13.2.1, the symbiotic nitrogen fixation involves specific associations between bacteria and plants (Table 13.2). Most important of these are the symbiotic association between rhizobia and legumes. Facultative organisms preferentially fix N_2under anaerobic conditions. Thus, the *Rhizobium*-legume association contributes significantly towards the nitrogen economy of soil. A typical legume-*Rhizobium* association is known to fix 25-60 kg ha^{-1} $year^{1}$ of nitrogen. In contrast, the contribution by nonsymbiotic nitrogen fixers is just 5 kg $ha^{-1}year^{-1}$.

Since more than 90% legumes form nodules, the process of nodulation is itself of great significance in the sequence of events of nitrogen fixation.

There are three main reasons why legumes are important in agriculture.

i) They fix nitrogen in the soil.

ii) Grains and forage legumes are rich in protein content thus nutritionally indispensable for humans and livestock.

iii) They provide nitrogen in flows to natural habitats such as tropical forest.

The development and functioning of nitrogen fixing nodules is a complex process which involves a series of multiple interactions between the bacteria and the legume host. Sequences of events have been studied from the

morphological, biochemical, and recently from molecular point of view where there is an exchange of signals in the form of chemical messages between the two partners.

The sequence of events has been subdivided into three principal stages.

1. Multiplication and Colonization of Rhizobia in the Rhizosphere

Rhizobia live in the soil as free living, saprophytic bacteria. Since the symbiosis between rhizobia and the legume roots is not obligatory, the latter may grow independently throughout their life span. These two partners, however, seek each other by a series of chemical signals **only** under nitrogen-limited conditions. The specificity of symbiotic interaction between *Rhizobium* and its host occurs at this very first stage and is called **recognition**. There are reciprocal signals between the host and symbiont for determination of host specificity for root nodule development. The rhizobia are attracted to move towards the legume roots by positive chemotaxis.

Flavonoids like **luteolin** secreted by the roots that are believed to act as chemotactic chemicals. In addition, many amino acids, organic acids, and sugars also contribute towards this chemotaxis, which help the bacteria to detect beneficial chemicals required for growth and reproduction. The rhizobia multiply and may soon get attached to the root hairs constituting the bacteria-rich rhizosphere (Fig.13.2).

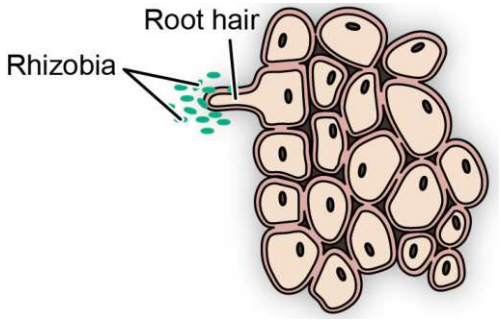

Fig.13.2: Binding of rhizobia to the emerging root hair in response to the chemotactic substances secreted by the root.

After colonizing the rhizosphere, the rhizobia now begin to synthesize special morphogenic signal molecules or **symbiotic signals** in response to the attractants. The attractants activate the transcription of ***nod* genes** which synthesize ***nod* factors/modulation factors** in rhizobia.The *nod* factors are lipochito-oligosaccharides (derivatives of chitin, a β – (1-4) linked polymer of N-acetyl-D-glucosamine. Many genes (viz., ***nod* A, *nod* B** and ***nod* C**) have been isolated which code for enzymes synthesizing the basic structure in ***nod*** factors. Different rhizobia have host-specific ***nod***-genes which allow a specific response from legume.

The ***nod***-factors are recognized by the host cells and induce many important changes in the growth and metabolism of the host roots before the bacteria enter the root hair and before nodulation is initiated. The roots become shorter and thicker. The root hair production increases, more branches appear in the root hairs. The root hairs start curling at their tip. The *Rhizobia* also release **mitogenic signals** which stimulate localized cell divisions in the root cortex (Fig. 13.3).

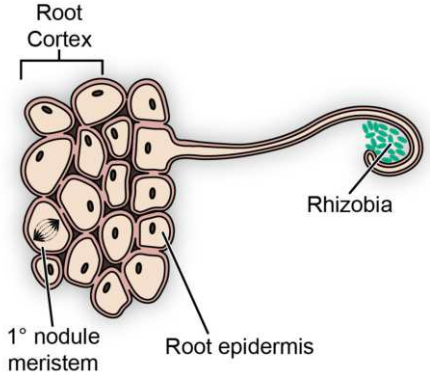

Fig. 13.3: After being chemotactically attracted to the root hair, the rhizobia stimulate the root hair (*nod* factor) to curl and send mitogenic signals for stimulating cell division in the cortex (from Hopkins & Hüner).

These cells divisions form the **primary nodule meristem,** which defines the region of future nodule development.

Attachment of rhizobia to the root hair of specific host involves chemical recognition. There are reciprocal signals between the host and symbiont for determination of host specificity for root nodule development. It is known that this recognition is a function of complementary molecules present on the surface of the two symbiotic partners. There are two types of molecules comprising this complementary group; one is the complex polysaccharide and the other **lectins**. The polysaccharide component is characteristic of both partners while the lectin molecules are produced only by the host (legume) concerned. Lectins are small, non-enzymatic proteins which are synthesized by the host. Lectins have many sites specific for binding to a variety of surface polysaccharides of the interacting partners. For example, as shown in Fig.13.4 clover root hair produces a lectin called trifollin with two specific sites, one for binding to polysaccharides at the surface of bacterium *Rhizobium trifolii* and other for binding to polysaccharide on the wall of root hair. Thus, lectin functions as an anchor to fix *Rhizobium* on the root surface.

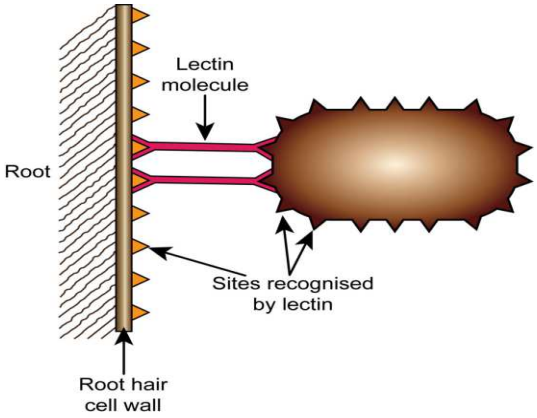

Fig. 13.4: Role of lectins in recognition of host and symbionts. The specific rhizobia attach to the root hair of the correct species because the bifunctional lectin molecules recognize antigenic sites of both partners.

2. Invasion of Root hair and Formation of Infection Thread

As a result of *Rhizobium* **nod**-factor induced curling of root hairs, the colonies of bacteria themselves get entrapped within the curls. The *nod*-factors are

thought to release enzymes like *pectinases*, *hemicellulases* and *cellulases*, which degrade the cell wall material of the host cell walls. As a result of degradation of some regions of the cell wall the bacterial cells gain access to the underlying plasma membrane of the root hair.

Upon the arrival of rhizobia on the outer surface of the plasma membrane the root hair stops growing and the plasma membrane starts invaginating. This internal tubular extension called the **Infection thread** is made by the fusion of membrane vesicles derived from Golgi to the end of the tube (Fig.13.5).

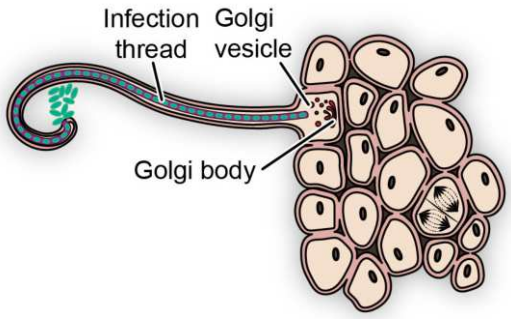

Fig. 13.5: Localized degradation of root hair wall results in formation of infection thread which is contributed by the Golgi activity. The nodule meristem is also actively dividing (From Taiz & Zeiger).

As the infection thread grows and elongates through the root hair cell, a thin layer of new wall material on the inner surface of the membrane is continuously being deposited which prevents the actual entry of the cells. In the meantime, cells at the primary nodule meristem start rapid cell division forming a distinct area within cortex. This zone is called a **nodule primordium,**a zone where nodule will develop. Position or site of this primordium is controlled by the action of signaling compounds like the nucleoside uridine and ethylene. Infection thread continues to elongate through the base of the root hair cell where its membrane fuses with the host cell plasma membrane (Fig. 13.6a). During this process, some bacteria get released into the apoplastic space. This initiates a new infection thread (Fig. 13.6 b). The infection thread continues its elongation into successive cells of the cortex while the bacteria also continue proliferating.

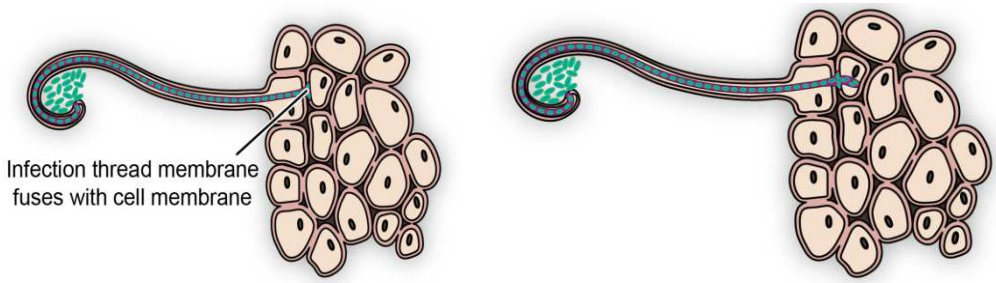

Fig. 13.6: a) Fusion of infection thread membrane and plasma membrane of the root hair cell; b) Release of *Rhizobia* into the apoplast. The bacteria penetrate the middle lamella to the subepidermal cell plasma membrane to initiate a new infection thread (From Taiz & Zeiger).

Finally, the infection thread reaches the developing nodule and its tip fuses with the plasma membrane. As a result, the bacterial cells, which were

enclosed by the membrane derived from the lost cell plasma membrane get released into the cytosol thereby infecting the going nodule (Fig. 13.7). The branching of the infection thread ensures spread of infection in many cells of host at the same time by the bacteria.

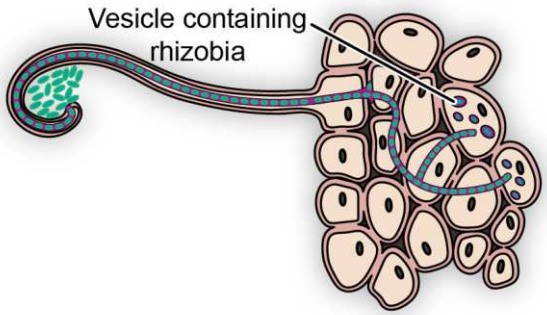

Fig. 13.7: Extension and arrival of the infection thread at the target cells, where membrane-enclosed bacterial cells are released (From Taiz &Zeiger).

The bacteria, after their release continue to divide. The surrounding membrane gradually increases in surface area to accommodate this growth.

3. Formation of Bacteroids

After some time, when the nodule is full of vesicles, the bacteria stop dividing. In turn, they start enlarging and finally differentiate into specialized nitrogen fixing endosymbiotic cells called **bacteroids**. These bacteroids are still enclosed by the peribacteroid membrane, which is derived from the infected root nodule cells. Main function of bacteroids is **nitrogen-fixation.** As the infection process continues, nodule gradually increases and finally with its specialized enzymes the nodule meristem establishes contact with the main root vascular system. This creates a route to export fixed nitrogen from the nodule to the plant as well as to import photosynthetic carbon into the nodule (Fig. 13.8).

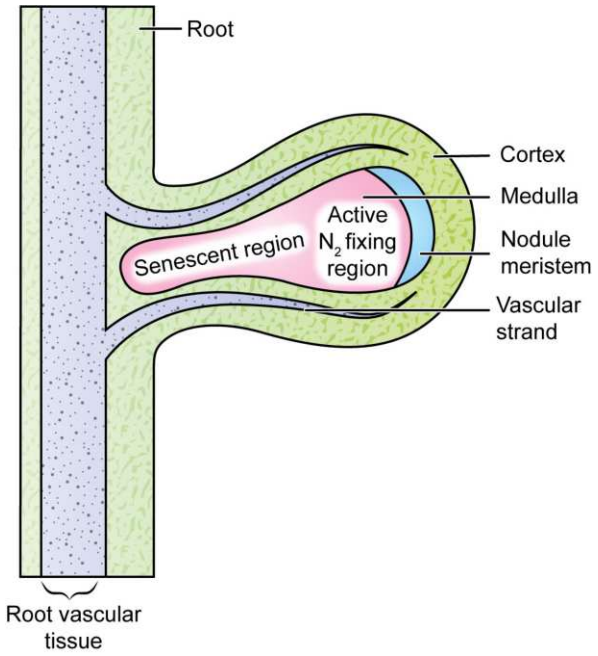

Fig. 13.8: Schematic diagram of a cross-section through a mature nodule showing vascular connections of the import and export of substances between the hosts and the microsymbiont (From Hopkins & Hüner).

The bacteroids have functional N_2-fixing apparatus and cytochrome component located in new bacteroid membrane that facilitates respiration at low O_2 concentration. During the time a special protein, leghemoglobin, is synthesized. It is also localized within peribacteroid membrane (Fig. 13.9).Several new proteins of enzymatic and transport nature is also produced exclusively by the host. Since these host-produced proteins are nodule specific, they have been given the name **nodulins.** Nodules are supplied with root vascular tissues.

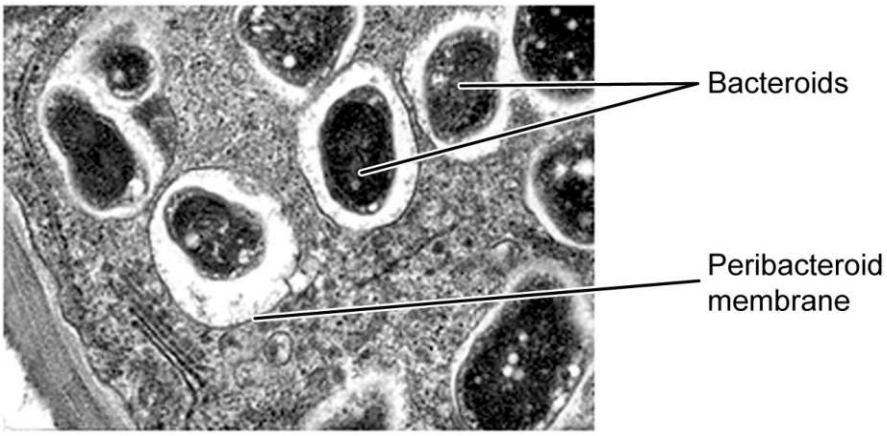

Fig. 13.9: An electron micrograph of soybean root nodules. Many bacteroids are in groups. Each group is surrounded by a peribacteroid membrane.

Mature bacteroids have enzymes for TCA cycle and oxidative phosphorylation. The bacteroids are active in ATP production and in supply of reductant for *nitrogenase* activity. The carbon requirement to produce ATP and reductant is met ultimately from photosynthetically produced carbohydrates translocated through phloem. The differentiation of bacterium into bacteroid is accompanied by inhibition of *glutamine synthetase* activity, so that the bacteroid cannot assimilate ammonia. In other words, the bacteroids function as ammonia producing factories for the host. Consequently, ammonia is released into the cytoplasm of the host cell and here it is assimilated into glutamine and asparagine in the case of pea and clover or it is assimilated into ureides as happens in soyabean. The reaction of conversion of NH_3 to glutamine has been already discussed in Unit 12.

SAQ 1

a) Given below in Column 1 are some N_2-fixing species. Match them with their symbiotic partner in Column 2.

	Column 1		Column 2
i)	*Anabaena*	a)	Certain leguminous plants
ii)	*Rhizobium*	b)	*Anthoceros*
iii)	*Nostoc*	c)	*Azolla* (fern)
iv)	*Frankia-actinorhizal*	d)	*Sorghum*
v)	*Azospirillum*	e)	*Casuarina*

b) Match the asymbiotic N_2-fixers in Column 1 with the characteristics given in Column 2.

Column 1		Column 2	
i)	*Nostoc*	a)	Anaerobic
ii)	*Azotobacter*	b)	Photosynthetic anaerobe
iii)	*Clostridium*	c)	Heterocyst
iv)	*Chromatium*	d)	Aerobic

c) Which among the following statements are true? Write T for true and F for false.

i) Only prokaryotes have the ability to fix nitrogen.

ii) Leguminous plants themselves cannot grow without *Rhizobium*.

iii) Enzymes necessary for the fixation of nitrogen are present in the bacteroids.

iv) Enzymes of TCA cycle and electron transport chain are present in bacteroids.

v) Bacteroid secretes aspargine into cortical cells.

vi) Infection thread consists of an infolded and extended plasma membrane of the bacteria.

d) Answer in one word

i) An enzyme produced by *nif* genes that converts N_2 to NH_3.

ii) The specificity of symbiotic interaction between *Rhizobium* and its host occurs at this stage.

iii) The transcription of these genes is activated by attractants.

iv) The molecules that help in recognition of both symbiotic partners during N_2 fixation.

v) A tubular extension made by fusion of membrane vesicles derived from Glogi body.

vi) They possess enzymes for TCA cycle and oxidative phosphorylation.

13.4 BIOCHEMISTRY OF NITROGEN FIXATION

Biological nitrogen fixation is a multistep process, and like the Industrial fixation, converts molecular nitrogen to diamide, hydrazine and finally to ammonia.

$$N_2 \longrightarrow \underset{\text{Diamide}}{HN{=}NH_2} \longrightarrow \underset{\text{Hydrazine}}{HN\text{-}NH_2} \longrightarrow \underset{\text{Ammonia}}{2NH_3}$$

Interestingly, very high temperature and pressure are needed to reduce the dinitrogen triple bond with lot of energy input. The reduction of dinitrogen by biological means is an equally high energy requiring process, utilizing a large proportion of the photosynthate provided by the host plant.

13.4.1 *Nitrogenase* Enzyme Complex

Nitrogen fixation is catalyzed by the enzyme ***dinitrogenase*.** The gene for coding this enzyme is present only in some prokaryotic cells. *Nitrogenase* is a multimeric protein complex and is composed of two proteins of different sizes. The larger protein of the *dinitrogenase* complex is a MoFe protein (a tetramer consisting of two pairs of identical subunits with Mo-Fe-S clusters. The two Molybdenum atoms are present in the form of an iron-molybdenum-sulfur cofactor. The number of clusters can vary depending upon the physiological status. The total molecular mass of the tetramer ranges from 180-235 kDa and a half decay time of 10 minutes in air.

The smaller protein, called Fe protein is a dimer which consists of two identical subunit polypeptides. The molecular mass of each subunit varies from 24-72 kDa in different species. An iron cluster (4 Fe and 4 S^{2-}) is present in each subunit. The Fe protein is O_2^- sensitive and gets inactivated irreversibly. It has a half-decay time of 30-45 seconds. The Fe protein participates in redox reactions involving conversion of N_2 to NH_3.

The overall nitrogen reduction reaction catalyzed by *dinitrogenase* can be represented in two steps (Fig. 13.10). In the first step ferredoxin serves as electron donor and reduces the Fe protein. The reduced Fe protein binds and hydrolyzes ATP. ATP is believed to cause a conformational change in the Fe protein itself, altering its redox potential to facilitate the transfer of electrons the two components of *dinitrogenase*.

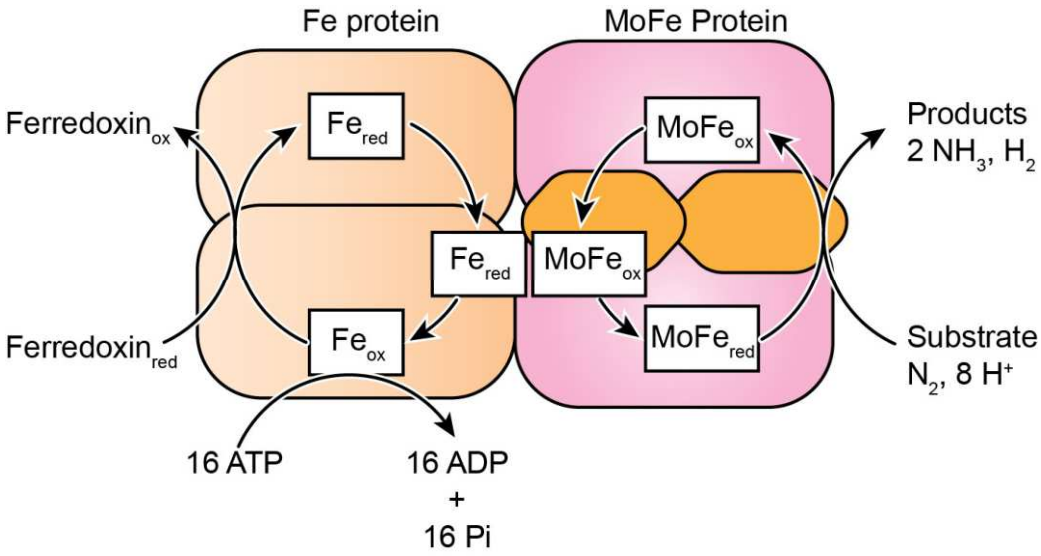

Fig. 13.10: The reactions catalyzed by Fe protein and MoFe protein of the *nitrogenase* enzyme complex (From Taiz & Zeiger).

The iron moiety serves as a carrier for electrons either in an oxidized ferric (Fe^{3+}) or a reduced ferrous (Fe^{2+}) state. You have already studied in Unit 6 that Fd acts as a very important electron carrier during the light reaction of photosynthesis.

The second step is facilitated by these redox reactions where the reduced Fe protein passes electrons to the MoFe protein, which in turn catalyzes the reduction of dinitrogen gas and hydrogen.

The overall reaction of *dinitrogenase* catalyzed reduction of dinitrogen to ammonia is summarized in the following equation.

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

In terms of energy, biological nitrogen fixation seems to be an energy requiring process since the process requires 8 electrons to reduce one molecule of dinitrogen along with 16 ATP. In addition, if we add another 9 ATP molecules which are required for reduced ferredoxin, the total requirement for 1 mol of N_2 reduction is 25 ATP, which is quit costly. In sharp contrast, fixation of one molecule of CO_2 in photosynthesis requires only 3 ATP molecules. Perhaps this is the reason why the N_2 fixation process is restricted to organisms where there is more efficient ATP production.

Dinitrogen =N_2

A better method for assessing the cost of nitrogen fixation is the measurement of the amount of carbon utilized. This is because the carbohydrates required to generate reducing potential ATP in the bacteroid come from photosynthesis of the host plant. Approximately 12 g of carbon are required to fix a gram of dinitrogen. The relationship of this costly process with respiration and photosynthesis is depicted in Fig. 13.11.

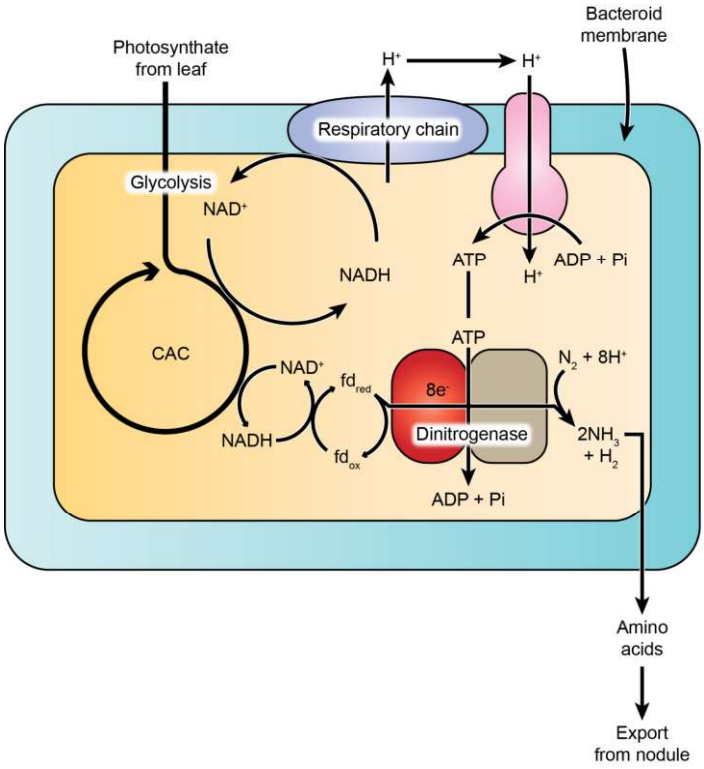

Fig. 13.11: Summary diagram illustrating the interactions between nitrogen fixation, respiration, and photosynthesis in bacteroids (From Hopkins & Hüner).

As is clear from Fig. 13.11 and also mentioned earlier, the high ATP requirement alone might have been the reason for restricting N_2 fixation process to organisms efficient in ATP production.You can see that hydrogen production during N_2-fixation seems to be a wasteful reaction energy-wise. It also affects *nitrogenase* activity because molecular H_2 is an inhibitor of N_2-

fixation. Attention, therefore, is being given to eliminate hydrogen generating reaction of *nitrogenase* during N_2-fixation.

13.4.2 Factors Influencing he Functions of *Nitrogenase*

Following are the factors that show considerable influence on the activity of *nitrogenase* enzyme.

i) Molybdenum (Mo) and Vanadium (Va)

Molybdenum must be available in nature to meet the demand of Mo for the formation of *nitrogenase* necessary for N_2-fixation. Now, it is known that Mo inhibits formation of Va containing *nitrogenase*. As you may know the relative distribution of Mo and Va in nature is in favour of Va. Therefore, it is quite likely that some ecological habitats could be deficient in Mo not in Va and N_2-fixers of such habitat are expected to produce Va containing *nitrogenase* for N_2-fixation Accordingly, there could be two nitrogen cycles functioning in nature: one mediated by Va system and the other mediated by Mo system.

ii) Molecular Hydrogen

As mentioned in equation for nitrogen fixation, atleast one molecule of hydrogen is evolved for every molecule of dinitrogen reduced. Thus, 25-30 percent of ATP and electrons supplied to *dinitrogenase* are wasted in hydrogen production.

N_2-fixing organisms also produce a membrane bound oxygen dependent enzyme called ***uptake-hydrogenase*** under N_2-fixing conditions. The physiological significance of the presence of uptake *hydrogenase* lies in its ability to consume hydrogen produced during *nitrogenase* activity through aerobic electron transport chain generating ATP in the process. The electrons are returned to the reductant pool. Such *nitrogenase uptake-hydrogenase* relationship in nitrogen fixing cells provides the following advantages:

i) By aerobic respiration it restores a major part of energy in the form of ATP.

ii) It offers protection of *nitrogenase* from oxygen.

iii) It prevents inhibitory effect of H_2 on *nitrogenase* activity.

The expression of genes (the ***hup*** genes) is responsible for uptake of *hydrogenase* have attracted considerable attention. Biotechnological studies are targeted towards making rhizobia strains with a higher capacity to recycle hydrogen thereby increasing overall efficiency of the process of nitrogen fixation in our principal crops.

Also, it has been observed that the *nitrogenase* enzyme catalyses exclusive production of hydrogen provided the N_2-fixer is in an atmosphere devoid of nitrogen. Efforts are being made to exploit this special property of the enzyme of photoproduction of H_2 by heterocysts of cyanobacteria on a commercial scale. This would require construction of special strains of cyanobacteria that would be genetically deficient in uptake *hydrogenase* enzyme. It is expected that these strains would continuously generate hydrogen in photosynthetic and N_2-fixing conditions.

iii) Oxygen

Molecular oxygen is a strong inhibitor of N_2-fixation as it blocks both the synthesis as well as the activity of *nitrogenase*. As you have seen in Table 13.1, there are N_2-fixers which are anaerobes, and many are aerobes. The problem of oxygen in anaerobes is taken care of by their very anaerobic mode of nutrition. If facultative, some organisms fix nitrogen only under anaerobic conditions. The problem is with aerobes like *Azotobacter* which require oxygen to fix nitrogen and grow at the expense of nitrogen. So how do the aerobic nitrogen fixers balance the conflicting demands of O_2 for their respiratory pathway on one hand and the sensitivity of *dinitrogenase* to oxygen on the other? Since both Fe protein and MoFe protein are irreversibly inactivated by molecular oxygen, the aerobic nitrogen fixers have evolved several strategies to regulate oxygen levels so that both the above-mentioned processes continue together.

Aerobic nitrogen fixers like some species of cyanobacteria fulfil this requirement of creating anaerobic or low O_2 conditions by isolating the nitrogen fixing apparatus in specialized cells called **Heterocysts.** Heterocysts are specialized cells formed from ordinary vegetative cells. They possess a thick, multilayered cell wall that restricts the diffusion of oxygen. Although heterocysts are photosynthetic, their photosystem II activity is lost during differentiation. Thus, they cannot produce any oxygen. They also lack *Rubisco* enzyme and consequently cannot fix CO_2. However, they still retain the capability to synthesize ATP by cyclic photophosphorylation or PSI (Fig. 13.12). Thus, necessary ATP for nitrogen fixation is provided,and at the same time the O_2 concentration is kept low by the formation of a new glycolipid layer. The heterocyst represents an early model of cellular specialization and division of labour normally associated with higher plants.

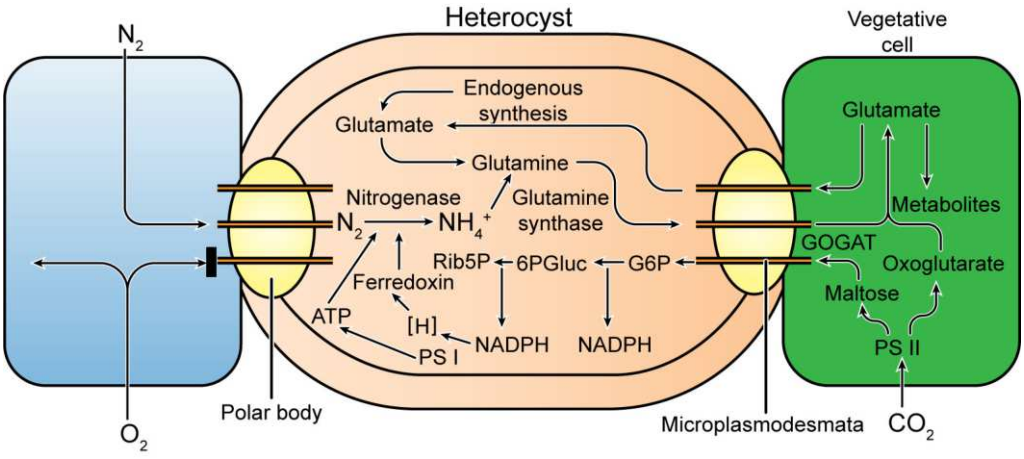

Fig. 13.12: Diagrammatic representation of carbon and nitrogen exchange between a heterocyst and vegetative cell of a cyanophycean nitrogen fixer (From Smith *et al* 1999).

iv) Leghemoglobin

An oxygen binding protein **leghemoglobin** is synthesized in the legume nodules (bacteroid injected host cell), the bacteroid and the host plant. While the bacteroid forms the haem part, the globin portion is synthesized by the host plant. Leghemoglobin gives a distinct pink color when the cut surface is exposed to air. Leghemoglobin is similar in structure to the hemoglobin of mammalian blood. Its function is also somewhat similar as it binds oxygen and

controls the release of oxygen in the domain of the bacteroid. The oxygen concentration in the bacteroid in kept at a level which is sufficient to produce ATP and reducing potential while at the same time preventing excess oxygen that could inactivate *dinitrogenase*. To continue aerobic respiration under low O_2 conditions, the bacteroids, use a specialized electron transport chain where the terminal *oxidases* have a much higher affinity for oxygen even higher than that of leghemoglobin.

13.4.3 Measurement of *Nitrogenase* Activity

There are various methods to find out whether an organism is a N_2-fixer or not. If an organism can grow in normal atmosphere without any provision of a nitrogen source like nitrate or ammonia, one infers the organism to be a N_2-fixer. It is important to mention here that N_2-fixer heterocysts containing cyanobacteria or *Rhizobium*-legume system are known to grow without any supply of fixed nitrogen.

The other more reliable method is the incorporation of ^{15}N isotope into cellular nitrogen containing compound which can be estimated in a mass spectrometer. This method is every costly and is, therefore, not easily accessible for day-to-day measurement of *nitrogenase* activity and N_2-fixation.

The most extensively used method for measuring *nitrogenase* activity is the acetylene reduction method. The method consists of incubating the N_2-fixing system with about 10% acetylene for a period of 10 to 30 min during which *nitrogenase* converts acetylene into ethylene depending on its efficiency. Gas samples are then taken out by syringe for the reaction sample and the acetylene reduced is assayed by injecting the gas sample into a Gas Chromatograph which separates quantitatively acetylene and ethylene and thus provides easy quantitative assay of ethylene. *Nitrogenase* catalyzed reduction of acetylene to ethylene is not accompanied by reduction of proton to molecular hydrogen as happens in the reduction of N_2 to NH_3.

The reduction of acetylene to ethylene involves two electrons and if one wants to equate the amount of ethylene produced with the amount of N_2 fixed then the amount of ethylene must be divided by a factor of 4 to find out the optimum level of N_2-fixing activity of *nitrogenase*.

The word diazotroph is derived from the words *diazo* ("di" = two + "azo" = nitrogen) meaning "dinitrogen (N_2)" and *troph* meaning "pertaining to food or nourishment", i.e., dinitrogen utilizing.

The word *azote* means nitrogen in French and was named by French chemist and biologist Antoine Lavoisier.

Diazotrophs are bacteria and archaea that fix atmospheric nitrogen gas into a more usable form such as ammonia. These microorganisms arecapable of growing without external sources of fixed nitrogen.

13.5 GENETIC REGULATION OF NITROGEN FIXATION

nif Genes

The genetics of nitrogen fixation is one of the most fascinating aspects and holds great promise in gene-transfer to the non-nitrogen fixers for higher productivity. The free-living nitrogen fixer ***Klebsiella pneumonieae*** has been investigated in detail for its nitrogen fixing genes. *Dinitrogenase* synthesis in this species is directed by a set of genes known as ***nif*** genes. Atleast 17 *nif* genes and a set of 7 operons have been described in this diazotroph. There are a few regulatory and structural genes which are involved in the different aspects of *nitrogenase* functioning. Two genes, the *nif* D and *nif* K encode far two different components of MoFe protein, *nif* H codes for Fe protein and ferredoxin by *nif* F.

nod Genes

Nodulation is regulated by *nod* genes and *nif* gens. A set of rhizobial *nod* genes get switched on prior to the infection of the root. The triggering factor is the flavonoids contained in the host root exudates. Three *nod* genes viz *nod* A, *nod* B and *nod* C are basic nodulation gens present in most rhizobia. *Nod* genes are important in determining host specificity.

fix Genes

These genes are switched on during later stages of nodule development. The *fix* genes are restricted to symbiotic nitrogen fixers. One *fix X* gene encodes for a ferredoxin while the others are involved in the transport of electrons to *dinitrogenase*.

The *fix* genes are required for the maintenance of N_2-fixation by the nodule. They control oxygen protection of *nitrogenase* as well as ATP and reductant supply.

nodulins

These are nodule-specific proteins encoded by *nod* genes located in the host cell genome. Two categories of *nodulins* viz., early and late, perform different functions. Early *nodulins* are involved with the infection thread plasma membrane and in the formation of nodule primordia. On the contrary, the late *nodulins* function at the time of nodule formation and nitrogen fixation. Leghemoglobin is a good example of late *nodulin*.

hup Genes

These are hydrogen uptake genes which make the nodules more energy efficient as compared to those which lack these genes. They control the activity of uptake *hydrogenase* for recovering some of the energy lost to hydrogen protection.

The *nif*, the *nod*, and the *fix* genes are located on a plasmid in the fast-growing rhizobia and on the nucleoid (chromosome) in slow growing rhizobia.

Efforts are on to introduce these genes into other rhizobia strains as well as non-nitrogen fixers to incorporate the nitrogen-fixing capability in cereals and other important crops.

SAQ 2

a) Match the characteristics given in Column 2 with enzymes given in Column 1.

Column 1	Column 2
i) *Dinitrogenase reductase*	a) Fe-Mo protein
ii) *Dinitrogenase*	b) requires ATP for activation
	c) transfers one electron at a time
	d) Transfer electrons to N_2
	e) Fe-protein

b) Fill in the blank spaces with appropriate words in the following statements.

i) In cyanobacteria *nitrogenase* is present in ……

ii) *Uptake-hydrogenase* utilizes hydrogen by passing it to…….under aerobic condition and generates…….

iii) In an atmosphere devoid of N_2 gas *nitrogenase* enzyme catalyses the production of …….only.

iv) A cyanobacterial strain deficient in uptake of *hydrogenase* will produce…….continuously in N_2- free atmosphere.

v) ……protects *nitrogenase* against oxygen and it also functions as a ……of oxygen.

c) Match the genes of N_2-fixation given in Column 1 with the functions given in Column 2.

Column 1	Column 2
i) *nif* genes	a) Maintenance of N_2-fixation
ii) *nod* genes	b) Functional *nitrogenase*
iii) *fix* genes	c) Formation of root nodules

13.6 SUMMARY

- Biological N_2-fixation is characteristic feature of prokaryotes and the process occurs in free-living and symbiotic state with various kinds of eukaryotic partners.
- *Nitrogenase* enzyme is made of two functional components *dinitrogenase reductase* (Fe-protein) and *dinitrogenase* (Fe-Mo protein).Both of these are essential for N_2 fixation, acetylene reduction, proton reduction and HCN reduction.
- *Nitrogenase* reaction occurs at the expense of 2-3 molecules of ATP consumed per electron transferred.
- Oxygen is inhibitor of both *nitrogenase* activity and its synthesis. The N_2-fixers have evolved a strategy to overcome the O_2 problem by having respiratory and conformational protection in *Azotobacter*, leghemoglobin in nodules of legumes and heterocysts in *Nostoc*.
- Nitrate and ammonia are strong inhibitors of N_2- fixation. In their presence N_2-fixing system is not functional at all. Uptake of *hydrogenase* activity is a mechanism of removing hydrogen from the site of nitrogen activity which concurrently carries out reactions of nitrogen fixation and hydrogen production.

- In *Rhizobium*-legume symbiotic N_2-fixing system, there are three classes of genes controlling N_2-fixation *in situ*. They are *nif* genes, *nod* genes and *fix* genes.
- Acetylene reduction technique is most common method of measuring *nitrogenase* activity.

13.7 TERMINAL QUESTIONS

1. How will you experimentally separate N_2-fixers from non-fixers?
2. Why is heterocyst essential for N_2-fixation in *Nostoc* and *Anabaena*?
3. What is the role of leghemoglobin in nodules?
4. Why is Mo an essential nutrient for N_2–fixation and nitrate assimilation?
5. What are Bacteroids? What roles do they play during nitrogen fixation?
6. Name few factors that influence the functioning of Nitrogenase.

13.8 ANSWERS

Self-Assessment Questions

1. a) i) *Azolla* (fern)
   ii) Certain leguminous plants
   iii) *Anthoceros*
   iv) *Casuarina*
   v) *Sorghum*

   b) i) Heterocyst
   ii) Aerobic
   iii) Anaerobic
   iv) Photosynthetic anaerobe

   c) i) True; ii) False; iii) True;
   iv) True; v) False; vi) False

   d) i) *Nitrogenase*
   ii) Recognition
   iii) *nod* genes
   iv) Lectins and polysaccharides
   v) Infection thread
   vi) Mature bacteroids

2. a) i) b, c and e;
   ii) a and d
   b) i) heterocyst
   ii) electron transport chain, ATP
   iii) hydrogen
   iv) hydrogen
   v) Leghemoglobin, reservoir.
   c) i) Functional *nitrogenase*
   ii) Formation of root nodules
   iii) Maintenance of N_2-fixation

Terminal Questions

1. The nitrogen fixers can be separated from non-nitrogen fixers by performing the following experiment. Take cyanobacteria like *Nostoc* and *Oscillatoria* and grow them separately in mineral growth medium which lacks NO_3^- or NH_4^+. Incubate these two cultures for growth under photosynthetic conditions. The culture which will keep on growing without external nitrogen source would be N_2-fixer and the other which will not grow would be non-N_2-fixer.

2. For N_2-fixation it is necessary to have O_2-protection mechanism for *nitrogenase* enzyme. In *Nostoc* and *Anabaena* this is provided by heterocyst which is a specialized structure that has lost PSII activity during differentiation. Hence in heterocyst *nitrogenase* is protected because of reduced oxygen tension. It would not be possible for these algae to fix N_2 without heterocyst because the green parts of algae evolve oxygen which is an inhibitor of N_2-fixing enzyme.

3. Oxygen is inhibitor of N_2-fixing enzyme *nitrogenase*. The primary role of leghemoglobin is to bind oxygen in the vicinity of the *nitrogenase* enzyme. It also serves as a reservoir of oxygen and supplies oxygen to meet the high energy demand during aerobic respiration.

4. Mo is part of component of enzyme *nitrogenase* and *nitrate reductase* and is involved in the reductive process of N_2 fixation. Like Fe, the Mo ion can also reversibly change from oxidized to reduced state and help in the transfer of electrons from reductants like ferredoxin, flavodoxin, and NAD(P)H to N_2 or NO_3^-

5. Refer to Subsection 13.2.3.

6. Refer to Subsection 13.4.2.

ACKNOWLEDGEMENT

Fig. 13.1 : https://o.quizlet.com/snUZABCUmA6jkPUEy8eAkA.jpg

Fig. 13.9 : From Wikipedia

UNIT 14

PLANT GROWTH REGULATORS

Structure

14.1 INTRODUCTION

In this unit and the coming unit15 we will deal with the control of plant growth and development. The growth and development of plants is largely controlled by the plant hormones. Plants produce these hormones by themselves to control the growth of stems, roots, branches, flowers and fruits and their development. Some hormones, for example auxins, gibberellins, cytokinins were discovered by scientists long time ago but now many new hormones are also being discovered from time to time.

Higher plants are complex organisms and their systematic development requires synchronization between cells. To coordinate various activities, the cells need to communicate with each other. Intercellular communication within plants takes place through hormones. Hormones are the signal molecules that individually or cooperatively direct the development of individual cells or transfer the information between the cells and thus, coordinate growth and development. The regulation and coordination of metabolism, growth and morphogenesis in higher plants depends upon the chemical signals present in the cells. Thus we can say that plant hormones are a group of naturally occurring, organic substances which influence physiological processes at low concentration. Plant hormones are also referred as phytohormones.

The present unit will discuss different group of plant hormones, how they were discovered and various roles they play in growth and development of plants.

Objectives

After studying this unit you would be able to:

- enlist major hormones in plants;
- appreciate the role of auxins, gibberellins, cytokinins, ethylene and abscisic acid;
- describe the new hormones discovered in plants;
- discuss the role of synthetic hormones; and
- explain the regulation of various growth processes by hormones.

14.2 PLANT HORMONES

Hormones are the chemical messengers that are produced in a cell. They modulate the cellular processes in another cell by interacting with specific proteins that function as receptors linked to cellular transduction pathway. The concept of hormone in plants can be traced back in 1758 from the work of **Duhamel du Moncaeu**. He noted swellings on the roots above the girdle wounds in woody plants. German botanist **Julius Sachs** postulated presence of organ forming substances in plants. He stated that root forming substances are produced in the leaves followed by their migration to stem. These substances assist in initiation of roots. Later **Charles Darwin**, through his experiments on canary grass, also mentioned about existence of such chemical substances. About half a century later, **F.W. Went** in 1926 discovered auxin as the growth hormone in plants.

Hormones that are transported to the sites of action in another tissue distant from the site of their synthesis are known as endocrine hormones. Some hormones act on the cells adjacent to their source of synthesis. These are referred as paracrine hormones. Hormones are organic molecules that influence the physiological processes in plants even at low concentrations. The development in plants is regulated naturally occurring hormones such as auxins, gibberellins, cytokinins, ethylene and abscisic acid.

14.2.1 Auxins

The discovery of auxin is a fascinating chapter in plant physiology. The hormone auxin was discovered first, through some experiments by **Charles Darwin** and his son **Francis Darwin** around 1880 (Fig 14.1). They found that if coleoptiles of a grass (*Phalaris*) were illuminated from one side they bent towards light. However, if the tip region was covered, no bending occurred.

In his book “The Power of Movement in Plants” published in 1880, Darwin wrote that some matter in the upper part, which is acted upon by light, transmits its effect to the lower part causing the latter to bend.

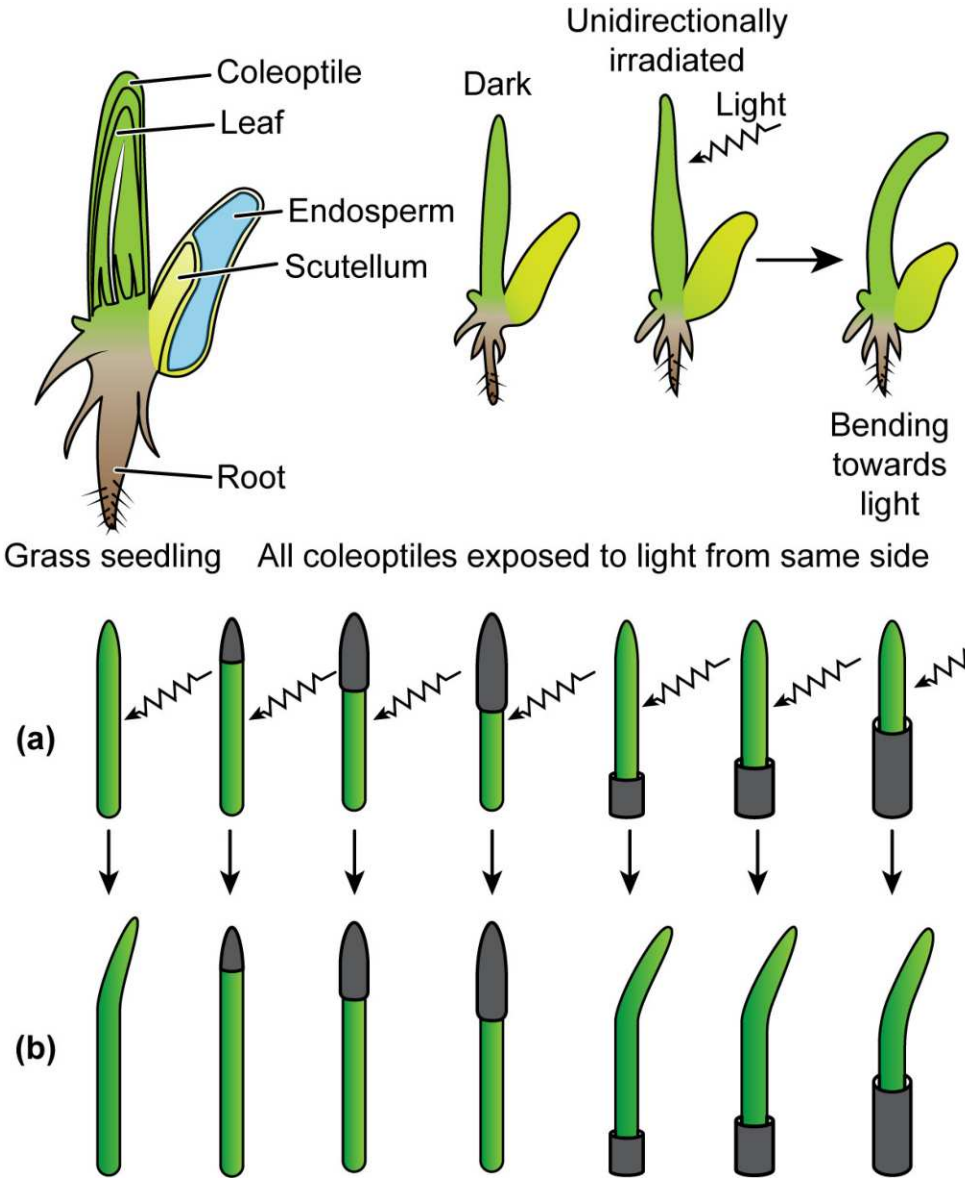

Fig 14.1: Darwin’s experiment on canary grass coleoptiles: a) various parts were covered with metal caps for determination of photosensitive region; b) Results observed in each case.

Darwin’s work was further explored by **Peter Boysen-Jensen** and **A. Paal**. They performed experiments to understand the movement of the stimulus. They decapitated coleoptiles of oat seedlings and (a) introduced a tiny gelatin block between the stump and cut tip and (b) a thin sheet of mica in the other (Fig. 14.2). The curvature in coleoptiles occurred in experiment ‘a’ but not in experiment ‘b’. This happened because the stimulus could pass down through gelatin but not mica. The curvature occurred only when the mica was inserted half way on the illuminated side.

The studies suggested that stimulus promotes elongation on dark side. **A. Paal** observed that in the dark portion, bending occurred in the opposite direction of the cut tip. This suggested that the stimulus in the tip affects cells present below.

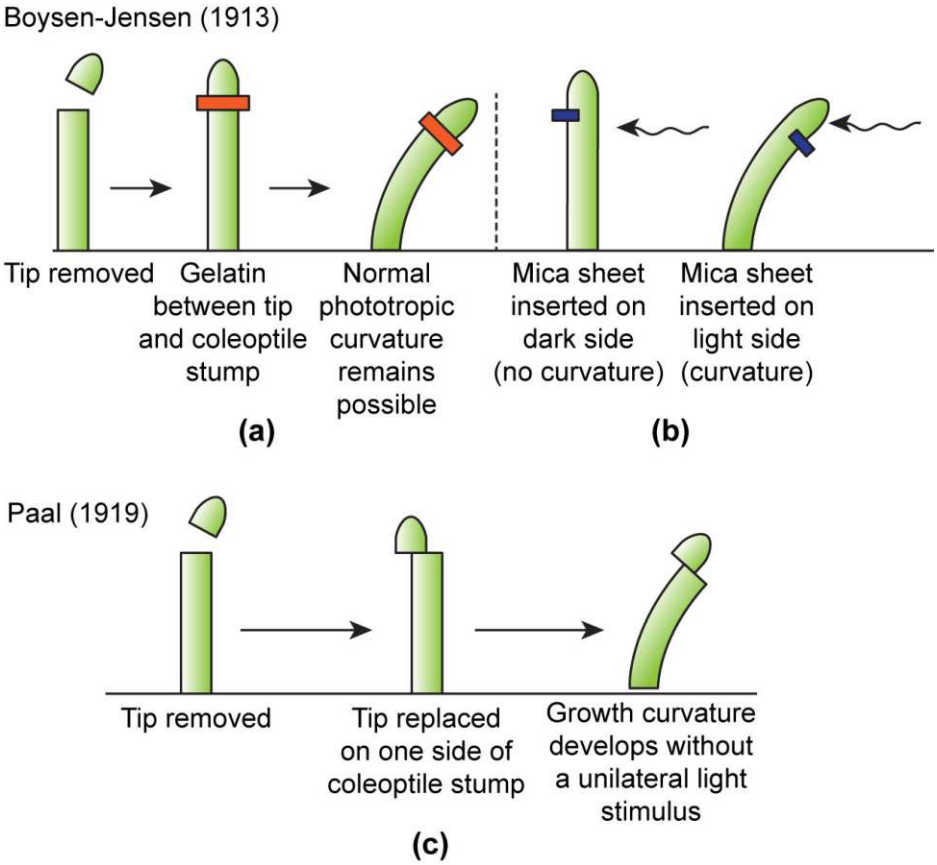

Fig. 14.2: a, b) Experiments on auxins by Peter Boysen-Jensen; and c) A. Paal experiment.

This stimulus was later discovered to be a diffusible substance by a Dutch scientist **F.W. Went** in 1926. He collected the substance in large amounts by placing decapitated coleoptiles tips on agar blocks. He then took tiny squares of the agar and placed them eccentrically on the cut end of the decapitated seedlings. Typically bending occurred within an hour after the blocks were applied (Fig. 14.3).

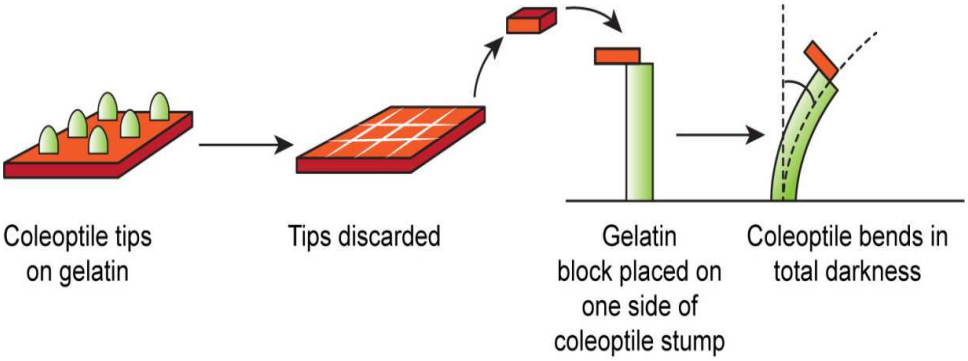

Fig.14.3: Went's experiment on auxins.

He concluded that the substance present in agar block diffused in the coleoptiles causing elongation of cells in that side resulting in curvature. Went named it auxin (from the Greek word ***auxein***, "to grow"). Later auxin was chemically characterized as indole-3-acetic acid or IAA by Thimann.

Auxin is the first plant growth hormone that was discovered. It plays a role in fundamental growth processes such as enlargement of plant cells. It is synthesized in the meristematic region and other actively growing regions such as coleoptile apices, root tips, germinating seeds and apical buds of

stem. Growing leaves, developing embryo, and developing inflorescence are also the sites of auxin synthesis.

Indole -3-acetic acid (IAA) is the most widely distributed naturally occurring auxin. Indole-3-butyric acid (IBA) is another compound showing auxin like activity. It has been isolated from maize and several other species. The chlorinated analogues of IAA (indoleacetic acid) have been found in extracts of legume seeds. The amount of IAA produced varies in different tissues. The vegetative tissues generally produce IAA in the range of 1 to 100 µg kg^{-1} of fresh weight. The amount is much higher in seeds.

Synthesis of Auxins

In 1930, K.V. Thimann first observed the synthesis of IAA in the mold *Rhizopus suinus* supplied with the amino acid tryptophan. The conversion of tryptophan to IAA was studied under *in vivo* conditions in more than 20 plant species. In most plants, synthesis of IAA occurs in three steps beginning with removal of amino group on the tryptophan side chain. The product is indole-3-pyruvic acid (IPA). This reaction is catalyzed by enzyme *tryptophan amino transferase*. This enzyme removes amino groups from the structural analogs of tryptophan such as phenylalanine and tyrosine. In the subsequent step, decarboxylation of IPA occurs to form indole- 3-acetaldehyde (IAAld). The enzyme that catalyzes this step is *indole-3-pyruvate decarboxylase*. Finally, IAAld is oxidized to form IAA by *NAD-dependent indole-3-acetaldehyde oxidase*. IAAld gets reduced to form indole-3-ethanol. IAA can reversibly get converted to IBA by the enzyme *indole-3-butyric acid synthase*. The synthesis of IAA via tryptophan independent pathway has been noted in mutants of maize and *Arabidopsis* (Fig. 14.4).

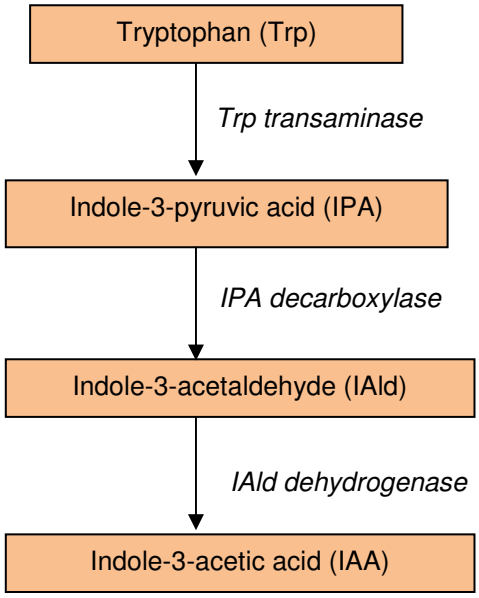

Fig 14.4: Auxin biosynthetic pathway in plants.

Studies have shown that *Arabidopsis* contains *nitrilase* enzyme that converts indole-3-acetonitrile to IAA. It has been suggested that indole-3-acetonitrile can be derived from **glucobrassicin**, a glucosinolate present in the members of family Brassicaceae.

Auxins can be present in free or bound form in plants. The auxin may be bound to the cell and can be extracted with solvents or by hydrolysis under alkaline conditions. Bound auxin forms chemical conjugates with sugars to form glycosyl esters. The conjugates are formed by esterification of a glucose or inositol molecule to the acid group of the side chain. These conjugates release active IAA upon solvent extraction or alkaline hydrolysis. The pool of IAA conjugates has been reported from seeds of maize and is formed in the endosperm. They act as the active hormone for the embryo. About 60% of the IAA requirement of the germinating seed is met through these conjugates.

Functions of auxin

Auxins are also involved in other developmental events such as secondary root initiation, vascular differentiation, and development of axillary buds, flowers and fruits. They play a crucial role in regulating various physiological roles in plants. Some of these include:

- Auxin stimulates cell elongation in stem and coleoptiles. It gets transported basipetally to the tissues below. Steady supply of auxin promotes cell elongation.
- The treatment of terminal shoots of fruit trees with α (alpha) naphthalene acetic acid prevents their elongation resulting in formation of dwarf shoots. This effect of auxin is due to shortening of internodes.
- The treatment of α- naphthyl acetamide treatment helps in the stiffening of cells and giving them a woody appearance. This prevents lodging.
- High levels of auxin promote the formation of roots in plants. Studies have established that auxins increase the formation of root initials. Thimann et al. showed similarity between the root forming substance and auxins. Formation of both lateral and adventitious roots get stimulated by auxins. Adventitious roots arise from the differentiated cells that begin to divide and develop into a root apical meristem. Some of the compounds that can be applied to cuttings to induce formation of roots include phenyl acetic acid, indole acetic acid, indole butyric acid. Hence auxin plays a major role in root initiation.
- Auxin is also involved in the vascular differentiation in shoots. The process is regulated by auxin concentration. Vascular differentiation is the regeneration of vessels and phloem sieve tubes around the wounds. Leaves are the source of auxin and removal of leaves above the wound limits vascular differentiation. It has been noted that differentiation of phloem sieve elements is favoured by low concentration of auxin (0.1%) while the xylem differentiation is favored by high auxin content (1%). Auxins promote cell elongation in the sub apical region of the stem.
- Auxins help in cell wall expansion by promoting efflux of protons. Increased activity of plasma membrane *H^{+}-ATPase* provides H^{+}. Binding of IAA to auxin binding protein present in the cytoplasm or cell surface induces the *H^{+}-ATPase* activity and increases proton pumping (activation hypothesis). Hydrogen ions play a role in cell wall expansion. Protons increase acidity of the cell wall. Increased acidity activates the activities of various hydrolyzing enzymes such as *cellulase*, *pectinase* and *hemicellulase*. These enzymes break the bonds of cellulose, pectin

and hemicelluloses and cell wall become loose. The proteins called expansions accelerate cell wall expansion by weakening the hydrogen bonds between the polysaccharide components of the wall.

- During cell wall expansion, auxin increases the supply of ADP promoting the synthesis of ATP, hence stimulating respiration.
- The development of floral mersitem depends on auxin transported to it from subapical tissues. The auxin transport from these tissues regulates leaf initiation and phyllotaxy (the pattern of leaf emergence from the shoot apex).
- Auxin delays the onset of leaf abscission (shedding). Addicott and Lynch (1951) showed that auxin gradient across the abscission zone controls abscission. Application of auxin to the cut surface of the petiole prevents the formation of the abscission layer. The premature fruit drop can be prevented to a great extent by spraying of 2,4D, IAA or NAA.
- Auxin promotes fruit development. Auxin is produced or mobilized from storage in pollen and the initial stimulus for fruit growth may result from pollination. The endosperm contributes auxin during initial stage of fruit growth and developing embryo takes over as the main auxin source at later stages.
- Auxins generally inhibit flowering but in pineapple a spray of dilute solutions of 2, 4-D, NAA has been found to promote flowering by synthesizing ethylene.
- Auxins promote development of fruits without pollination and fertilization. Auxins promote growth of the ovary wall leading to formation of fruit. The fruits that develop from parts of the flower without involving the process of fertilization are referred as parthenocarpic fruits.
- Very high concentrations of auxins such as 2,4D affect the growth promoting activities of the root cell. The root cells get distorted, sieve tubes get blocked and the cell divisions are disturbed. The roots dry and the plant die. Thus application of auxins can help in eradication (removal) of weeds.
- Auxins promote cell division. In case of a wound, the proliferation of cells results in the formation of callus. The callus formation is linked with cambial activity which is stimulated by auxin. Studies have shown that cambial activity is promoted by auxin
- With the growth of shoot, the apical meristem forms new primordia. A group of cells in the axil of the primordial become isolated from the apical meristem which form the axillary bud. In some plants the bud continues to grow but in many plants, mitosis and cell expansion in the bud is arrested and hence the axillary bud fails to grow. The removal of shoot apex stimulates the growth of axillary bud. It is believed that apical bud exerts a dominant influence and suppresses the cell division and enlargement in the axillary bud. This phenomenon is referred as **apical bud dominance**. K.V. Thimann and F. Skoog tried to find out the relationship between auxin and apical bud dominance and stated that axillary bud development remains suppressed in the presence of auxin.

Bioassays

The effect of a biologically active substance on living materials can be checked by performing certain experiments. These are known as **bioassay**. Bioassays for auxins include

- *Avena* curvature test
- *Avena* section test
- Split pea stem curvature test
- Cressroot inhibition test

Avena curvature test

The assay was proposed by F.W. Went. The measurement of auxin activity can be done by studying its polar transport. The seedling of *Avena* is decapitated. An agar block containing the auxin is placed on the decapitated stump (Fig. 14.5). Auxin from the agar is transported down the coleoptiles and causes the side to grow faster than the opposite side, resulting in curvature. Bending depends upon the hormone concentration. The more of the concentration of hormone more is the curvature.

Avena section test

The studies suggest that auxin stimulates cell elongation. *Avena* grains are germinated in dark and 25-30 mm long coleoptiles are harvested. The apex is removed and a section of 3-5 mm in length is cut from the coleoptile. The length is measured. The sections are soaked in distilled water for an hour and distributed in petriplate having the test solution with various concentrations. The sections are incubated for 12, 24 and 48 h and the sections are measured.

Split peas stem curvature test

This test depends upon the differential growth response. It was reported by Went in 1934. A stem section of pea seedling is split longitudinally and floated on the test solution. A negative curvature is noted because of uptake of water by cortical cells. The epidermal cells show increase in length because of auxin while the cortical cells how increase in width. If the concentration of auxin is increased, a positive curvature will result.

The pea seeds are grown in dark but exposed to red light for some time (3 h). The stem is decapitated and the portion between node and internode is selected. The selected portion is soaked in distilled water and the cut stem is slit longitudinally and placed in a Petri dish a having auxin solution. The curvature of the stem is noted.

Cress root inhibition test

The roots show more sensitivity to auxins. Low concentrations of auxin stimulate root growth. The test is used for measuring low concentrations of auxin. Seeds are sterilized and germinated on a moist paper. After emergence of roots (about 1 cm long), they are placed in test solution. The length of the root is measured in control and treated.

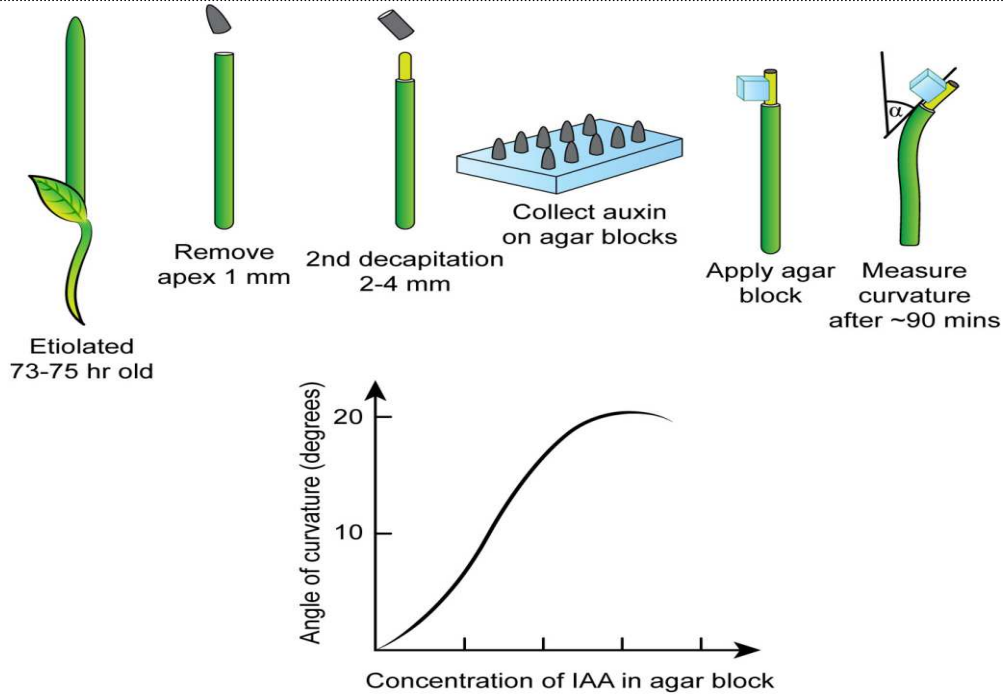

Fig.14.5: Bioassay of auxin. The extent of bending as measured by angle α, is a function of auxin or IAA in the agar block (Source: Leopold 1955).

Transport of auxins

Auxin generally moves from shoot to root. The polar movement i.e., either up or down has been noted in coleoptiles, stem and roots (Fig.14.6).

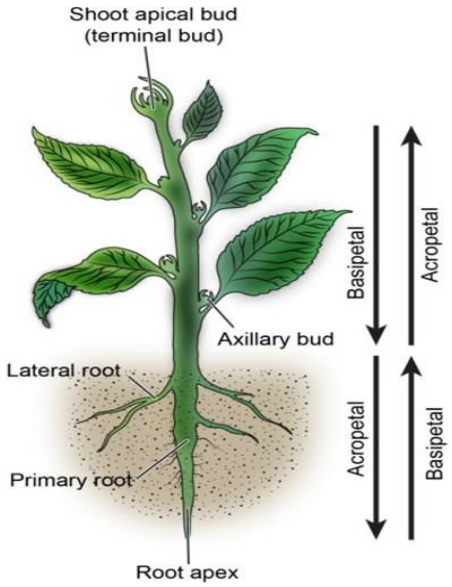

Fig. 14.6: Polar transport of auxin in plants.

When the movement of a compound occurs from the apex towards the base, the movement is called **basipetal** while the movement towards the morphological apex is known as **acropetal**. The movement of auxins is generally basipetal i.e. it moves away from the root tip. The movement of auxin is important because auxin plays a role in various growth and developmental responses in plants. The polar transport of auxin is a carrier mediated active transport and depends upon oxidative metabolism in mitochondria. The polar transport is inhibited by respiratory poisons such as cyanide and 2, 4 -dinitrophenol. Inhibitors block auxin transport by binding to discrete carrier molecules which are involved in polar transport system.

BOX.14.1: Auxin transport in a cell

A chemiosmotic model for auxin transport has been proposed by **Rubery** and **Sheldrake** (1974) and **Raven** (1975). According to this model

1) A pH gradient or proton motive force across the plasma membrane provides the driving force for uptake of IAA,

2) An IAA influx carrier and efflux carrier is located at the base of auxin transporting cells.

IAA is a weak acid and depending upon the pH, it exists in either protonated form (IAAH) or the unprotonated anionic form (IAA-). The cell wall is moderately acidic with pH of 5.5. At this pH, 20% of the IAA occurs in the protonated (IAAH) form. The cell wall space contains both protonated and anionic IAA. A small proportion of the uncharged IAAH molecule diffuses slowly across the plasma membrane from the cell wall space into the cell. Inside the cell, IAAH dissociate to form IAA^- and H^+. The auxin is now trapped inside the cell because the IAA^- cannot diffuse across the membranes. The position of the efflux carrier (generally located in the membrane of the cell) establishes the polarity in auxin transport. The influx carrier identified as AUX1 plays a role in auxin metabolism and transport. This protein has been supposed to function as amino acid/proton symport carrier. *PIN* genes that encode putative auxin efflux carriers have been identified in plants. *PIN* proteins direct the transport of auxin from one cell surface to another. *PIN1* protein has been found to be localized in the basal membranes of xylem parenchyma cells.

Mechanism of action of auxin

Auxins act in cell by getting adsorbed to the hormone binding site. They induce acidification of membrane by bringing a change in nucleic acids, proteins or plasticity of wall. The auxin plays a role in cell growth by increasing rate of cell expansion. According to **R. Cleland** and **D. Rayle** (1970) auxin causes acidification of the cell wall by stimulating cells to excrete protons. The proton secretion lowers pH and this activates one or more wall loosening enzymes. This results in cell enlargement. Studies with *Avena* coleoptiles have shown that apoplastic pH decreases from 5.7 to 4.7 on application of auxin.

A. Hager (1970) proposed that auxin stimulates proton excretion by activating plasma membrane bound H^+-*ATPase*. Auxin induced increase in cell growth has been explained through **acid growth hypothesis** proposed by **Cleland** and **Hager** (1971). According to this hypothesis auxin activates ATP proton pumps located in the plasma membrane. Enhancement in the activity of H^+-*ATPase,* pumps more protons inside resulting in acidification of the cell wall, hence lowering the pH**.** Lowering of pH supports turgor induced cell expansion and cell wall extensibility. The activation of enzyme occurs after binding of auxin to specific transmembrane protein receptors such as ABPI (Auxin binding protein I).

14.2.2 Gibberellins

They are plant hormones that belong to a large family of plant constituents called terpenoids. They are biosynthetically related to carotenoid pigments, sterols, and other isoprene derivatives. More than 135 gibberellins have been identified in plants. GA_1 and GA_4 are the two most active commonly occurring gibberellins reported in higher plants. All the gibberellins are diterpenes and

possess a 20-carbon structure. A C20 gibberellin is referred as gibberellic acid (GA_3). The gibberellin biosynthesis occurs mainly in the developing seeds, fruits, young leaves and apical region of roots. They are also produced in developing endosperm, cotyledons of legumes or scutellum of cereals.

During late nineteenth century, the Japanese rice farmers found a disease that reduced the yield of their crops. Plants were infected with the ***bakane byo*** (foolish seedling disease) which exhibited weak, elongate stems and produced little or no grain. It was found that the disease is somewhat related to the fungi *Gibberella fugikuroi*. In 1926, **E. Kurosawa** reported the appearance of symptoms of the disease in uninfected rice plants treated with the filtrates from the cultures of the fungus. The compound was isolated, crystallized and named as gibberellin. He isolated three gibberellins viz. gibberellin A_1, gibberellin A_2 and gibberellin A_3. Gibberellin A_3 was found identical to gibberellic acid.

Synthesis of gibberellins

Gibberellins are synthesised through the normal isoprenoid pathway of terpene biosynthesis. The biosynthesis of gibberellins occurs mainly in the root apex, elongating shoots, young leaves, seeds and fruits.

Gibberellins are either synthesised in the regions where they act or are transported there from some other part of the plant. The translocation of gibberellins is not polar. It is believed that gibberellins synthesized in the root tip get translocated to the other aerial parts of the plant through xylem. Gibberellins can move in several different ways in the plant in a manner which is similar to other organic metabolites such as the carbohydrates, amino acids etc. They can either be transported by simple diffusion, or through the phloem or the xylem. Their transport is not polar like that of auxins.

The synthesis of gibberellins involves three steps- First -formation of two phophorylated derivatives from acetyl Co A, viz., Isopentenyl pyrophosphate and Dimethylallyl pyrophosphate. Second- One molecule of Isopentyl pyrophosphate condenses with the molecule of Dimethylallyl pyrophosphate to form geranylgeranyl pyrophosphate (C20) (GGPP) through geranyl pyrophosphate (C10) and farnesyl pyrophosphate (C15) (Fig. 14.7). GGPP gives rise to GA 12-7, which acts as the source of all gibberellins.

A number of growth retardants also known as **antigibberellins** have been applied to potted plants as foliar spray or soil supplement. These retardants reduce stem elongation, resulting in plants which are shorter, compact with dense foliage. In roots application of **antigibberellins** such as **2-chloroethyl trimethylammonium chloride** (CCC) stops the synthesis of gibberellins. AMO-1618 and phosphon-D are the antigibberellins that inhibit enzymes involved in the synthesis of kaurene. Ancymidol blocks the oxidation of kaurene to kaurenoic acid. The effect of these compounds can be reversed by the application of gibberellins such as GA_{20} or GA_3.

The translocation of gibberellins is not polar. It is believed that gibberellins synthesised in root tip get translocated to the other aerial parts of the plant through xylem.

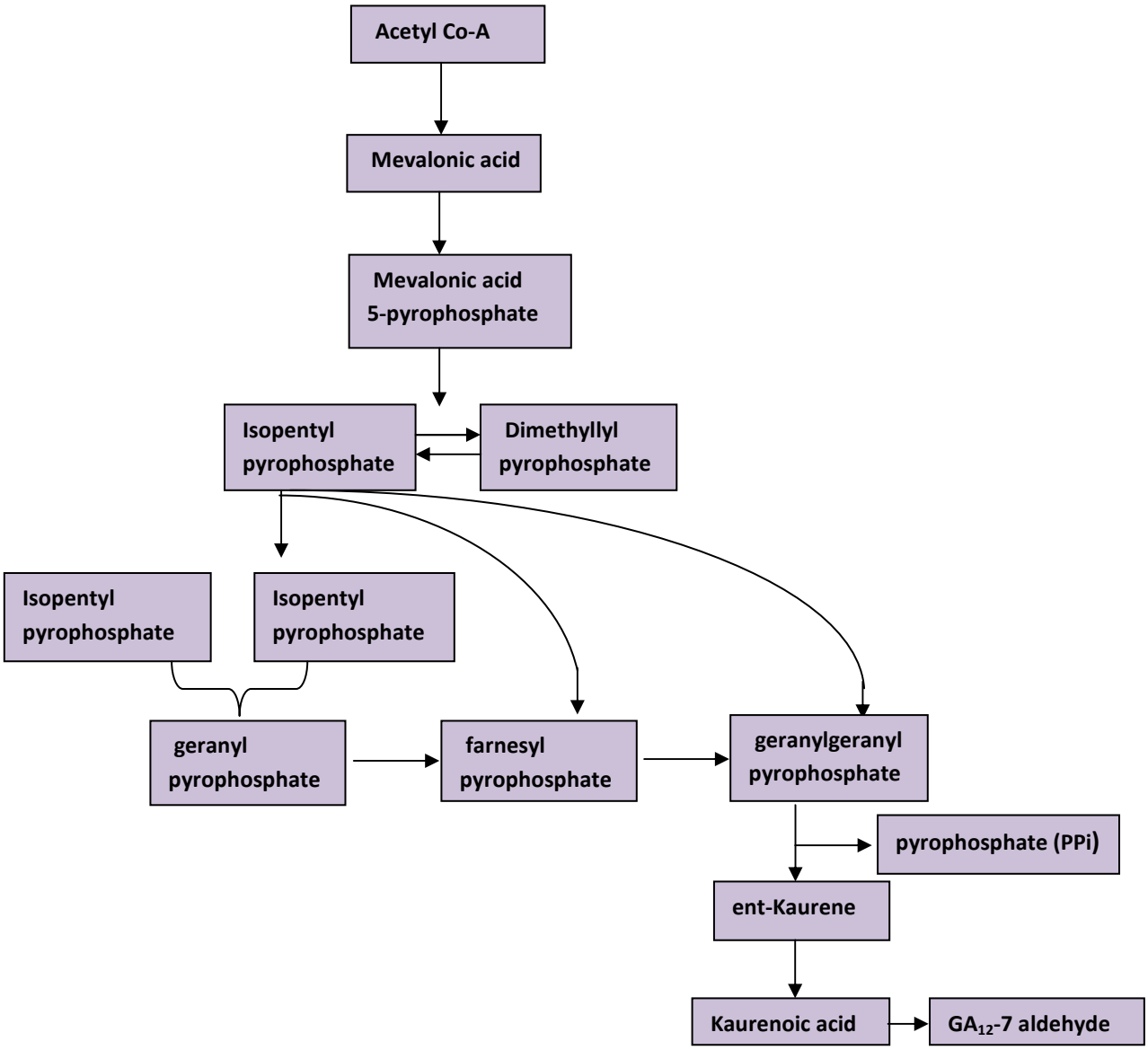

Fig. 14.7: Giberellin biosynthesis in plants.

Functions of gibberellins

Various responses in plants have been observed after treatment with gibberellins. Some of the major physiological roles of gibberellins include:

- Gibberellins promote seed germination. When the concentration of GA is increased it removes dormancy and promotes germination. During germination, they induce the synthesis of hydrolytic enzymes such as *amylases* and *proteases* in cereal grains. These enzymes degrade the stored food reserves accumulated in the endosperm or embryo. The degradation of carbohydrates and storage proteins provide nourishment and energy to the growing seedling. Gibberellins are also involved in mobilization of endosperm reserves during early stages of embryo growth, leaf expansion, pollen maturation, flowering and fruit development.
- GA possesses the capacity to stimulate hyper elongation of stem, especially in dwarf plants and rosette plants. Hence, gibberellins are involved in the regulation of stem elongation. **B.O. Phinney** and **P.W. Brian,** while working on maize and garden pea found a direct

relationship between the dwarfing or internode length genes and gibberellins. Application of exogenous gibberellins to the dwarf mutant restores the normal length in phenotype. Gibberellins are also supposed to induce elongation in length of internode as noted in maize. You will also see a demonstration of this effect of gibberellins (Demonstration 1) in your practical classes.

- The gibberellins increase the size of leaves in number of plants. The leaves of pea, beans, cucumber, lettuce and cabbage showed increase in size after spray of gibberellins. The increase in the photosynthetic area increases the productivity of plants.
- GA regulates the transition from juvenile to adult phase. In many woody perennials, the juvenile phase (phase in which no flower or fruits are produced) can be shortened by treatment with GA_3.
- GA promotes flowering in long day plants. GA promotes formation of staminate flowers and inhibitors of GA promote formation of pistillate flowers.
- GA promotes pollen development and pollen tube growth in plants.
- GA promotes fruit set and fruit growth in plants. GA induced fruit set may occur in absence of pollination resulting in **parthenocarpic** fruits. In grapes the seedless variety produces small fruits which are enlarged by treatment of GA_3.
- Gibberellins play a role in synthesis of RNA and proteins. Exogenous application of GA causes an increase in level of *α amylase* and RNA. Application of GA results in organization of endoplasmic reticulum into stacks. These are the site of synthesis of *α amylase*. According to Butcher et al. (1970) the hormone, acts as the first messenger which helps in the synthesis of 3, 5 cyclic AMP (the second messenger). This messenger activates enzyme precursors for producing active enzymes required for various physiological functions.

Bioassays

The bioassays for the hormones are based on study of plant responses like reversal of stem dwarfism, synthesis of *α amylase* in barley endosperm. A germinating seed is given gibberellin treatment. The length of the epicotyls and growth of hypocotyls is measured.

In rosette plants, there is a lack of internode elongation resulting in compact growth with densely spaced leaves. This condition results from mutation in the step in the GA_1 biosynthetic pathway. Hyper-elongation of stems has been noted in rosette plants after application of gibberellins. Some plants such as spinach and cabbage do not flower in rosette form. They undergo internode elongation before flowering. This is known as **bolting**. The process is triggered by an environmental signal, change in photoperiod or low temperature. Zeevaart et.al (1979) found that spinach contains six gibberellins including GA_{19}, GA_{20}. The gibberellin GA_{20} causes bolting in rosette plants of spinach under short day conditions. Spinach contains high levels of inactive form of GA_{19} and low levels of active GA_{20}. The level of GA_{20} increases while GA_{19} decreases under long day conditions.

The gibberellins initiate the mobilization of nutrient reserves stored in the endosperm in cereal grains. In cereals gibberellins are released by the hydrated embryos. Cereal grains such as rye, barley, wheat have a protein rich layer of cells called **aleurone** which surrounds the starchy endosperm. During germination of seed, the cells in the aleurone secrete hydrolytic enzymes such as *α amylase* and *proteases* (Fig. 14.8).

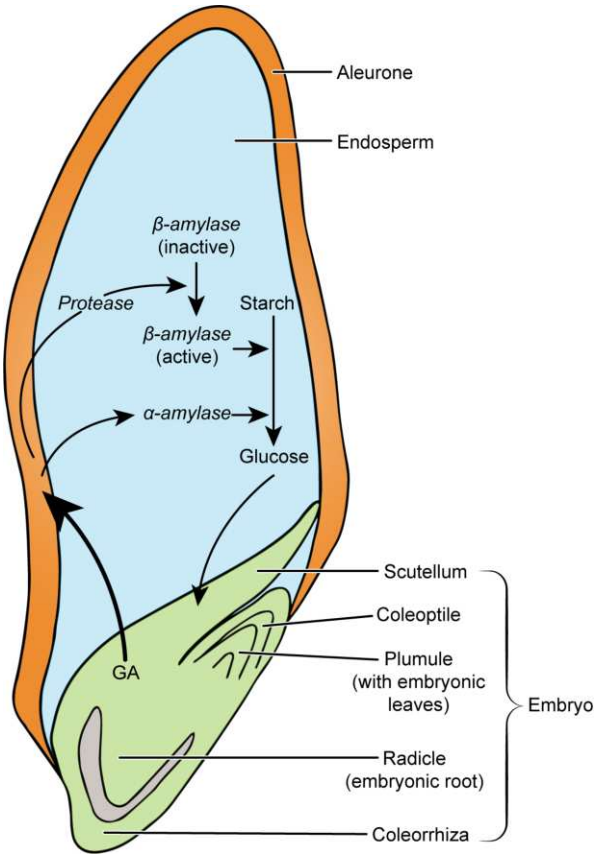

Fig. 14.8: Gibberellin induced seed germination.

These enzymes are involved in the hydrolysis of carbohydrate and proteins stored in the endosperm. The starch and proteins provide nourishment to the developing embryo. It is suggested that the germinating embryo sends a signal which is probably a gibberellin to the aleurone cells. The gibberellins activate or repress the transcription of genes encoding for hydrolytic enzymes. It has been demonstrated that GA induced secretion of *α amylase* could be blocked by inhibitors such as actinomycin D and cycloheximide. These chemicals inhibit RNA and protein synthesis.

Spray of gibberellins increases the length of floral stem. Larger fruit size is obtained by the spray of gibberellin at the time of pollination and fruit set. Gibberellins enhance seed germination and seedling emergence in species such as grapes, citrus, apples, peach and cherry. The GA induced *α amylase* in barley aleurone led to its widespread use of GA in the malting industry. The growth of stems can be reduced by using growth retardants or antigibberellins. The blocking specific steps in gibberellin biosynthesis reduce endogenous levels of gibberellin suppressing internode elongation. These are used mainly for ornamental plants. This is done to get shorter, compact and darker green foliage.

SAQ 1

a) State whether the statements are 'True 'or 'False'

i) Hormones influence the physiological processes in plants even at low concentrations.

ii) Auxin is synthesized mainly in the non-meristematic regions of the plant.

iii) Auxins can be found free or bound form in plants.

iv) Low levels of auxin can promote the formation of roots in plants.

v) The translocation of gibberellins is polar.

vi) Gibberellins promote flowering in long day plants.

b) Answer in one word:

i) Who discovered auxin as the growth hormone in plants.

ii) The precursor of auxins in plants.

iii) The mode of auxin transport in plants.

iv) The chemical substances when applied to plants as foliar spray or soil supplement reduce stem elongation.

SAQ 2

Fill in the blanks:

a) The auxin was discovered first through experiments on …… of a grass (*Phalaris*) illuminated from one side.

b) …….. is the most widely distributed naturally occurring auxin.

c) Auxin stimulates cell elongation in ……. and …… .

d) Auxins promote the cell wall expansion by promoting of ……protons which increase …….. of the cell wall.

e) Auxins promote development of fruits without ……. and ……..

f) Gibberellins promote …….. of stem in dwarf plants.

14.2.3 Cytokinins

The first naturally occurring cytokinins were isolated from milky endosperm of maize and developing plum fruit.

Cytokinins are the derivatives of nitrogenous base, adenine with isoprene related side chain or aromatic side chain. Accordingly, the cytokinins are called isopernoid cytokinins or aromatic cytokinins. The most common cytokinins are N6-Δ^{2}-isopentenyl-adenine (iP), trans-zeatin (tZ), dihydrozeatin (DZ). Kinetin is a synthetic derivative not found in plants.

The first experimental evidence for cytokinins came through **Haberlandt** in 1913. He found that a chemical controls the cell division in plants. He

demonstrated that phloem sap could cause the non-dividing parenchymatous tissue to revert to an actively diving meristematic tissue. **F. Skoog** while studying the nutritional requirement of tissue cultures found that stem tissue explants containing vascular tissue proliferate on auxin containing medium. These explants if freed from vascular tissue exhibited cell enlargement. They showed cell division if extracts of vascular tissue, coconut milk and yeast were added to the media. Later **C.O. Miller** was able to identify the active material as adenine.

Synthesis of Cytokinins

Two mechanisms have been proposed for cytokinin biosynthesis. One is tRNA induced cytokinin synthesis and the other is synthesis of free cytokinins. Studies have shown that significant amount of free cytokinin in plants is derived from the degradation of tRNA. The isopentenyl groups are transferred from isopentenyl pyrophosphate (IpPP) using an enzyme *prenyl transferase*. This enzyme synthesizes tRNA, and is required for the synthesis of isoprenoid compounds. It recognizes a specific base sequence in the tRNA molecule and transfers the isopentenyl group to the adenosine adjacent to the 3′ end of the anticodon. The *cis*-zeatin formed subsequently gets converted to active *trans*-zeatin by the enzyme *cis-trans-isomerase*.

During the synthesis of free cytokinins, an enzyme *isopentenyl pyrophosphate* plays an important role. *AMP isopentenyl transferase*, also known as *cytokinin synthase* catalyzes the conversion of 5-AMP (adenosine-5'-monophosphate) and isopentenyl pyrophosphate into i^6ADE (adenine). The enzyme is a type of prenyl transferase and removes phosphate to form isopentyl adenosine. The enzyme converts AMP and dimethylallyl-diphosphate (DMAPP) to the active cytokinin isopentenyladenosine-5′-monophosphate (iPMP). The products of the enzyme activity are isopentenyladenosine-5′-triphosphate (iPTP) and isopentenyladenosine-5′-diphosphate (iPDP), which subsequently get converted to zeatin (Fig.14.9).

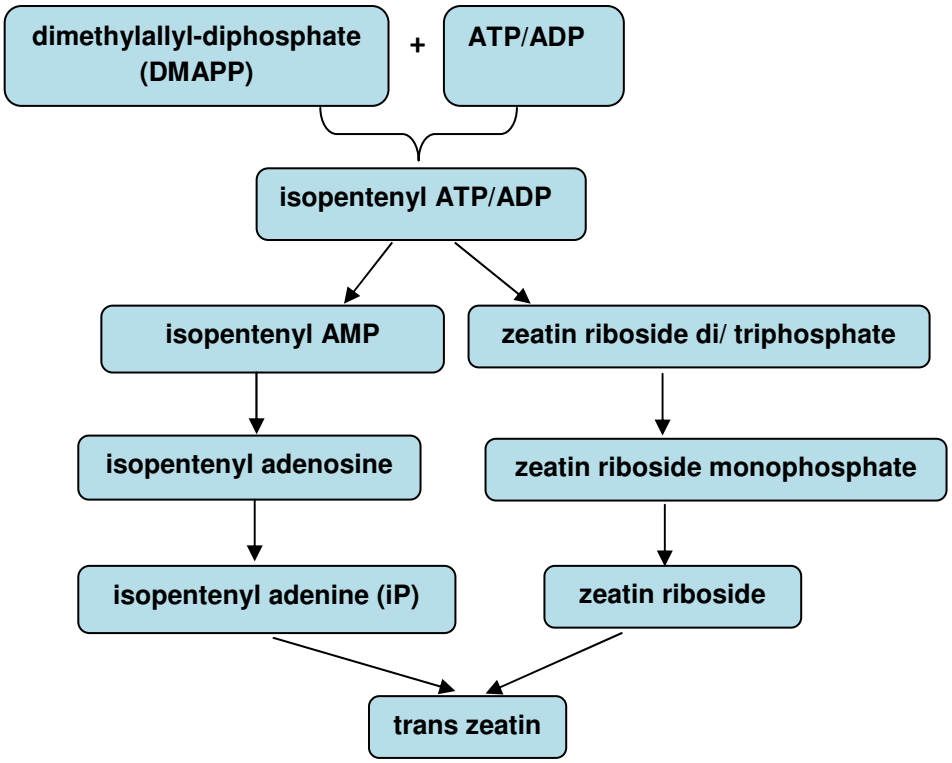

Fig. 14.9: Cytokinin biosynthesis in plants.

Zeatin and Ip are biologically active cytokinins in plants. Cytokinins are synthesized in both root and shoot meristems during active growth period. Cytokinins produced in shoot meristem get distributed to long distances whereas those synthesized by root meristems get transferred to tissues lying close to the root. In roots, cytokinins are synthesized primarily in the root tip. They get translocated to the aerial parts of the plant through xylem. Cytokinins are transported from the root to the shoot via xylem as zeatin riboside. The presence of cytokinins in the xylem sap proves the movement of hormone is polar. Immature seeds and developing fruits also contain high levels of cytokinins but do not show any apparent movement. After reaching the leaves, some amount of zeatin ribosides either get converted to free base or to cytokinin glucosides. These glucosides get compartmentalized within cells and are not available for physiological functions.

Functions of cytokinins

- Cytokinins regulate cell division or cell cycle. The absence of hormones causes the cells to be arrested in G1 or G2 phase of the cell cycle. The cells showing arrested division in G2 phase do not possess *cyclin – dependent kinases* (CDK). The activity is reduced due to high level of phosphorylation on the tyrosine residue. If cytokinin supply is restored the tyrosine gets dephosphorylated, enzyme gets activated and cell division is resumed. Thus cytokinin plays a role in generation of active CDK complex that initiates cell division by catalyzing the transition from the G2 phase of mitosis or M phase in cultured cells.

  Cytokinins are also known to possess high level of D-type cyclin (CycD3) which controls the transition from G1 to S phase. Cytokinins initiate the cell division at the G1 to S phase transition through the induction of CycD3.

- Cytokinins have the capacity to initiate cell division in quiescent and non-dividing cells. Cytokinins promote shoot growth by increasing cell proliferation in the shoot apical meristem. The hormone initiates cell proliferation and division in tissues cultured in media having both auxin and cytokinin.

- Cytokinins induce organ formation in tissue culture conditions. The ratio of auxin and cytokinins controls root, shoot initiation in callus tissues and growth of axillary buds. If the ratio of auxin to cytokinin is high, the cultures initiate root formation. Conversely, low auxin to cytokinin ratio promotes shoot formation. Equal proportion of the two causes continuous proliferation of the callus.

- Kuraishi and Okumara (1956) found that cell enlargement in mature leaves occurs in the presence of kinetin.

- Cytokinins antagonize the effect of auxin in regulating the growth of axillary buds or apical dominance. Recent studies have demonstrated that elevated auxin levels show increased apical dominance while the overproduction of cytokinins reduces apical dominance. The application of the cytokinins to shoot apex or axillary bud directly releases the bud from inhibition.

- Cytokinins are also considered as the inhibitors of senescence. Exogenous application of cytokinins delays onset of senescence, maintain protein levels and prevents chlorophyll breakdown in detached leaves. Richmond and Lang (1975) reported delay of senescence (disappearance of chlorophyll and protein degradation) in the detached leaves of *Xanthium* treated with kinetin. The hormone proved effective in removing the effect of ageing which was referred to as **Richmond-Lang effect**. Cytokinins are supposed to direct nutrient mobilization and help in retention of nutrients by stimulating the metabolism in the area of cytokinin application.
- Cytokinins have been found to be very effective in breaking the dormancy of seeds and other plant organs. They substitute the red light requirement for breaking the seed dormancy.

Bioassays

Cell division test

These tests are performed in tissue cultures of tobacco pith tissue and soyabean callus tissue. The cultures were maintained in media for about 2 to 4 weeks and their fresh and dry weights were compared with controls. The changes in growth tell about the role of cytokinins.

Chlorophyll preservation test

The effect of delay in senescence was studied using *Xanthium pennsylvanicum* by Osborne and McCalla (1961). The leaves of the plants were allowed to senescence by keeping them in dim light. The leaf discs were kept in dark for 48 h on the filter paper soaked in the test solution. The chlorophyll was extracted and their levels were measured. A linear relationship between the amount of chlorophyll retained and the log of the concentration of cytokinins was noted.

Germination test

In this bioassay, the promotive effect of cytokinin on seed germination was checked. The lettuce seeds were kept in the test solution in the dark at 25^{o} C for 8 h. The seeds were then allowed to germinate for 24 to 60 h. The effects on seed germination and plant growth were recorded.

Table 14.2: Summary of the effect of Auxins, Gibberellins and Cytokinin.

Activity	Auxin	Gibberellins	Kinin
Cell elongation	Yes	Yes	Yes
Cell division	Yes	Yes	Yes
Polar transport	Yes	No	No
Avena curvature test	Yes	No	No
Apical dominance and Dormancy	Yes	No	No
Root initiation	Yes	No	No

Prevention of abscission	Yes	-	-
Preventive of lodging	Yes	No	No
Selective herbicide	Yes	No	No
Flowering in pineapple and Litchi	Yes	No	No
Parthenocarpy	Yes	Yes	No
"Bolting"	-	Yes	-
Flowering of long day plants	-	Yes	-
Substituting vernalisation	No	Yes	No
Dwarf Maize test	-	Yes	-
Breaking dormancy	No	Yes	Yes
Seed germination	-	Yes	Yes
Flowering of short day plants	No	No	Yes
Morphogenesis	No	No	Yes
Richmond-Lang effect	No	No	Yes
Tobacco pith culture test	No	No	Yes

SAQ 3

Fill in the blanks:

a) …….. is a synthetic derivative which is not found in plants.

b) Immature seeds and developing fruits contain high…….. of…….. ..

c) For initiation of root the ratio of auxin to cytokinin should be…….. .

d) Over production of cytokinins will reduce…….. .

e) Cytokinins are very effective in ……of seed dormancy.

14.2.4 Abscisic Acid

The compound was discovered accidently as an interfering substance in the experiments of auxin response in *Avena* coleoptiles. **Bennet-Clarke** and **Kefford** found that the substance inhibited the growth of coleoptiles sections and called it as inhibitor β. The compound was supposed to be involved in apical bud dominance and maintaining dormancy. In 1964, **P.F. Wareing** proposed the term 'dormin' for the endogenous dormancy inducing substance. The substance that accelerated abscission was isolated from the senescing leaves and was named abscission II. The compound was later named as abscisic acid.

Two pathways have been proposed for the synthesis of abscisic acid (ABA). In most of the plants, ABA is synthesized directly from a 15 carbon terpenoid precursor, farnesyl phosphate. Indirect pathway suggests its synthesis from cleavage of carotenoid such as β carotene via 40 carbon terpene violaxanthin.

Enzyme *nine-cis-epoxycarotenoid dioxygenase* (NCED) cleaves the 40 carbon violaxanthin to produce a 15 carbon product xanthoxin. Xanthoxin gets converted to abscisic acid aldehyde by an enzyme *alcohol dehydrogenase* which further gets oxidized to abscisic acid by *abscisic aldehyde oxidase*. The synthesis of enzymes *NCED* and *xanthoxin* occurs in the chloroplast while the rest of the reactions occur in the cytosol (Fig. 14.10).

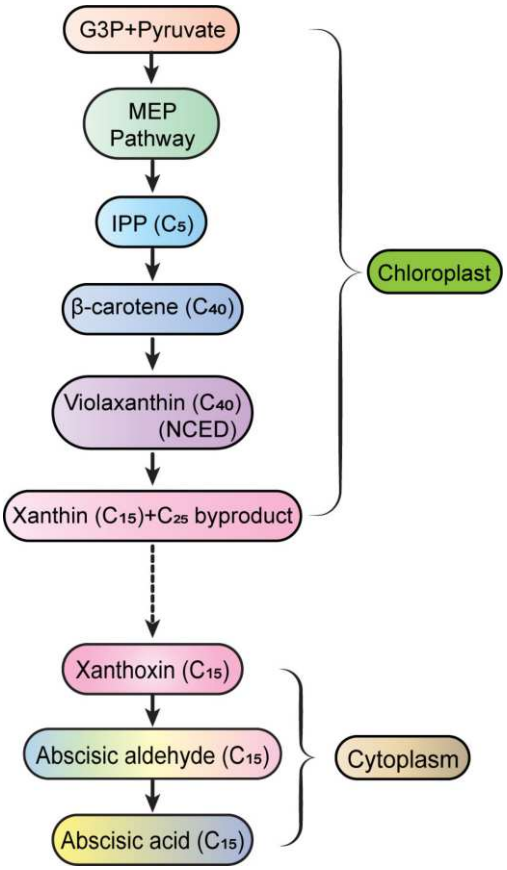

Fig. 14.10: Schematic representation of ABA biosynthesis in plants.

Studies suggested that ABA precursors originate in the chloroplasts but ABA is formed in the cytosol. *NCED* expresses itself in the guard cells under conditions of senescence in leaves and cotyledons, while the enzyme *abscisic acid oxidase* gets induced in guard cells under conditions of water stress. Thus, the site of ABA synthesis is guard cells under stress conditions, while vascular tissues are the site of ABA synthesis in non-stressed plants. ABA is highly mobile and rapidly gets transferred to other parts of the plant.

Functions

- ABA regulates maturation of embryo and seed germination. In seeds, the cytokinin levels are high during early stages of embryo development (when cell division is fast). During phase of cell enlargement, the IAA and GA levels increase but at later stages of embryo development (maturation) the ABA levels increase. During this stage there is cessation of embryo growth, accumulation of nutrient reserves in the endosperm and development of tolerance to desiccation.

  ABA plays a critical role in maturation of embryo. It prevents precocious germination or vivipary. This has been established through viviparous mutants in maize and *Arabidopsis*. The mutants show low levels of ABA

in seeds and they germinate prematurely on the cob itself before the seeds enter the dormancy. ABA stimulates the protein accumulation in the later stages of embryo development and prevents *α amylase* biosynthesis in cereal grains. This shows the role of ABA in seed maturation and prevention of premature or untimely germination.

- ABA accelerates senescence of leaves.
- It inhibits germination of seeds such as those of lettuce.
- It inhibits gibberellin induced growth in various tests. It is suggested to be an antagonist to gibberellin. It inhibits gibberellin induced *α amylase* in barley aleurone.
- It promotes ageing and abscission of leaves.
- It promotes root growth but inhibits shoot growth.
- ABA accumulates in high amounts in leaves during wilting. Increased concentrations of ABA have been noted in leaves showing stomatal closure during water stress conditions. ABA is known to act as an antitranspirant since it possesses the capacity to induce stomatal closure under water stress conditions. This reduces water loss through transpiration. Inhibition of electron transport and photophosphorylation in chloroplast disrupts the proton accumulation in the thylakoid lumen which lowers the pH of the stroma. The pH of the apoplast surrounding the mesophyll cell increases at the same time. The pH gradient stimulates release of ABA from mesophyll cells into the apoplast which gets carried to guard cells via transpiration stream.
- ABA is also supposed to play a role in lateral or secondary root development. Application of hormone to the lateral meristem at early stages of root growth promotes lateral root formation. Studies suggested that endogenous ABA inhibit or delay flowering in plants like *Arabidopsis*.

It has been suggested that ABA inhibits biosynthesis of several growth hormones or inactivates them. It inhibits RNA and protein synthesis but at the same time stimulates the activity of hydrolytic enzymes.

14.2.5 Ethylene

It is a hormone which is represented by a gaseous hydrocarbon. It is synthesized primarily in response to stress and is produced in high amounts by tissues undergoing senescence or fruit ripening. It is commonly used to induce ripening in bananas. It occurs in all plant organs-roots, stems, leaves, bulbs, fruits seeds, though the rate of production varies in each organ.

The effect of ethylene on plants was described by **Dimitry Nikolayevich Neljubow** in 1886. He found abnormal growth of dark grown pea seedlings due to ethylene emanating from illuminating gas. Later studies reported the role of this hormone in ripening of fruits. **H.H. Cousins** reported a volatile substance to be responsible for fruit ripening in plants. He discovered that ripe oranges released a volatile product that accelerates ripening of bananas.

Synthesis of ethylene

M. Lieberman and **L.W. Mapson** in 1964 demonstrated that methionine gets converted to ethylene non-enzymatically. **D. Adams** and **F. Yang** (1979) showed that S-adenosylmethionine (SAM) is the intermediate compound formed during synthesis of ethylene from methionine. Under aerobic conditions, 1-aminocyclopropane-1-carboxylic acid (ACC) gets converted to ethylene. The enzyme *ACC synthase* catalyzes the oxidation of ACC to ethylene (Fig. 14.11).

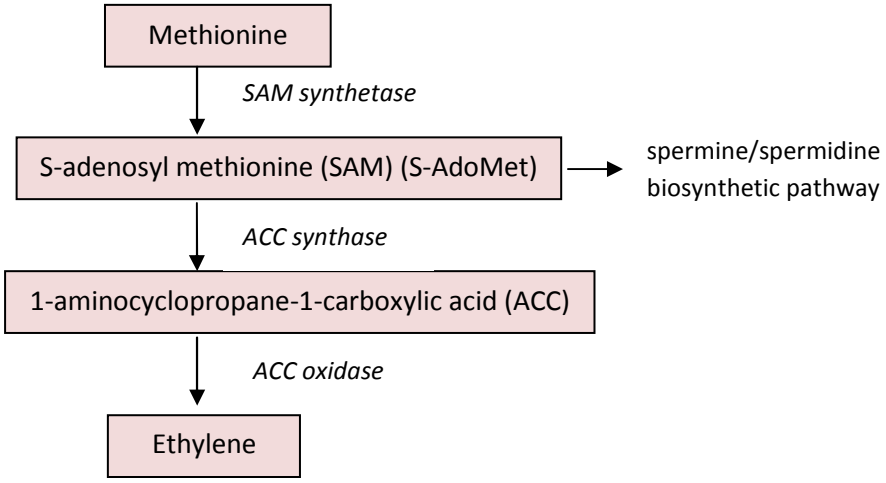

Fig. 14.11: Ethylene biosynthesis in plants.

The cleavage of SAM to give rise to 5 methylthioadenosine (MTA) and ACC is mediated by enzyme *ACC synthase* and is the rate limiting step in ethylene biosynthesis. Ethylene production is promoted by factors such as IAA, wounding and water stress. These factors induce the synthesis of enzyme *ACC synthase*. Control of the ethylene production is supposed to be regulated primarily through transcription regulation of the *ACC synthase* gene.

Functions

Ethylene produces many effects on growth of plants.

- It mainly promotes fruit ripening and senescence. Application of ethylene promotes climacteric effect and induces ripening. The role of ethylene in fruit development has been studied in tomato, a climacteric fruit. When the fruit matures, chemical and physiological changes begin which help in ripening of fruit. It involves a significant increase in the rate of respiration followed by a decline. This period is referred as **climacteric rise** and it signals the onset of degeneration or senescence. During climacteric phase, the concentration of ethylene increases several fold. The complex carbohydrates break into simple sugars, cell walls become soft due to action of hydrolytic enzymes (cellulase and pectinase). Chlorophyll degradation occurs and volatile compounds for the flavour and aroma are produced. This has been noted in fruits such as bananas, lemons, oranges.

- Generally a compound 2-chloroethanephosphonic acid (CEPA) is sprayed on the plant to induce the effect.

  The ethylene production is followed by expression of set of genes that enhance ripening related activities such as development of fruit colour, flavour and texture. In mutant called as *never ripe,* ethylene is non-

responsive because of defective ethylene receptor protein. In this plant ripening genes are not expressed and fruit never develops red color and has no flavor and texture as of a ripe tomato.

- The hormone has been shown to stimulate elongation of stems, petioles, root and floral structures in aquatic and semi-aquatic plants. The submergence reduces gas dispersion and maintains high ethylene levels.
- Ethylene stimulates many inhibitory and abnormal growth responses such as swelling of stem tissues and downward curvature of leaves called **epinasty**. Epinasty occurs due to excessive elongation on adaxial side of petiole. It is a common response of plants to anoxia under conditions of water logging. The vertical orientation of epinastic leaves reduces the absorption of sunlight and transpirational water loss.
- Ethylene also plays a role in seed germination, apical dominance, fruit ripening, cell death and pathogen responses.
- It stimulates the initiation of root and regulates the formation of root nodules in legumes. It inhibits the formation of bulbs, tubers in many flowering plants. The hormone induces several genes related to the ethylene response factor (ERF).
- Auxin induced ethylene formation Inhibition of stem elongation and swelling in the cells. Ethylene inhibits transport of auxin.
- One of the effects of the ethylene is called '*triple response'*. It is noted in etiolated (grown in dark) dicot seedlings. The response is characterized by inhibition of hypocotyl and root cell elongation. The response may be produced due to ethylene mutation. In presence of endogenous ethylene, the triple response is not seen.

SAQ 4

a) Cytokinins play a role in cell division. Explain.

b) State whether the following statements are True and False:

i) P.F. Wareing proposed the term 'dormin'.

ii) Abscisic acid delays senescence in leaves.

iii) ABA does not play any role in maturation of embryo.

iv) ABA prevents vivipary in fruits.

v) Ethylene is a gaseous hydrocarbon.

c) Fill in the blanks:

i) ……. mainly promotes fruit ripening.

ii) Ethylene is responsible for……..…… in leaves.

iii) During climacteric phase the concentration of ethylene ……. several folds.

iv) Ethylene also regulates …..……..formation in legumes.

v) Ethylene inhibits transport of ……..…… .

14.3 NEW CLASS OF PLANT HORMONES

Apart from the five hormones which we have described above, there are several newly discovered hormones. We have discussed these hormones in coming section. Some of the important ones are brassinosteroids, salicyclic acid, strigolactones and jasmonic acid. Many new naturally occurring substances in this category are still being discovered.

Brassinosteroids

These are steroid hormones. They were discovered in 1970. A group of agricultural researchers showed that pollen possess a rich source of growth promoting substances. These are a complex mixture of lipids and have shown to capacity to stimulate elongation of second internodes in bean. The active substances were isolated from rape plant i.e. *Brassica napus* and called as **Brassins**. In 1979, the active component was identified as brassinolide (BL). More than 40 analogs of BL have been isolated from 60 plant species and from tissues such as pollen, seeds, leaves, stem, roots and flowers. The hormone is active even at very low concentrations (micromolar). It has been suggested that responses of brassionosteroids (BR) are controlled by auxins.

Brassinosteroids play a role in various developmental activities in plants. These include:

- stem growth
- pollen tube elongation
- increase in rate of cell division
- seed germination
- leaf morphogenesis
- apical dominance
- inhibition of root elongation
- vascular differentiation
- acceleration in senescence and cell death.
- increase in extensibility of the cell wall by regulating the genes encoding enzymes such as *expansins* and *cellulose synthase*.
- mediate responses to both abiotic and biotic stresses including salt, drought, temperature and pathogens.

Salicyclic acid

Salicylic acid (SA or 2-hydroxybenzoic acid) is the phenolic compound synthesized by plants. It plays direct and indirect roles in regulating many aspects of plant growth and development such as thermogenesis and disease resistance.

The first evidence that SA is a plant hormone came from studies of voodoo lily (*Sauromatum guttatum*). SA initiates thermogenesis in lily. The thermogenic response was initiated by calorigen which was later characterized as SA.

Thermogenesis is a process that generates heat by uncoupled electron flow through *alternative oxidase* pathway in mitochondria. The levels of SA increase several fold (~100) prior to such event. Externally applied SA also induced thermogenesis. The hormone induces thermogenesis primarily by stimulating the mitochondrial alternative respiratory pathway. This pathway generates one ATP and releases the energy from electron flow as heat. SA treatment also induces the expression of *alternative oxidase* and/or the alternative respiratory pathway in non-thermogenic plant species.

Functions

- The hormone plays a role in defense signaling. Earlier studies established that application of SA to tobacco plants induces defense gene expression and enhances resistance to viral infection. It plays a role in disease resistance. It prevents local pathogen infection but mediates Systemic Acquired Resistance (SAR) at the sites far from the site of infection. Rise in levels of SA were detected prior to the development of local and/or systemic disease resistance in tobacco and cucumber.
- SA also acts as a signal for flowering. The SA-deficient mutants of *Arabidopsis* showed delay in flowering.
- Exogenous SA has been shown to affect resistance to biotic (pathogen-associated) stress and tolerance to many abiotic stresses (drought, chilling, heat, heavy metal, UV radiation, and salinity/osmotic stress).
- The hormone affects many aspects of plant growth and development including seed germination, vegetative growth, flowering, fruit yield, senescence, stomatal closure, root initiation/growth, photosynthesis, respiration, glycolysis, Krebs cycle, and the alternative respiratory pathway. Some of these processes are induced by SA in a concentration-dependent manner. They get activated by treatment with a low dose of SA but inhibited by a high dose.
- SA retards senescence of petals by lowering the ethylene levels.
- SA plays a role in regulating cellular redox status. Low concentrations of SA induce accumulation of ROS which serve as secondary signals to activate biological processes. In contrast high concentrations of exogenous SA result in accumulation of ROS at high levels leading to oxidative stress and cell death.

Strigolactones

Strigolactones (SLs) are the new group of hormones discovered in plants. Studies have shown that plants can transport strigolactones internally. Recent studies suggested that they play a major part in the interaction between plants and their environment.

Low concentrations of strigolactones prove very effective in altering the growth and development in plants. These hormones have been found to play a crucial role in branching of plants. They play an important role in interaction

between plants and symbiotic fungi. The fungi live in a symbiotic relationship with plants and this interaction is mutually beneficial. Fungi transports minerals from the soil to the plant and in return the plant gives sugars to the fungi. SLs released by roots of host plants serve to attract arbuscular mycorrhiza to the root surface and stimulate branching of fungal hyphae and facilitate the phosphate uptake from soil. The hormones assist the seeds of parasitic plants to germinate. The seedlings of the parasite attach to the root of the plant and use the plant's nutrients for their own growth and reproduction. The mutant plants showed low seed germination in parasitic plant.

Jasmonic acid

The jasmonates (JAs) including jasmonic acid and its derivatives are plant hormones. They control plant defenses against herbivore attack and pathogen infection, provide tolerance to abiotic stresses (ozone, ultraviolet radiation, high temperatures, freezing), regulate various aspects of development, including root growth, stamen development, flowering, and leaf senescence.

Exogenous application of JAs inhibits various aspects of seedling growth, including primary root growth, leaf expansion, and hypocotyl elongation. They also repress leaf expansion by inhibiting the activity of the mitotic cyclin CycB1,2 and cell division.

Nitric oxide

Nitric oxide (NO) is a reactive gas known to be produced by plants. It has been synthesized during nitrate assimilation but under conditions of low oxygen availability, the nitric oxide is produced by reduction of nitrite. It can also be produced non-enzymatically from nitrite in acidic condition found in plant cell walls.

The main role of NO in plants include regulation of stomatal closure, lateral root initiation, flowering, seed germination, responses to abiotic stresses and plant defense against pathogen attack by stimulating production of H_2O_2.

$$NO_2^- + 2e^- + 2H^+ \longrightarrow NO + H_2O$$

$$NO_2^- + H^+ \longrightarrow HNO_2 \longrightarrow NO + H_2O + 2R \text{ (biological reductant)}$$

Karrikins

These are plant growth regulators produced from burning plant material. Some karrikins might be produced by processes such as chemical or microbial degradation of vegetation in response to disturbance of the soil or removal of the plant canopy. Karrikins comprise C, H, O and belong to the class of butenolide compounds. They contain two ring structures, one of which is a pyran and the other one is a lactone comprising of five membered ring known as butenolide.

Karrikins are potent in breaking dormancy of seeds of many species adapted to environments that regularly experience fire and smoke. They trigger seed germination and control seedling growth in taxa that would rarely experience fire. Endogenous karrikins present in plants control of seed germination.

SAQ 5

a) Make a list of new plant hormones that play a role in plant development and growth.

b) List the developmental activities in which brassinosteroids have a role to play.

c) Write a short note on Karrikins.

14.4 SYNTHETIC GROWTH HORMONES

Synthetic auxins have commercial importance. Some of the important synthetic auxins include indole-3-acetic acid (IAA), indole-butric acid (IBA), beta naphthoxy acetic acid (NOA), 1-naphthaleneacetic acid (NAA), (2,4-dichlorophenoxy) propionic acid (2,4-D). Examples of some commonly used synthetic cytokinins are 6-benzylaminopurine (BAP) and 6-furfurylaminopurine or kinetin.

Synthetic auxins such as 2, 4 -D and and 4-chlorophenoxy acetic acid (4-CPA) are used as herbicides. Synthetic auxins are highly effective because they are not metabolized by the plant as quickly as IAA. They are retained in shoots for long time. They induce excessive cell expansion and subsequent cell death. Indole butyric acid and naphthalene acetic acid are used in vegetative propagation (through stem and leaf cuttings) and rooting.

4-CPA is sprayed on tomatoes to increase flowering and fruit set while NAA is used to induce flowering in pineapple. This happens due to auxin induced ethylene production.

NAA is also used to prevent fruit drop in pear and apples. Spraying in early stages of fruiting enhances abscission of young fruits while spraying after fruit maturation prevents premature fruit drop and keeps fruit attached to the tree till maturity.

14.4 SUMMARY

- Hormones are the chemical messengers produced in one cell and modulate the cellular processes in another cell by interacting with specific proteins that function as receptors. Major hormones that regulate growth and developmental activities in plants include auxins, gibberellins, cytokinins, ethylene, and abscisic acid.
- Auxin was the first growth hormone discovered in plants. It plays an important role in enlargement of plant cells. It is synthesized in the meristematic region and other actively growing regions. Auxin synthesized in shoot apex gets transported basipetally to the tissues below. The hormone stimulates cell elongation in stem, coleoptiles and promotes vascular differentiation in shoots. High levels of auxin promote the formation of lateral and adventitious roots.

- Gibberellins are diterpene compounds synthesized mainly in the developing seeds, fruits, young leaves and apical region of roots. They promote seed germination. They initiate the mobilization of nutrient reserves stored in the endosperm in cereal grains. They possess the capacity to stimulate elongation of stem.
- Cytokinins are the derivatives of adenine with isoprene related side chain or aromatic side chain. The regulate cell division. They have the capacity to initiate cell division in quiescent, non-dividing cells and have been considered as the inhibitors of senescence. Exogenous application of cytokinins delays onset of senescence, maintain protein levels and prevents chlorophyll breakdown in detached leaves.
- Abscisic Acid (ABA) is a growth regulator synthesised from cleavage of carotenoid such as β carotene to produce 40 carbon terpene violaxanthin. ABA plays a role in maturation of embryo and seed germination. It prevents precocious germination. It plays a role in closure of stomata during water stress and acts as an antitranspirant.
- Ethylene is a hormone which is represented by a gaseous hydrocarbon. It is synthesized primarily in response to stress and is produced in high amounts by tissues undergoing senescence and ripening. It is commonly used to induce ripening in fruits and senescence.
- Some new hormones have been discovered recently in plants. They include brassinosteroids which play a role in pollen tube elongation, leaf morphogenesis and apical dominance. They also mediate responses to both abiotic and biotic stresses. Salicylic acid (SA) is the phenolic compound synthesized by plants. It plays direct or indirect roles in regulating many aspects of plant growth and development such as thermogenesis and disease resistance. Strigolactones have been found crucial for the branching of plants and play a major role in interaction between plants and symbiotic fungi.

14.5 TERMINAL QUESTIONS

1. Fill in the blanks.
   a) Transport of auxin in plants is …….. .
   b) The most commonly found form of gibberellin ……. .
   c) The precursor of ethylene in plants is…… .
   d) The steroid hormone found in plants is ……. .
   e) The precursor of abscisic acid in plants is …… .
   f) ……. hormone plays a role in opening and closing of stomata.
2. Enlist the major role that auxins play in plants.
3. How do gibberellins promote seed germination?
4. What is epinasty? It is linked with which hormone in plants.

5. Give the full form of abbreviations.
   a) IAA
   b) IBA
   c) tZ
   d) iP
   e) ACC
   f) SA
6. Discuss in brief :
   a) Auxin and cell elongation
   b) Ethylene and ripening of the fruit
   c) Discovery of auxins
   d) Discuss functions of Gibberellins.
7. Describe the effect of Auxins, Gibberellins and Kinins in a tabular form.

14.6 ANSWERS

Self-Assessment Questions

1. a) i) True; ii) False; iii) True; iv) True; v) False; vi) True
   b) i) F.W. Went
      ii) Tryptophan
      iii) Basipetal
      iv) Antigibberellins
2. a) coleoptiles
   b) Indole -3-acetic acid (IAA)
   c) stem, coleoptiles
   d) efflux, acidity
   e) pollination, fertilization
   f) elongation
3. a) Kinetin
   b) levels, cytokinins
   c) high
   d) apical dominance
   e) breaking

4. a) Cytokinins regulate cell division or cell cycle. In the absence of gibberellins, cells division gets arrested in G1 or G2 phase of the cell cycle. The cells showing arrested division in G2 phase do not possess cyclin –dependent kinases (CDK) activity. The activity reduced due to high level of phosphorylation on the tyrosine residue. In presence of cytokinin, tyrosine gets dephosphorylated, enzyme gets activated and cell division is resumed. Thus cytokinin also plays a role in generation of active CDK complex that initiates cell division by catalyzing the transition from the G2 phase of mitosis or M phase in cultured cells. Cytokinins are also known to possess high level of D-type cyclin (CycD3) which controls the transition from G1 to S phase. Cytokinins initiate the cell division at the G1 to s transition through the induction of CycD3.

   b) i) True; ii) False; iii) False; iv) True; vi) True

   c) i) Ethylene

      ii) epinasty

      iii) increases

      iv) nodules

      v) auxins

5. a) Few newly discovered hormones that play a role in plant growth and development are

      - brassinosteroids
      - salicyclic acid
      - strigolactones
      - jasmonic acid
      - nitric oxide

   b) Refer to Section 14.3.

   c) Refer to Section 14.3.

Terminal Questions

1. a) polar

   b) GA_3

   c) methionine

   d) brassinosteroid

   e) β carotene

   f) abscisic acid

2. The major roles of auxins in plants are

   - stimulation in cell elongation in stem and coleoptiles.
   - secondary root initiation, vascular differentiation, development of axillary buds, flowers and fruits.
   - vascular differentiation in shoots. The production of xylem strands depends upon the amount of IAA moving through the petiole. The extent of vascular regeneration is directly related to the supply of auxin. The differentiation of phloem sieve elements is favored by low concentration of auxin (0.1%) while the xylem differentiation is favored by high auxin content (1%). In culture conditions, the callus differentiates into vascular tissue in the presence of auxin.
   - formation of lateral and adventitious roots.
   - delays onset of leaf abscission (shedding). Application of auxin to the cut surface of the petiole prevents the formation of the abscission layer.
   - promote fruit development.

3. Gibberellins promote seed germination. The gibberellins are released by the hydrated embryos. Gibberellins initiate the mobilization of nutrient reserves stored in the endosperm. During germination the cells in the aleurone secrete hydrolytic enzymes such as α amylase and proteases. These enzymes are involved in the hydrolysis of carbohydrate and proteins (stored food reserves) accumulated in the endosperm or embryo. The degradation of carbohydrates and storage proteins provide nourishment and energy to the growing seedling. This happens in cereal grains such as rye, barley, wheat which have a protein rich layer of cells called aleurone surrounding the starchy endosperm. The starch and proteins produced provide nourishment to the developing embryo.

4. Ethylene stimulates growth responses such as swelling of stem tissues and downward curvature of leaves called epinasty. Epinasty occurs due to excessive elongation on adaxial side of petiole. This response occurs in response to anoxia under conditions of water logging. The vertical orientation of epinastic leaves reduces the absorption of sunlight and transpirational water loss.

5. a) IAA - Indole -3-acetic acid

   b) IBA - Indole-3-butyric acid

   c) tZ - trans-zeatin

   d) iP - isopentenyladenine

   e) ACC -1-aminocyclopropane 1-carboxylic acid

   f) SA - Salicylic acid

6 a) Refer to Section 14.2.1.

   b) Refer to Section 14.2.5.

   c) Refer to Section 14.2.1.

   d) Refer to Section 14.2.2.

7.

Auxins	Gibberellins	Cytokinins
Synthesised in the root tip	Synthesised in root apex, elongating shoots, young leaves	Synthesised in the root tip
Transport is basipetal	Transport is both basipetal and acropetal.	Transport is acropetal
Promote branching of roots	It has no role in rooting	Inhibit root branching
Has no role in flowering	Promote flowering in long day plants	Promote flowering in short day plants
Has no role in seed germination	Plays a role in seed germination	Has no role in seed germination
Brings about apical dominance	Has no role in apical dominance.	Has no role in apical dominance.
Inhibits growth of lateral buds	Does not affect growth of lateral buds	Promotes growth of lateral buds
Promotes for cell elongation	Does not play role in cell elongation or division	Mainly responsible for cell division and differentiation
Play no role in bolting	Help in bolting of rosette plants	Does not play any role in bolting
Does not play any role in growth of shoot segments	Promotes growth of shoot segments	Does not play any role in growth of shoot segments
Essential for growth of callus	It has no role in callus growth.	It has no role in callus growth.
Does not help in breaking seed and bud dormancy	Help in breaking seed and bud dormancy	Does not play any role in seed and bud dormancy
Involved in the vascular differentiation in shoots	Play no role in vascular differentiation in shoots	Play no role in vascular differentiation in shoots
Delays the onset of leaf abscission	Does not play any role in leaf abscission	Does not play any role in leaf abscission
Promotes development of fruit	Does not play any role in development of fruit	Does not play any role in development of fruit
Does not play any role in formation of leaves	Increase the size of leaves in plants	Does not play any role in formation of leaves

UNIT 15

PLANT RESPONSE TO LIGHT AND TEMPERATURE

Structure

15.1 INTRODUCTION

The developmental phases of a flowering plant involve seed germination, vegetative growth, flowering, fruiting, and senescence. These phases are precisely timed and are under strict internal and external controls. You must have noticed that a seed remains dormant all winter long buried in the sand, suddenly germinates, develops into a mature plant and flowers in the spring. A short rainfall in desert brings life to many plants and the dormant winter buds become active in the spring. What external or internal factors initiate germination which involves resumption of all the metabolic activities? How does a seed sense that all the environmental conditions are favourable for its germination and growth and how do buds know that the winter is over?

You also know that most fruits, vegetables, and flowers are seasonal. *Chrysanthemums* bloom in winters while Gulmohar and Amaltas bloom in the summer. Why do some plants bloom in the spring and others wait for summer, fall or winter? Many flowers open and close on a fixed schedule as if a clock is present in their cells. Now it would be interesting to find out whether it is possible to grow *Chrysanthemums* throughout the year or make Morning glories bloom at dusk? You can ask many such questions regarding development of a plant.

In the previous unit you have learnt that hormones coordinate growth and development in different parts of an organism by triggering cellular reactions in the target cells. In this unit we will discuss the phases of plant development that are under the control of specific environmental signals. You will see that plant cells receive environmental signals which governs various developmental events. The signals are received by some special molecules and the genes are turned on at a precise time to regulate specific activity.

Objectives

After studying this unit, you should be able to:

- describe seed formation and seed germination and the factors that control these processes;
- list the factors that are responsible for flower induction and discuss the nature of the receptor that perceives external stimuli;
- explain the role of phytochrome in controlling the period of light and dark cycles in flowering;
- discuss the role of phytochrome in regulating plant development;
- list the various factors that affect onset of senescence;
- describe the principle and uses of tissue culture technology; and
- Give example of circadian rhythms regulated by biological clocks in plants.

15.2 PHOTOPERIODISM

One of the major changes that occur during the life cycle of a plant is the transition from vegetative stage to the reproductive stage. In this transition the vegetative meristems change into flowering meristems which form sepals, petals, carpels, and anthers instead of branches and leaves. It is a major morphogenetic change. In some plants this change occurs once in lifetime and after flowering, fruit and seed set, the plant dies. Whereas, in others, the flowers are produced every year, as in trees. Now the question arises, is it due to an internal annual rhythm in the plants or a requirement for environmental conditions? In this section we will discuss what controls flowering and how this transition occurs.

One of the major factors that affects flowering, as we know today is related to the effects of the environment and to the duration of light/dark 24 hours cycle or temperature. However, it took several years for scientists to realize this fact.

We will describe two experimental observations made by **Garner and Allard** (1920) which suggested that the length of the day determines the behaviour of plants with respect to flowering.

The phenomenon of photoperiodism was discovered by two American scientists, **Garner and Allard** in 1920, working at the U.S. Department of Agriculture at Beltsville, Maryland.

In temperate regions the day length is more during summer.

The seeds of soyabean (*Glycine max*) were planted at different times in the month of May, June, and August. Even though planted at different times, all of them flowered in the month of September. The first planted seeds took 125 days to flower whereas the last set flowered in only 58 days. These observations show that at a particular time of the year the day length was suitable for plants to flower. The second observation was made on a mutant of tobacco called *Maryland mammoth*, because of the large size of its leaves. In fields in winter, however, in glass houses when light duration was provided like that of summer, it was possible to induce flowering even in winter in these plants. The term **photoperiodism** was suggested by Garner and Allard to categorize plants based on their response to relative length of day and night as manifested by their flowering.

15.2.1 Long day, Short Day and Day Neutral Plants

Based on their requirement for day length (number of hours of light) for flowering, plants were grouped under three major categories, 'Short-day'(SDP), 'Long-day'(LDP) and 'Day-neutral' plants. (Fig. 15.1)A SDP requires more than a critical period of darkness. In this case SDP needed more than 8 hours of darkness or less than 16 hours of light to flower. A LDP requires less than a critical hour of darkness or more than a certain hour of light. In this case, LDP required more than 10 hours of light to flower.

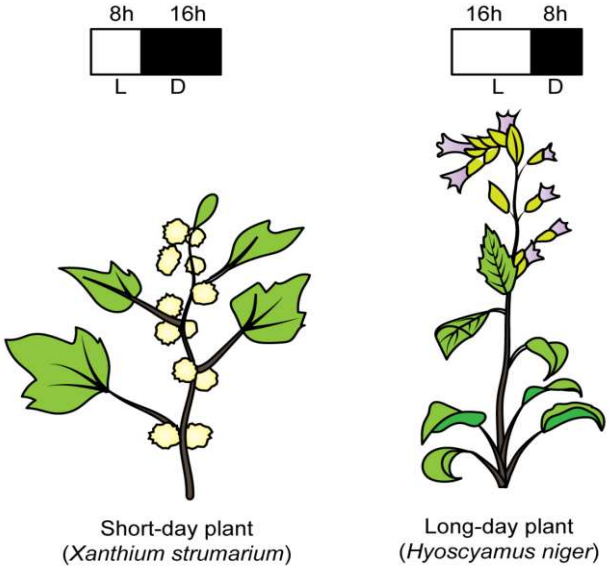

Fig. 15.1: A diagrammatic representation to show the difference between a short-day plant (SDP) and a long-day plant (LDP).

1. **Short-Day plants (SDP):** These plants only flower or flower more profusely and rapidly when given less than a certain (critical) number of hours of light in a day (24 hours cycle). The ***critical period***, let it be clear, is not 12 hours. It can be for example 10 hours. In that case a plant given less than 10 hours of light (say 8 hours light + 16 hours of darkness) will flower. And this critical period is determined experimentally and varies from plant to plant. Short-day plants are found in tropical regions where day length varies comparatively little during the year. If the short-day plants are to flower, the day length must not exceed a critical value. The photoperiodic requirement of some plants is

controlled by temperature. Some of them have a qualitative photoperiod requirement at one temperature, while at another temperature; their requirement for photoperiod becomes quantitative.

Thus, the short-day plants can be further sub grouped into:

i) ***Obligate short-day plants*****:** Such plants have an absolute requirement for long nights and short days. They are also called obligates. Any other condition given to them inhibits flowering. They are also called **Qualitative short-day plants**.e.g., *Amaranthus candatus, Chenopodium album* (pigweed) (Fig. 15.2a), *Coffea arabica* (Coffee), *Euphorbia pulcherimma, Frageria* (Strawberry), *Glycine max var. Biloxi* (Soyabean)(Fig.15.2b), *Ipomoea hederacea* (Morning glory), *Lemna perpusilla* (Duckweed), *Nicotiana tobacum* Var. Maryland mammoth (Tobacco), *Xanthium strumarium* (Cocklebur).

(a) **(b)**

Fig.15.2: Obligate short-day plants: a) *Chenopodium album*; and b) *Glycine max var. Biloxi.*

ii) ***Facultative short day plants*****:** are those whose flowering gets accelerated by short days. Such plants are also known as **Quantitative short-day plants**. e.g., *Saccharum officinarum* (Sugarcane), *Gossypium hirsutum*(Cotton) are some of the examples (Fig. 15.3).

Interestingly, *Allium cepa* (Onion) and *Chrysanthemum morifolium* are quantitative SDPs which flower better by low temperature pretreatment. There are many of the above-mentioned plants that show a transition between different photoperiods with varying temperature.

(a) **(b)**

Fig.15.3: Facultative short-day plants, a) *Saccharum officinarum*; and b) *Gossypium hirsutum*.

iii) ***Short-long day plants (SLDP*)**: These plants flower only when a sequence of short days is followed by an exposure to long days (e.g., June-July). e.g., *Campenula medium, Trifolium repens* (Clover) (Fig. 15.4).

(a) **(b)**

Fig.15.4: Short-long day plants a) *Campenula medium*; b) *Trifolium repens*.

2. Long-Day plants (LDP): The definition of this is exactly opposite to short-day plant. That is those plants which flower when given more than a certain (critical) number of hours of light (day length). These plants are found in temperate region where they flower during summer.

The LDPs are also of three types:

i) ***Obligate/Qualitative long day plants*:** which must necessarily have long days and short nights as their absolute requirement to flower? The day length must always exceed a certain critical photoperiod. They could even flower even when there is continuous light. e.g., *Hyoscyamus niger*(Henbane), *Spinacea oleracea* (Spinach) (Fig. 15.5), *Lolium temulentum* (Ryegrass), *Avena sativa* (Oat) and more.

(a) **(b)**

Fig.15.5: Obligate/Qualitative long day plants: a) *Hyoscyamus niger* (Henbane); b) *Spinacea oleracea* (Spinach).

ii) ***Facultative/Quantitative Long Day Plants*:** accelerate their flowering in long days e.g., *Brassica rapa* (Turnip), *Beta vulgaris* (Beet), *Lactuca sativa* (Lettuce), *Pisum sativum* (Garden pea), *Triticum aestivum* (Spring wheat), *Secale cereale* (Spring rye),*Petunia hybrid* (Fig. 15.6), *Hordeum vulgare* (Spring barley).

(a) (b)

Fig.15.6: Facultative/Quantitative Long Day Plants: a) *Secale cereale*; b) *Petunia hybrid*.

iii) ***Long-Short day plants (LSDP)*:** These long day plants will flower when they are exposed to long days prior to exposure to short days (i.e., September-October). For example, *Aloe bulbifera* (Aloe), *Cestrum nocturnum* (Fig. 15.7), and *Bryophyllum.*

(a) (b)

Fig.15.7: Long-Short day plants: a) *Aloe bulbifera*; b) *Cestrum nocturnum.*

3. **Day-Neutral plants/Photoneutrals /Indeterminate plants:** Besides SDP and LDP, those plants that flower irrespective of the length of light are called day-neutral plants. For such plants there is no specific requirement of light duration or temperature. They can flower in any day length. e.g., *Lycopersicon esculentum* (Tomato), *Mirabilis jalapa* (4O'Clock plant) (Fig. 15.8), *Oryza sativa* (Rice), *Zea mays* (Corn), *Pisum sativum* (Garden pea).

(a) (b)

Fig.15.8: Long-Short day plants: a) *Mirabilis jalapa*; b) *Lycopersicon esculentum*.

4. **Intermediate day length plants:** These plants flower only within a narrow range of long photoperiod i.e., 12 to 14 hours. Shorter or longer photoperiods than this will not result in flowering. e.g., *Coleus hybrida, Chenopodium album* (Fig. 15.9*)* and *Saccharum spontaneum.*

(a) (b)

Fig.15.9: Intermediate day length plants: a) *Coleus hybrida;* b) *Chenopodium album.*

5. **Ambiphotoperiodic plants:**These plants are inhibited by intermediate day lengths. They would flower in short days or long days. *Chenopodium rubrum* and *Setaria verticillata* (Fig. 15.10) belong to this category.

(a) (b)

Fig.15.10: Intermediate day length plants: a) *Chenopodium rubrum*; b) *Setaria verticillata*.

The day length requirements vary from species to species (Fig. 15.11). Even within a single species, different varieties of long-day plant may show a range of critical day length. It is reported that rice plants can detect changes in day length of only 15 minutes. Therefore, it seems that plants must have an extremely accurate time-measuring mechanism within the tissues of the plant. Flower induction in some plants can be stimulated by day length only if the plant has been previously chilled, for example daffodil and henbane. While some plants, for example, primrose flower solely in response to pre-chilling, otherwise they are day neutral (see Section 15.5).

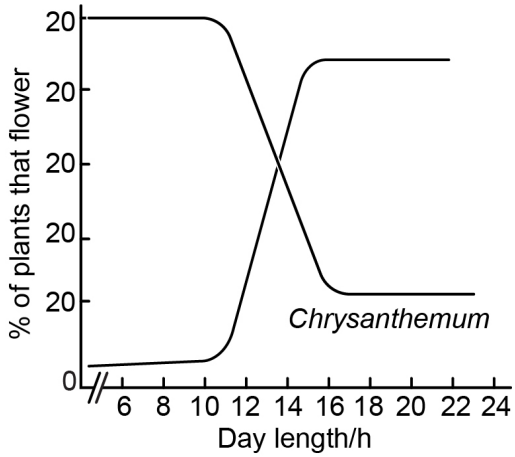

Fig. 15.11: Day length and percentage of flowering in *Chrysanthemum* and in spinach.

15.2.3 Importance of Dark Period

For quite some time the role of light period (photoperiod) was emphasized in flowering. However, based on certain experiments it was realized that it is the dark period that is more important than the light period for inducing a plant to flower. In fact, as early as 1912, from his experiments on flowering, **Julien Tournois** concluded that flowering occurred "not so much by shortening of day as by lengthening of nights". Much later **Hammer and Bonner** (1936) proved through their experiment that dark period is more important than the light period for inducing flowering in plants. They took a short-day plant, *Xanthium* required 16 hours of darkness and 8 hours of light to flower. If the dark period was reduced, plants did not flower. Interestingly, if the dark period of 16 hours was interrupted by light, again there was no flowering. However, if the light period was interrupted by dark period, or if plants were kept less than 8 hours in light there was no effect on flowering. It was clear that altering the light period had no effect but if the dark period were less than 16 hours, the plants would not flower. A look at Fig. 15.12 will further clarify this experiment and confirm that dark period is more important for inducing plants to flower. The role of dark period is to bring about some changes which trigger the development from the vegetative to the flowering state. The role of light period is to realize this change and help in bringing about maximum flowering.

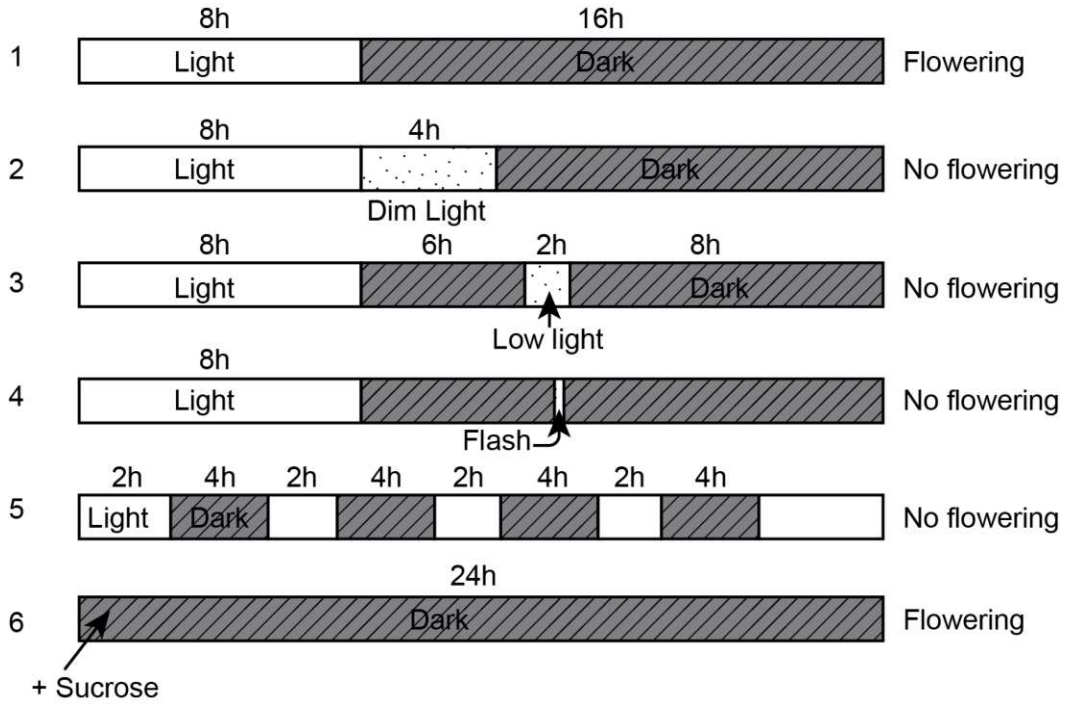

Fig. 15.12: Experiments to show that dark period is important for flowering. If dark period is interrupted by diffuse light (3) or even a flash of light (4) during the long dark period SDP, no flowering.

For short day plants (SDPs), it is the uninterrupted long period of darkness which is more important than the length of the day. Such plants may thus also be called **long-night plants**. On the other hand, the long day plants require either a relatively small period of darkness or no darkness at all, as is clear from Fig. 15.13 that continuous light exposure to them may result in the best flowering. Thus, such plants can also be called **short-night plants**.

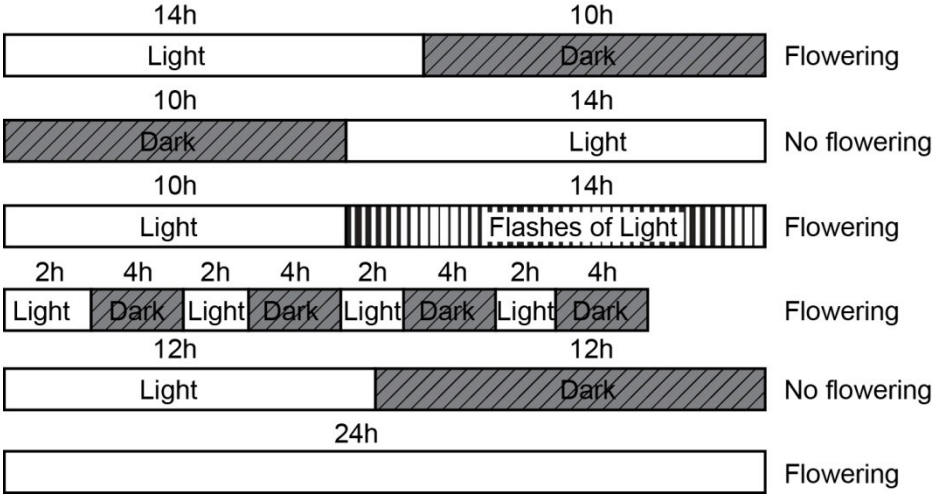

Fig. 15.13: Long day plants are in fact short night plants.

15.2.3 Site of Perception of Light Stimulus

It has been recognized since long that the photoperiodic stimulus is first perceived by leaves of a plant. Experiments on *Chrysanthemum* plants by the Russian physiologist **Chailackhyan** demonstrated that even 1/8 of a mature leaf, when exposed to light, was sufficient to induce flowering. Later work by **Borthwick** and **Hendricks** in the 1950s at the USDA established that the quality of light exposure was also important in the induction of flowering Both SDPs and LDPs were sensitive to red light in the region of 580 nm - 680 nm in causing night break. On the other hand, wavelengths in the far-red region (730 nm) had the ability to *reverse* the red light induced inhibition.

Photomorphogenesis is light-mediated development, where plant growth patterns respond to the light spectrum. Light induces various changes in gene-expression seed germination, seedling development, and the switch from the vegetative to the flowering stage. Phytochromes, cryptoc hromes and phototropins are photochromic sensory receptors that operate in the different portions of the electromagnetic spectrum to show the morphogenetic responses.

Plants follow a different developmental program in dark with altered growth patterns. These changes are studied under **skotomorphogenesis**. A seedling may switch over to either of the modes depending on the availability of light.

SAQ 1

a) Fill in the blank spaces in the following statements.

i) The major morphogenetic change in flowering is the change of …… meristems into…….meristems.

ii) Photoperiodic control of flowering depends on the length of uninterrupted……period given.

iii) …….. plant is likely to flower at the same time of the year whether grown in the house, field, or green house.

iv) A short-day plant requires more than a critical period of……

b) State whether the following statements are true (T) or false (F)?

i) The day neutral plants can flower irrespective of day length but require a specific temperature to do so.

ii) Ambiphotoperiodic plants are inhibited by intermediate day lengths.

iii) Even a single young leaf when exposed to light is enough to induce flowering.

iv) An SDP will flower if the light given to it is less than the critical period.

v) *Coffea arabica* is an example of an obligate short-day plant.

15.3 PHYTOCHROME

You know that plants capture light energy during photosynthesis, now you are familiarized with another important and interesting role of light in developmental phenomena. Just as for photosynthesis light is absorbed by pigments chlorophylls and carotenoids, similarly, to bring about developmental response light must be absorbed by some pigments. One of the pigments that detects the quality of light in the range of 600-800 nm region is **phytochrome**. It may also be mentioned that some developmental changes do exist even in total darkness. These processes would be independent of active form of phytochrome. Such changes are called **skotomorphogenetic** as against **photo morphogenetic** changes that occur in light. There also exists an unidentified blue light absorbing pigment which brings about phototropism and nastic movements.

15.3.1 Discovery

We have earlier described the experiment that proved the importance of the dark period in flowering. In that experiment if dark period was interrupted with light, the flowering was not induced in a short-day plant. In further experiments instead of white light, dark period was interrupted by lights of various wavelengths like red, blue, yellow, and far-red light. These experiments showed that red light alone was sufficient to inhibit flowering. However, the effect of red light was not always inhibitory. For different developmental response, its effect was different. For example, and experiment done to check effect of red light on lettuce seed germination, it was found to have stimulatory effect. Another interesting observation made by **Borthwick**, **Hendricks** and **Parker** (1952) was that any response to red light was nullified by far-red light. The results for percentage germination of lettuce seed under different irradiation conditions are shown in Table 15.1.

Table 15.1: The germination (%) of moist lettuce seed irradiated with Red and FR (far-red) (After Borthwick et al. 1952)

Sequence of light exposure / Irradiation	Final Irradiation	Germination (%)
Control (Dark)		8.5
R (660 nm) 1 min.	R	98
R (1 min) + FR (730 nm) 4 min.	FR	54
R + FR + R	R	100
R + FR + R + FR	FR	43
R + FR + R + FR + R	R	99
R + FR + R + FR + R + FR	FR	54
R + FR + R + FR + R + FR + R	R	98

From several such experiments, it was concluded that some developmental response brought about by red light could be reversed if followed by far-red light and vice versa. Based on these physiological experiments, it was proposed that both red and far-red lights must be absorbed by the same pigment. This pigment was discovered by **Hendricks and Borthwick** at USDA-ARS during a period from 1940 to early1960s.

Phytochrome, thought to be present on the plasma membrane, exists in two forms: a red-light absorbing form (***Pr***), which after absorbing red light gets converted to far-red light absorbing form (***Pfr***). When *Pfr* form absorbs far-red light, it gets converted back to *Pr* form as shown in Fig. 15.14. So, the two forms are interconvertible.

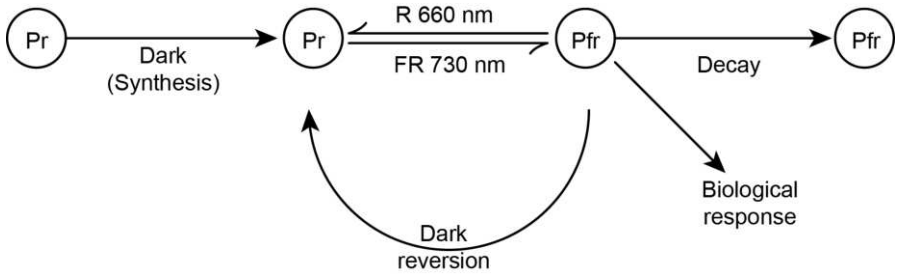

Fig. 15.14: Schematic representation of photoconversion of phytochrome. Phytochrome is synthesized in dark. On receiving light in red range (*R*) it is converted from *Pr* (red light absorbing form) to *Pfr* (far-red light absorbing form). The *Pfr* form is biologically active and induces a response. It also undergoes decay (*Pfr*) i.e., it degrades with the passage of time. When plants are left in dark for long, *Pfr* form in some cases can also return to *Pr* state. It also can be brought back to *Pr* state by irradiation with far-red light.

15.3.2 Properties of Phytochrome

The experiments on reversible effect of red and far-red light to study developmental responses were done by Borthwick and Hendricks at the same place where Garner and Allard had discovered the phenomenon of photoperiodism.

Phytochrome is a chromoprotein: this means it is composed of a protein and a chromophore. The chromophore is attached to the protein and it responsible for its color and light absorption. It is possible to isolate phytochrome in pure form by following the procedures of protein purification. It absorbs red light (maximum absorption occurs at 660 nm wavelength) and on conversion, far-red light (maximum absorption occurs in far-red light region at 730 nm). A typical absorption spectrum of phytochrome is shown in Fig. 15.15. It is a soluble protein and is present in the cytoplasm. It is believed that ***Pfr*** form may be associated with the membranes. The intact phytochrome protein has a molecular weight of 124,000 Daltons. The total amino acid sequence of the protein is also known and, using recombinant DNA technology, the gene coding for phytochrome has been isolated.

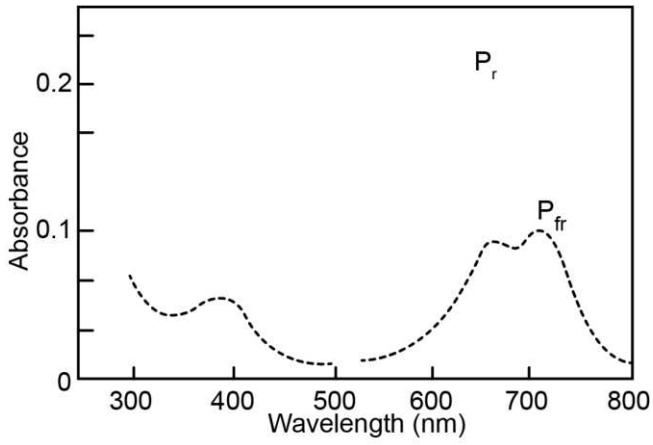

Fig. 15.15: Absorption spectrum of phytochrome *in vitro.* Phytochrome was purified and its absorption spectrum was measured by a spectrophotometer. When *Pr* was irradiated with red light, the absorption spectrum (dotted line) was obtained. This shows the conversion of *Pr* to *Pfr*. Note that not all *Pr* can be converted to *Pfr* form and one sees two peaks.

The chromatophore is a tetrapyrrole molecule (Fig. 15.16) like chlorophyll, but unlike chlorophyll it is an open tetrapyrrole and contains no metal ion. It is covalently attached to the protein. It has been shown that it is *Pfr* form of phytochrome that is biologically active. Changes in the structure occur during conversion of *Pr* to *Pfr* which probably make the *Pfr* form active.

Proteins cannot absorb visible light. They absorb only UV-light. So chromatophore part is essential for the absorption of light in the visible range.

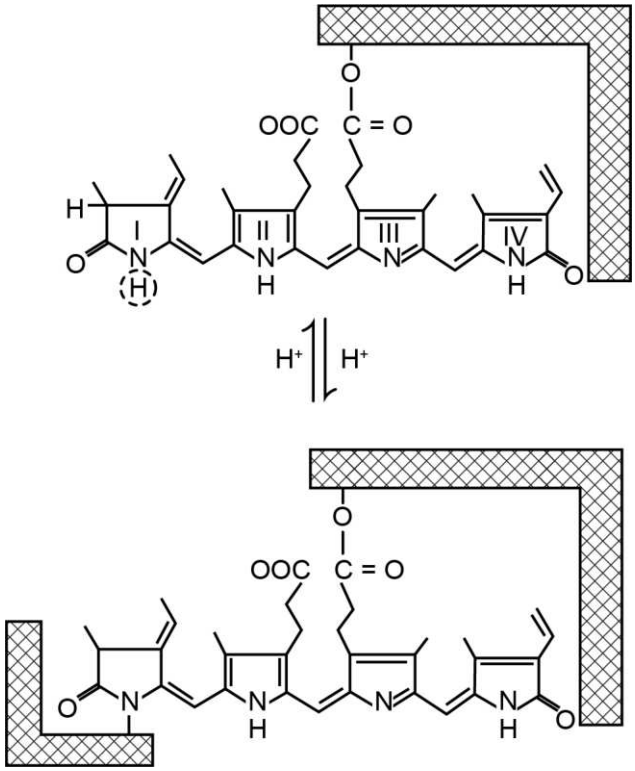

Fig. 15.16: The structure of chromatophore and its attachment to protein. Phytochrome chromophore is an open tetrapyrrole. It is attached to the protein at a specific site and undergoes proton shift on conversion from P*r* to *Pfr.*

The chromophore of phytochrome in higher plants is tetrapyrrole **phytochromobilin** which is covalently linked to the apoprotein and helps the phytochrome to absorb *R/FR* light. Phytochrome has several structural domains (viz., PAS domain, GAF domain and PHY domain) which are responsible for its synthesis, assembly, and functioning. Phytochrome has now been shown to be a light regulated *protein kinase* which is capable of autophosphorylation. The inactive *Pr* form is dimerized in the cytosol and at the time of conversion to *Pfr* undergoes a conformational change in the domains and moves from the cytosol into the nucleus. Inside the nucleus the phytochrome mediates change in gene transcription, serving as a light activated switch for altered gene activity.

There are two types of phytochromes viz., **Type I** and **Type II**. Their concentration varies. However, type I phytochrome degrades in light as the dark grown seedlings have nearly 9 times more Type I than Type II. Phytochromes are encoded by a multigene family whose number varies from three (Rice) to five (*Arabidopsis*).

Box 15.1: A method to estimate phytochrome

Since there is no chemical test, the only method available to test and estimate phytochrome is the spectrophotometric method. Firstly, the difference in the absorbance between 660 and 730 nm is measured after red light irradiation (ΔOD red, No, 1). Next, the sample is irradiated with far-red light and again the difference in

ΔOD is measured (ΔOD far-red, No. 2). The content of total phytochrome is measured by subtracting the value obtained after red light irradiation from far-red light irradiated value.

Phytochrome total = Δ (ΔOD) = [ΔOD far-red - ΔOD red]

1) Δ (ΔOD) = $[OD_{660} - OD_{730}]$ after red irradiation

2) Δ (ΔOD) far-red = $[OD_{660} - OD_{730}]$ after far-red irradiation.

Nowadays special spectrophotometers are also available which can measure the absorption difference between two wavelengths. These are called dual wavelength spectrophotometers.

15.3.3 Phytochrome-Mediated Responses

Phytochrome responses are those which are controlled reversibly by red and far-red light.

i) **Fast responses:** those which occur within a time span of seconds to minutes and

ii) **Slow responses:** those that take hour to days to manifest themselves.

Some of the fast responses are discussed below:

i) It was found that when mung bean root tips were kept in a specific solution (containing ATP, IAA, ascorbic acid, $MnCl_2$, KCl) the root tips adhered to a glass beaker when irradiated with red light. This effect was reversed by far-red light within 30 seconds. It is suggested that the quick response is probably due to change in electric charges on the root tips in response to red light, so the root tips adhere on to the negatively charged glass surface. Such a response can be classified as a fast response and is called **Tanada effect** (after Takuma Tanada).

ii) Similarly, it was shown that if alga, *Mougeotia* was irradiated, its single chloroplast turned in response to red light, but far-red light alone had no effect (Fig. 15.17). The red-light effect was reversed by far-red light. This movement was also noticed within minutes of irradiation. In fact, in these experiments, the light was irradiated on the cytoplasm or membranes barrier and not onto the chloroplast directly.

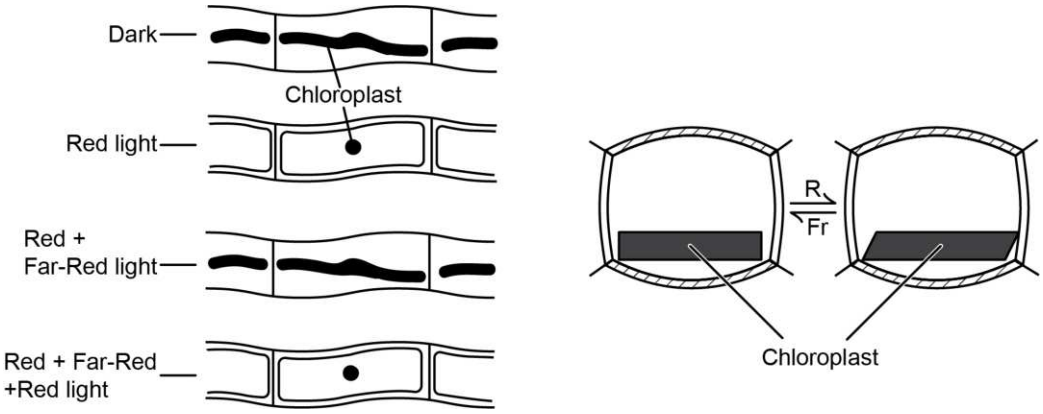

Fig. 15.17: An experiment with *Mougeotia* to demonstrate a fast response. Filaments of *Mougeotia* are made up of long cells each of which contains a single flat plate like chloroplast which can turn inside the cell in response to light. The light induced chloroplast movement was shown to be red/far-red light reversible response as shown above. The movement starts with a lag phase of only a few minutes.

It is believed now that apart from processes involving wavelengths exceeding 550 nm and photosynthesis, all are phytochrome mediated.

The phytochrome mediated plant response can be categorized in various ways.

a) Based on time of response — the response are either Developmental (Slow) or Rapid.

   i) Developmental responses are slow as they also involve other physiological processes like growth and differentiation. As a result, they take a longer time to manifest. e.g., Flowering, seed germination, anthocyanin synthesis, chlorophyll synthesis, etiolation, de-etiolation, hypocotyl hook opening, unfolding of leaves in seedlings and an array of developmental responses in lower plants like spore germination in ferns, rhizoid development from gemmae in liverworts and bud differentiation in protonema of mosses.

   ii) The rapid responses occur within seconds of light irradiation. This may not involve growth but changes in the permeability to water, changes in biometric potential between different regions of roots as well as change in turgidity of motor cells in the pulvinus of leaflets and orientation of the chloroplasts.

b) Phytochrome responses can also be categorized/classified based on the amount of light required. The amount of light (number of photons falling on a unit surface area) required to induce a response is called **fluence**.

Fluence is measured in terms of micromoles or quanta per square meter (μmol^{-2}). The light that is incident on the object refers to irradiance and not the intensity, as generally believed. Irradiance is also called the fluence rate (measured as micromoles of quanta per square meter per second — $\mu mol\ m^{-2}s^{-1}$).

Since total fluence = fluence rate x length of time of irradiation, the phytochrome responses can be divided into three types:

i) ***Very low fluence responses* (*VLFRs*):** Which can be initiated by fluence as low as 0.0001 µmole $m^{-2}$and saturate at 0.05 µmol m^{-2}. For example, *Arabidopsis* seeds can be induced to germinate by an exposure of red light of 0.001 to 0.1µmol m^{-2}(which is many times lower than even the light emitted by a firefly in a single flash). These responses are *non-photoreversible* as significant amounts of *Pfr* are not made.

ii) ***Low fluence responses* (*LFRs*):** Can be initiated only after the fluence reaches 1.0µmol m^{-2} and get saturated to 1000μmol^{-2}. Such response are *photoreversible* and obey the law of reciprocity which means that the product of the fluence rate and the time of irradiation regulated the magnitude of response. For example, lettuce seed germination, regulation of leaf movements, promotion of de-etiolation.

iii) ***High irradiance response* (*HIRs*):** Require prolonged or continuous high irradiance exposure. In this case the response is proportional to the

irradiance until the response saturates. Additional exposure will now have no effect. Such responses are time-dependent and *non-photoreversible* and do not obey the law of reciprocity. Some of the examples are production of ethylene in *Sorghum*, enlargement of cotyledons in mustard, induction of flowering in henbane and synthesis of anthocyanin in apple skin segments and various dicot seedlings.

iv) In many leguminous plants it was found that leaves closed if they were transferred from light to darkness. It has been shown that this response is under phytochrome control.

v) Several other responses, which could be classified as slow responses like seed germination, hypocotyl elongation, leaf expansion, abscission, flowering, fruiting have been shown to be under phytochrome control.

Several other responses, which could be classified as slow responses like seed germination, hypocotyl elongation, leaf expansion, abscission, flowering, fruiting have been shown to be under phytochrome control.

15.3.4 Mechanism of Action

You have learnt that biochemical and molecular changes are brought about by phytochrome. However, what is not yet clear is the relationship of these changes to a specific developmental event. Since phytochrome controls many processes even in one plant (Table 15.18). This makes the analysis of molecular action a difficult task.

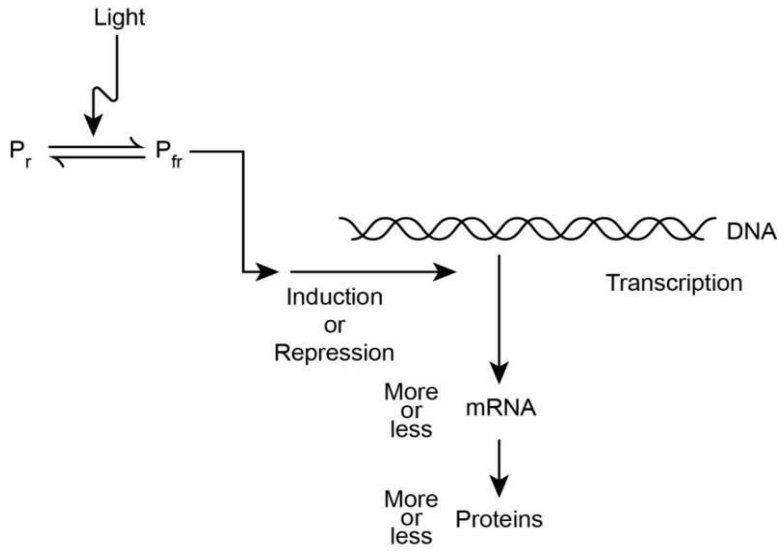

Fig. 15.18: Hypothesis for differential regulation of gene expression. The biologically active form *Pfr* can affect the transcription process by either inducing the synthesis of new mRNA or by repressing the synthesis of mRNA already being synthesized in dark. By altering the level and quality of enzymes and proteins, developmental responses can be controlled by phytochrome.

Table 15.2: List of multiple effects of light on mustard seedlings grown in dark

1.	Inhibition of hypocotyl lengthening
2.	Enlargement of cotyledons
3.	Opening of the hypocotylar (“Plumular”) hook

4.	Development of primary leaves
5.	Synthesis of anthocyanin
6.	Increase of the rate of chlorophyll accumulation (in white light)
7.	Changes in the rate of cell respiration
8.	Changes in the rate of degradation of storage protein Increase of negative geotropic reactivity of the hypocotyl
9.	Increase of protein synthesis in the cotyledons
10.	Decrease of RNA contents in the hypocotyl
11.	Increase of RNA contents in the cotyledons
12.	Differentiation of stomata in the epidermis of the cotyledons
13.	Increase in the rate of carotenoid synthesis
14.	Increase in the level of mRNA, increase in *nitrate reductase,* increase in *RuBP carboxylase*

Of the various changes brought about by phytochrome many of them occur within a few minutes and some other have a time-lag of 1-2 h or more. It has been suggested that responses which occur rapidly, say within 0-15 minutes, may be considered as **'membrane associated events',** and the responses with a lag period of few hours may be considered as **'gene expression events'** (Fig. 15.19). The responses which are measurable as changes in growth may be considered final **'developmental responses'.** It has been found in several systems that phytochrome does affect membrane related phenomena. First, it has been reported in a few cases that phytochrome may be associated with membranes. For example, in *Mougeotia*, if the membranes were irradiated with light, chloroplast movements was detectable. Also, experiments suggest that phytochrome binds to the membranes, but the result need to be confirmed. However, it has been clearly established for example in *Albizzia* that phytochrome controls transport of K^+ ions across the plasma membrane. Similarly, it has been shown that phytochrome also affects the movement of calcium ions. The exact mechanism by which phytochrome affects membrane transport proteins is not yet known.

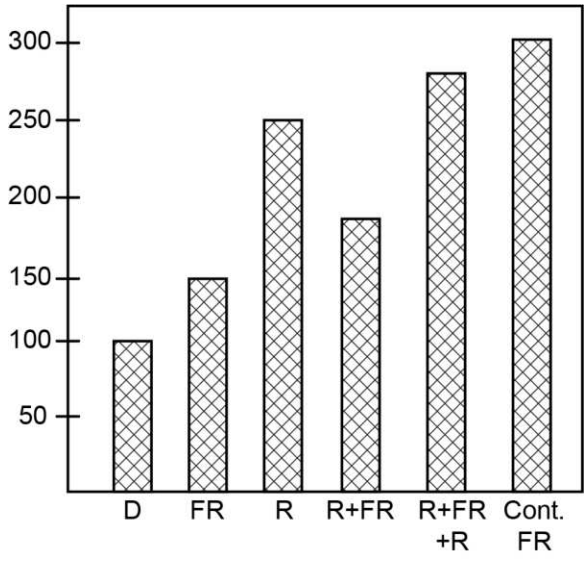

Fig. 15.19: Photoreversible effect of R and FR light on the induction of *nitrate reductase*. R and FR lights were given for only 5 minutes to etiolated (plants grown in the absence of light) maize leaves.

More work has been done on gene expression events. It has been shown that phytochrome does affect the level and activity of enzymes. In some cases, however, the pigment increases the enzymes levels as for example, in *phenyl alanine ammonia lyase*, *nitrate reductase*, *RuBP* c*arboxylase*, light harvesting chlorophyll a/b protein, whereas in other the phytochrome decreases the enzyme level, e.g., NADH *protochlorophyllide oxidoreductase*. An example of phytochrome regulation of *nitrate reductase* enzyme is shown in Fig. 15.19.

Recently, it has been shown that phytochrome affects the level of enzymes by affecting the process of transcription. An increase in the level of mRNA of several proteins in response to light irradiation has been shown.

A major question that remains to be answered is how does phytochrome which is present in the cytoplasm affect gene expression in the nucleus? It is suggested that probably some signals or second messengers are generated by phytochrome which affect at the level of transcription as shown. Lately the role of calcium ions as messenger of light mediated responses has been emphasized. This is based on the findings that in many instances the light affect could be mimicked by calcium ions.

The molecular properties of phytochromes undergo a change after absorbing light. As a result, the phytochrome protein interacts with other cellular component to bring about changes. The signal transduction pathways lead to response in term of ion fluxes causing rapid turgor changes a slower low term alteration in gene expression.

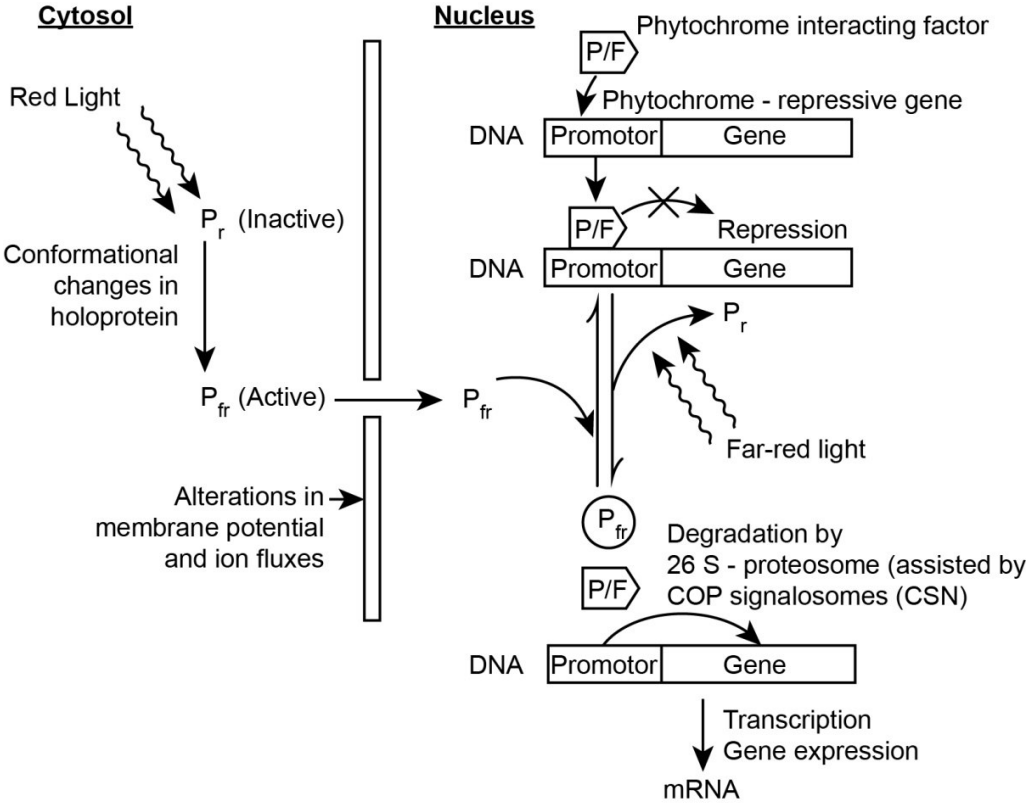

Fig. 15.20: A suggested model explaining the transport of floral stimulus and the induction of flowering genes (after Taiz *et al*).

It has now been demonstrated that phytochrome A enters the nucleus through the mediation of Far-red light whereas red light triggers the import of phytochrome B. Some phytochrome interacting factors (PIFs) present in the

nucleus bind to the promotor region of the phytochrome-repressive genes. This causes repression of transcription. Entry of *Pfr* into the nucleus triggers degradation of PIF by a 26S-proteosome system. Gene transcription is now activated, and mRNA is formed. Increase in the concentration of *Pr* in the nucleus helps in reassociation of PIF with the promotion region thereby causing gene repression Fig. 15.20). There are **primary response** genes which encode the transcription factors get regulated by a shift from dark to light. On the other hand, the secondary response genes require new protein synthesis and are late acting. Thus, a transition from dark modulated development (skotomorphogenetic) to a light modulated (photomorphogenetic) on requires a reprogramming of gene expression. In addition, various phytochrome Interacting factors (***PIFs***) have been identified. These proteinaceous transcription factors modify the phytochrome action by acting as negative regulators of phytochrome response.

15.4 FLOWERING HORMONE

It was in 1880, that Julius Sachs suggested that there is a chemical basis for flowering. Later experiment indicated that some hormone was produced which controlled flowering. The hormone was named **florigen** by **Mikhail Chailakhyan**. Before we find out what the florigen is, let us first see the experiments which suggested that a chemical is involved in flowering and that it is produced in leaves.

The concept of florigen-a flowering hormone was given by a Russian Botanist named Chailakhyan in 1930s.

The light dark conditions which induce plants to flower are called **inductive conditions.**

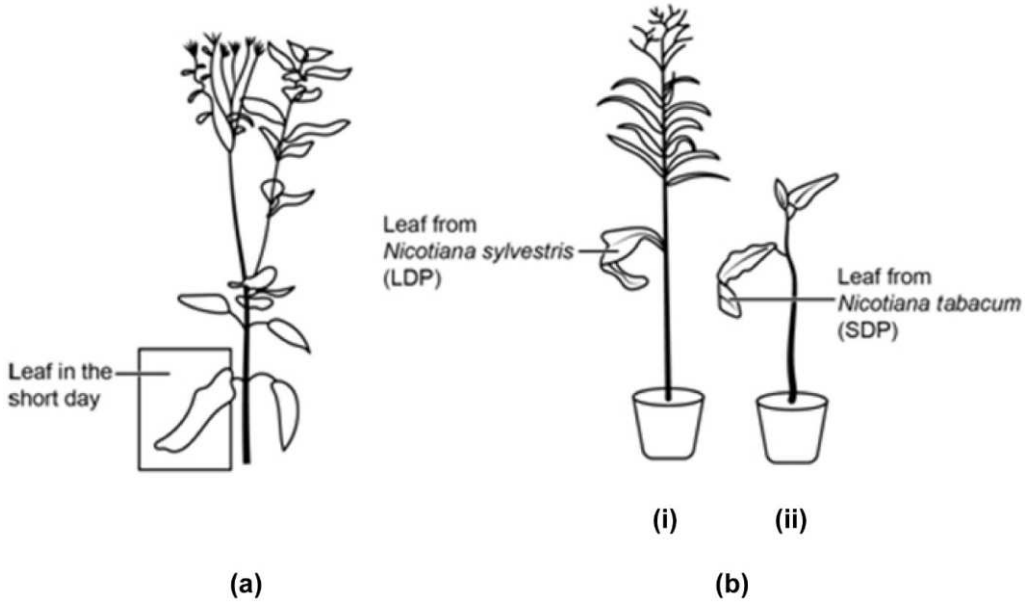

Fig. 15.21: Experiments to demonstrate that photoperiodic stimulus is perceived by the leaf. In a) *Kalanchoe blossfeldiana* plants begin to flower even if one single leaf is maintained under short-day conditions. In experiment b) a leaf from LDP, *Nicotiana sylvestris* was grafted on SDP, *N. tabacum.* The plant started flowering even under long-day conditions (i). However, if a leaf from *N. tabacum* was grafted on *N. tabacum* itself in a control experiment ii, flowering was not induced.

In an experiment only the leaves of *Kalanchoe***,** a short-day plant, were given the inductive conditions and not the whole plant. Even then the plant flowered (Fig. 15.21). More such experiments were done where treatment of light-dark cycle was given only to the leaves and the plants were induced to flower.

Some scientists performed experiment with a twig, a single leaf of which had been given the inductive condition. It was grafted onto a plant which was not given any inductive condition. Interestingly, it was found that the grafted plant also started flowering (Fig. 15.21). In some cases, grafts were taken from plants which were induced by short-days, for example from *Xanthium canadense* and were put on plants which required long-day conditions e.g., *Rudbeckia bicolour*. Even in such graftings the recipient plants, in the above case *Rudbeckia*, started flowering.

If you have understood the above experiments perfectly well, you will come to the following conclusion.

i) the leaves perceive the light stimulus, and

ii) some chemical moves out of the leaves (as must be occurring in grafts) and goes to the meristems and changes them from vegetative to flowering. What is this chemical?

However, some observations are contrary to this view. It has been shown that florigen can move readily between tissues, but it cannot pass between two tissues if separated by a strip of agar while a hormone can travel easily through agar. Further, experiments have suggested that the flowering stimulus moves through the phloem. This suggests that some chemicals, other than hormones may also be involved in flower induction *in vivo.* We can hope that soon it would be possible to isolate and identify florigen by analyzing and comparing the content of phloem sap before and after flower induction.

15.4.1 Biochemical Changes

Many workers have tried to follow the biochemical changes that precede flowering and result in meristems which give rise to flowers instead of vegetative structures. In *Pharbitis*, which is a short-day plant and requires only one dark period for flowering it was found that soon after the dark period, the flowering stimulus begins to move out of the leaves. In this experiment, the plant was given inductive conditions and after specific time intervals, biochemical changes were given inductive conditions and after specific time intervals, biochemical changes were measured in meristem. An increase in metabolic activity around 40thhour at the floral apex was manifested by an increase in the level of RNA, proteins, and ribosomes. Electron microscopic observations also revealed extensive formation of endoplasmic reticulum. These activities were followed by an increase in DNA synthesis and mitotic activity. At about 88th hour after floral induction, the rate of cell division was noticed particularly in the central zone and peripheral zone of apical meristems.

Such experiments have also been done in other plants. However, it has not been possible yet to identify which of the RNA or proteins are responsible for the onset of flowering. With the application of newer techniques, it has been possible to suggest that there some specific flowering genes which get **switched on** after receiving specific light-dark cycle. Although we do not know the products of all these genes, some of them have been shown to code for proteins which regulate transcription.

SAQ 2

a) In the following sentences, fill in the space with appropriate words:

i) *Pr* absorbs……. nm wavelength of light and *Pfr* absorbs at…… .

ii) The effect of red light could be completely reversed by …….. light.

iii) Phytochrome is a …….. protein. It is present in the ……. of cell.

iv) The biochemical signal to the nucleus by phytochrome for making new mRNA is suggested to be …….. .

v) Biological active form of phytochrome …….. can affect ……. process by induction or repression.

b) List the biochemical changes that precede flowering.

15.5 PLANT RESPONSE TO TEMPERATURE-VERNALIZATION

Vernalization is a Latin term, it means "to make springlike".

Some plants flower only after passing a winter season. For example, winter wheat is sown in the autumn for harvest in the following summer. It needs exposure to cold. If winter is mild, plants do not flower, and the crop fails. It has been shown that winter wheat and many other plants require a period of chilling 0°-2°C for about a week for flower formation. The cold treatment given to geminating seeds for flower induction is called **vernalization.** The technique of vernalization was developed in Russia where winter crop required chilling for successful cultivation. The seeds are soaked for small period to initiate germination and then they are buried in the snow. Later, they are planted in the spring when severely cold conditions are over. Thus, chilling is also a stimulus for flower induction.

Which part of the plant requires chilling stimulus for floral induction? Experiments show that only the shoot apex receives the stimulus (needs vernalization) which then is passed on to the other parts of the plants. Similar conditions have been made with the help of grafting experiments (in Henbane). When the shoot apex that has received the stimulus is pinched off, the lateral shoot flowers. And if the apices of lateral shoots are also pinched off, the side shoots develop and flowers. Moreover, when extracts of a vernalized plant are applied to a long-day plant growing under short-days, the recipient plant flowers. Like light induction, the stimulus can also pass through a graft to a non-vernalized plant. These results show that flowering stimulus is transmitted from shoot apex to other tissues. The chilling stimulus was named **vernalin** by Melchers (1939). The nature of this compound has yet not been identified, although it is believed that it could be a gibberellin like substance as the latter is capable of inducing flowering in some biennials without any cold pretreatment.

The term vernalization was coined by Russian scientist **Lysenko** in the 1920s. A detailed study on the Petkus rye (*Secale cereale*) was made by Gregory and Purvis in the 1930s where the cold pretreatment could mimic the flowering behavior of a spring crop. If a vernalized seed on plant is exposed to high temperature, it loses its earlier effects, and the effect of low temperature is lost (Fig.15.22). This is called **devernalization**. Treatment once again with low temperature can result in vernalization.

15.5.1 Mechanism of Vernalization

There is growing conception of some hormonal involvement in vernalization. The responsible substance, **Vernalin** is believed to be produced in some biennials in response to low temperatures. According to some workers two chemicals viz., gibberellins and anthesins - are responsible for flowering. Anthesins are already abundant in LDPs. So cold treatment to LDPs induces vernalin which now combines with anthesins to produce gibberellins which induces flowering (see scheme in Fig. 15.22).

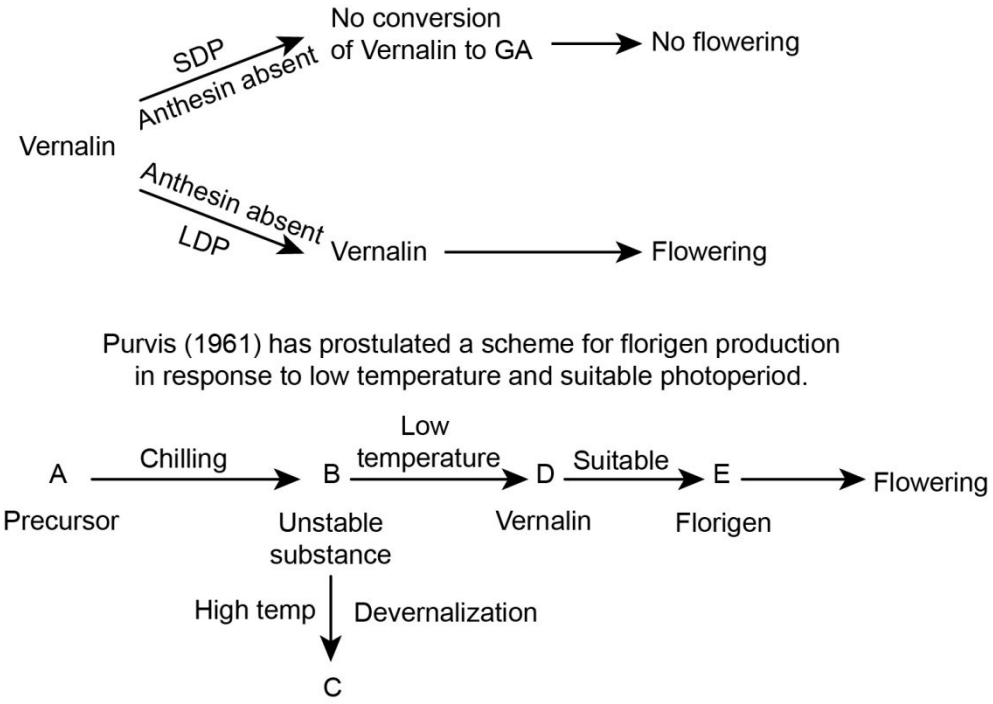

Fig. 15.22: A schematic depiction of low temperature induced flowering.

Vernalization is also known to shorten the flowering time. Hence more than one crop can be obtained per year. Crops like cotton and maize which require long stimulus can now be grown in temperate areas which have short summers.

Recent investigations on *Arabidopsis* (LDP and low temperature requiring type) have revealed changes in the pattern of gene expression in the meristem in response to cold treatment. Such changes in DNA which can pass to the next generation of cells by mitosis or meiosis are called **Epigenetic changes.** A gene flowering locus *C* (*FLC*) has been identified which acts as a repressor of flowering. Vernalization switches off this gene for the remainder of the plant's life cycle. Thus, flowering occurs in response to long day conditions. Three genes (*VRN1, VRN2* and *VRN3*) are thought to determine the vernalization stimulus requirement in cereals.

Vernalization has been practiced extensively in Russia and other countries with severe winters so that winter crops can be grown in spring season to protect them from freezing injury.

SAQ 3

Fill in the blanks with appropriate words/terms.

a) ……. is the site of perception of cold treatment.

b) A cold temperature treated grain could be …… by exposing to high temperatures.

c) The existence of a transmissible stimulus for vernalization called ……. is strongly suggested.

d) In many plants …….. can stimulate vernalization.

e) Both ……..….. followed by …….. are required for flowering.

15.6 RHYTHMIC CHANGES/ BIOLOGICAL CLOCK

Lastly, we are going to learn about a phenomenon which is still not very well understood by scientists. Like animals, which show rhythmicity in their behavior, it has been found that some developmental events in plants also occur with regular periodicity in time. These processes are rhythmic and if these occur in the absence of external disturbing factor, these are called **endogenous rhythms.** This would be clear if you have ever observed sleep movements of leaves in plants like *Phaseolus, Mimosa, Albizzia* and *Samanea*. In these plants during the daytime the leaves are open and during the night they droop down. In fact, these movements can be recorded. This was done by tying a thread to the leaf that shows periodicity. The thread is rolled over a pulley and then attached to a stylus which is touching a rotating drum as shown in Fig. 15.23.

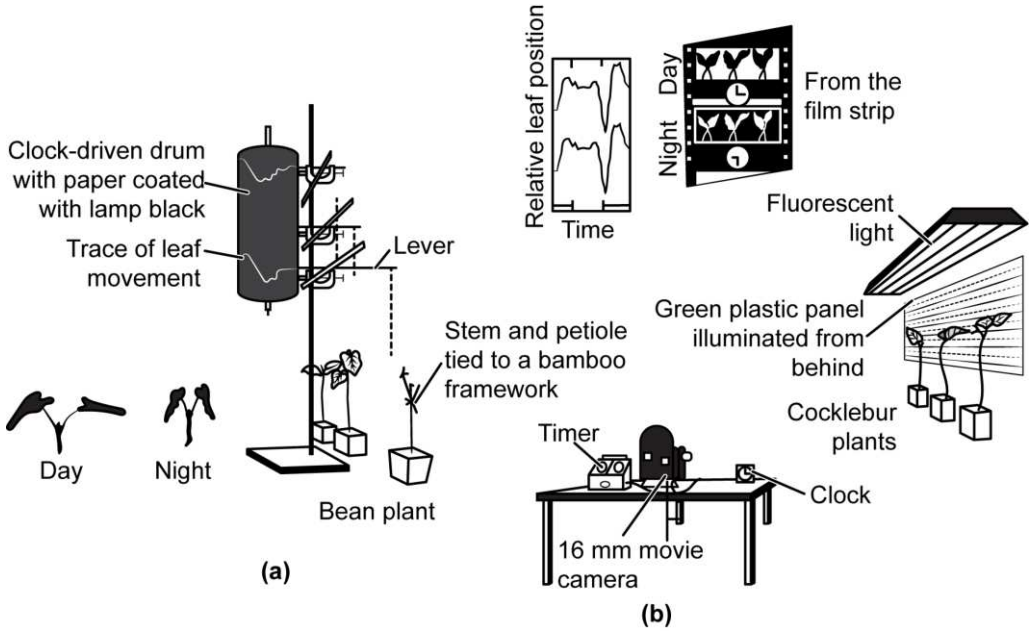

Fig.15.23: Methods for recording leaf movements. The leaf of the plant is tied with a thread to a lever that touches the marker running on a clock driven drum a) Since the drum is moving the trace of leaf movement can be recorded over a period of day and night. A time lapse photography camera is fitted which takes pictures of the plant after short time intervals; b) From the developed film as shown in above right side, the leaf position with respect to time can be recorded.

How do we know that these movements are independent of external factors? An experiment was done where after the rhythm was induced by light exposure, the plants were kept in total darkness for days. It was found that the leaves would open and close as they would have done under normal conditions. These oscillations were about 23 h apart. Such rhythms with oscillations of 20-23 h are called **circadian rhythms** and the cellular machinery which helps the plant remember and keep these rhythms in time is called the **'Biological clock'.** Interestingly, when plants were put on satellites, the rhythms persisted.

There are some good examples of plants where circadian rhythms have been noticed and studied. These are sporulation in *Oedogonium cardiacum;* spore discharge in *Pilobolus sphaeroporus*; leaf movement in *Phaseolus multiflorus* and CO_2 fixation in darkness in *Bryophyllum fedtschenkoi*.

Very pretty floral clocks have been assembled in tourist places in various parts of the world. One in Canada near the famous Niagara Falls tells the time from 6.00 a.m. to 6.00 p.m., beautiful flowers open at sharp 6.00 a.m., 7.00 a.m., 8.00 am., and so on, a glorious sight worth seeing. Fig. 15.24 depicts a floral clock found in a garden in Europe.

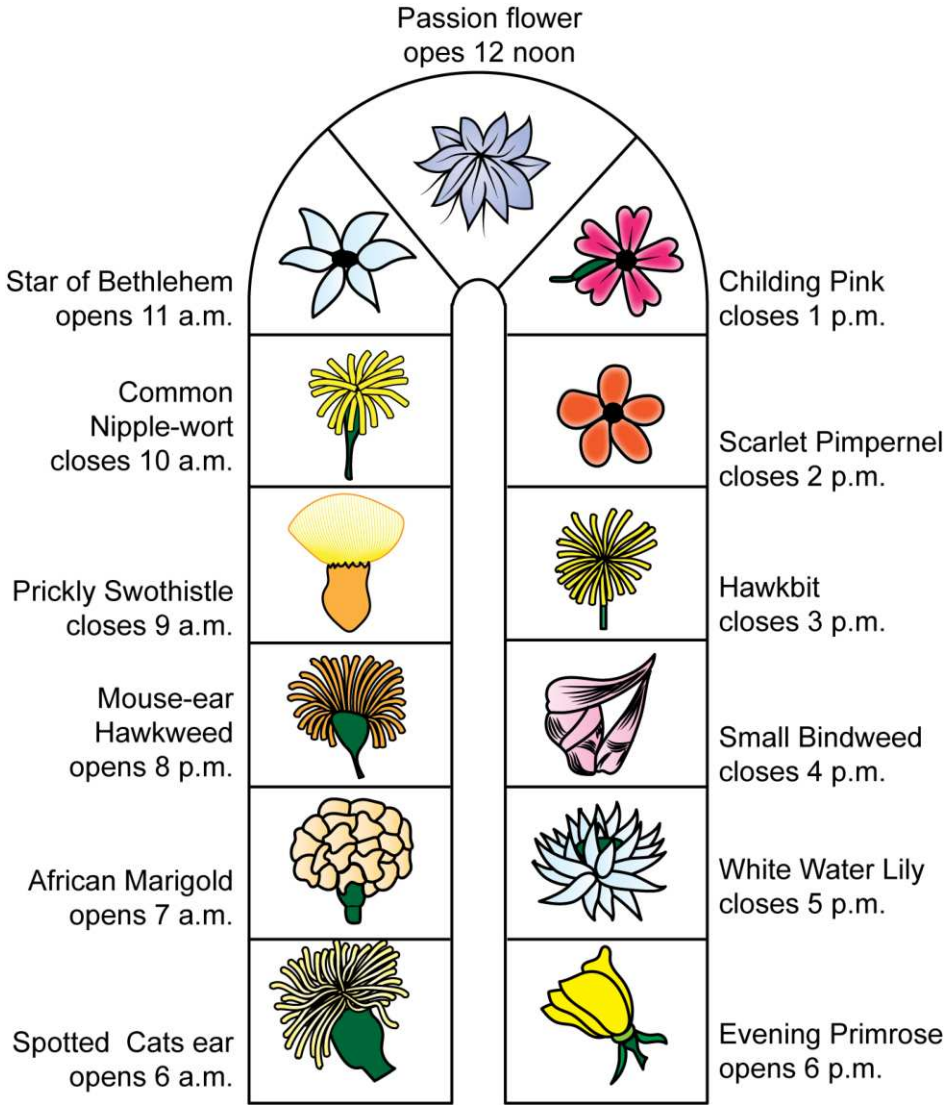

Fig. 15.24: The flower clock.

15.6.1 Factors Affecting Rhythms

Two factors, more important than others which regulate or affect the time period and the intensity of a rhythm are temperature and light. It has been found that once the rhythm is set, many circadian processes are independent of temperature over a wide range. The effect of light in many cases has been shown to be regulated by phytochrome, the pigment-protein complex about which you have studied in section 15.3. The amount of $\boldsymbol{P_{fr}}$ form changes during the day, i.e., from sunrise to sunset. At sunset, when red light is less, its level would decrease which would further go down in the following darkness. In the morning when red light is more, the ***Pfr*** level would rise again. This daily change could be one of the factors to set the biological clock.

One of the founders of biological clock research in plants, Erwin Bunning of Botanical Institute in Tübingen, Germany, worked on leaves of different varieties of soyabean and found some correlation between their photoperiodic behaviour and sleep movements. He further found that leaf movement was less pronounced. These observations do suggest that light does influence rhythmic phenomenon in plants.

At present it is still not clear what is the physical basis of the biological clock. Certainly, it is not any specialized timing organ. Some processes get affected at the nuclear, protein and membrane level. However, the exact nature is not known with certainty. Probably some chemical inside each cell works as a clock. Although recent work seems to suggest the involvement of membrane bound glycoproteins which intercept environmental signals and set the clock.

SAQ 4

Match the contents of column I with those given in column II.

	Column I		**Column II**
i)	Biological clock setting	a)	Spore discharge in *Pilobolus*
ii)	Sleep movements	b)	Absence of external factor
iii)	Niagara Falls	c)	Level of P_{fr}
iv)	Circadian rhythm	d)	Floral clocks
v)	Endogenous rhythms	e)	*Phaseolus*

15.7 SENESCENCE

Plants follow a common pattern: growth, flowering, senescence and death. The entire period from the beginning to death is called the longevity or age or life span. Life span varies from species to species. Some plants like annuals, complete their life cycle within a few months whereas others may live for a few centuries. For example, the life of *Juniperus scophularium* is around 3,000 years. The giant *Sequoia* also has a long life-span.

The period just before death is called the senescent period. Senescence is age-dependent degradation and degeneration of cells, organs or the entire organism, leading to death. During this period deterioration occurs because there is a consistent decrease in viability and increase in vulnerability. This phase can be prolonged but cannot be reversed.

Senescence may occur either very quickly or may be a slow process. Sometimes the individual organs senesce while the whole plant may remain healthy. Hence, senescence in plants occurs at various levels, both at the organ and organism level. The senescence at the organ level is evident by the change in leaf color and the subsequent death of autumn leaves. Cell death in leaf starts from mesophyll cells and proceeds to other cell types. The most notable change seen during senescence is the change in the cellular structures of leaves which occur due to disintegration of intracellular organelles particularly, the chloroplast.

The process of senescence shows variation in various types of plants (Fig. 15.25). In annuals, the whole plant dies; in biennials, the plant dies only after two years, whereas in perennials, year after year the leaves and fruits are shed but the main plant survives. Broadly, senescence has been categorized into following types.

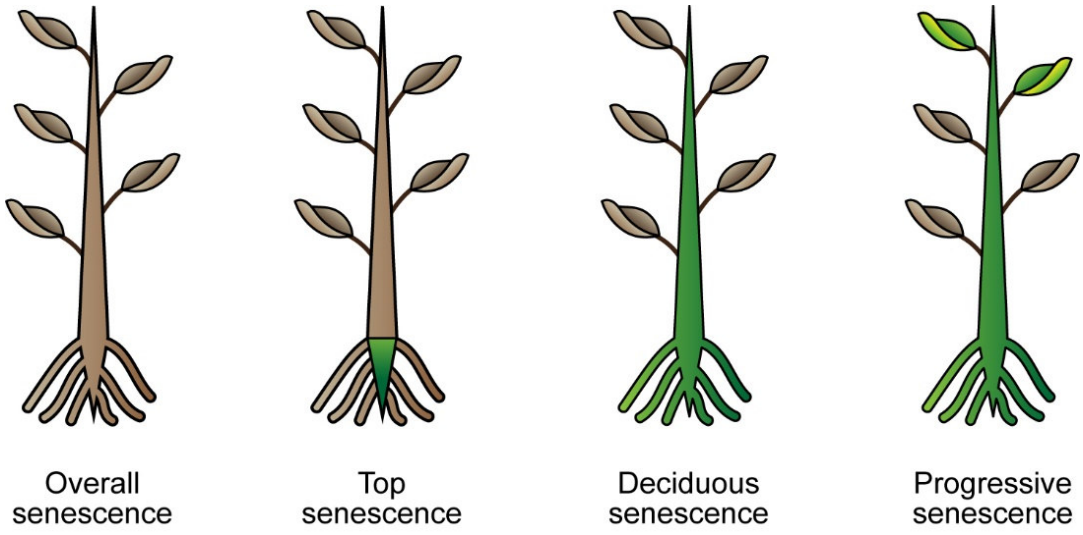

Fig. 15.25: Diagrammatic representation of different senescence patterns noted in plants. The dying parts are shaded brown.

Overall senescence (whole plant senescence): Only the parts above the ground level i.e., the aerial parts die whereas underground parts survive, for example- potato, rice, wheat, gram.

Deciduous senescence (simultaneous or synchronous senescence): Only the leaves senesce as in many trees such as elm and maple. The senescence of leaves, or abscission occurs when at the base of a leaf a layer of cells is laid which is called abscission layer. An abscission zone or separation zone formed at the base of the petiole is composed of a top layer that has cells with weak walls. The weak walls of the cells in the top layer break which allows the leaf to be shed (Fig. 15.26).

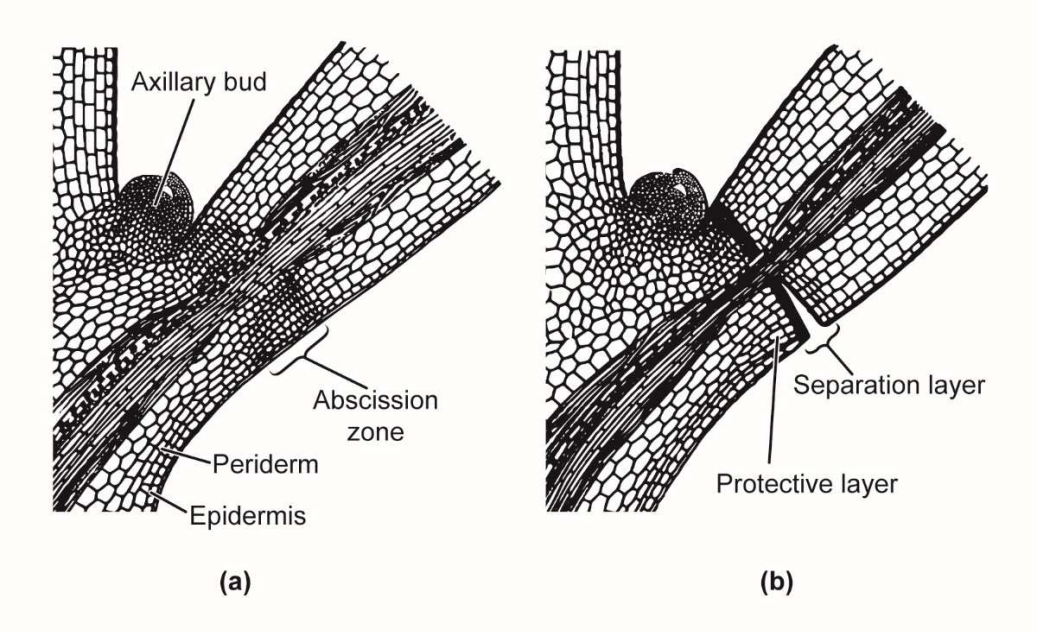

Fig. 15.26: L.S of leaf base showing a) abscission zone; b) formation of separation layer.

The bottom layer expands in the autumn. The cells of abscission layer show high activity of *cellulase* enzyme which degrades cellulose of cell wall. Another enzyme that increases during abscission is *polygalacturonase* (which hydrolyses pectin, a major component of the middle lamella region of the wall). So, the cells get separated and the leaves fall. Below this layer, the plant makes a protective layer which has many lignified cells. When a leaf falls, this layer protects the tissue from desiccation.

Progressive senescence: Here also the leaves are shed but it is gradual senescence of leaves up the stem, for example- palm trees.

Sequential senescence: It is found in many perennial plants. The tips of the main shoot and branches continue to produce new buds and leaves. The older leaves and branches senescence and die, for example-*Eucalyptus, Pinus*.

15.7.1 Biochemical Changes Associated with Senescence

During senescence many physiological and biochemical changes take place in plants. One of the important changes noted in plants is the decrease in chlorophyll content. The degradation of chlorophyll results in yellowing of leaves. The overallstructure of chloroplast shows disorganization as the thylakoids gets disturbed. As a result CO_2 fixation capacity of chloroplast declines. Chloroplast degeneration is accompanied by loss of proteins such as *ribulose biphosphate carboxylase* (Rubisco) and chlorophylla/bbinding protein (CAB) from the chloroplast. The organelles such as mitochondria and nucleus remain intact. It has been suggested that *pheophytinase* (PPH), an enzyme plays a role in chlorophyll degradation. Enzymes such as *phospholipase D*,*phosphatidic acid phosphatase*, *lytic acyl hydrolase* and *lipoxygenase* get involved in hydrolysis and metabolism of the membrane lipid in senescing leaves. The lipid peroxidation and membrane leakiness also increases during the process due to increase in generation of reactive oxygen species (ROS).

A dramatic metabolic transition from anabolism (carbon assimilation) to catabolism has been noted in leaves. This transition to nutrient remobilization has been suggested as major reason for degradation of cellular structures. Increased catabolic activity is responsible for converting the cellular materials accumulated during the growth phase of leaf into nutrients that are supplied to developing seeds or to other growing organs.The nutrients produced in the leaf either show degradation or get redistributed to developing seeds during senescence. The rate of respiration also decreases with time and supply of ATP is reduced.The hydrolysis of macromolecules such as proteins, lipids, nucleic acids and pigments occurs as number of degradative enzymes like *proteases*, *ribonucleases*, P-*glucan hydrolases*(for loosening of cell wall), *chlorophyllase* (for degradation of chlorophyll) are produced. These enzymes increase the rate of catabolism and finally over a period of time, the plant succumbs and dies. In annual plants, hydrolyzed molecules are relocated to developing seeds and fruits. In trees, the nutrients that are degraded eventually get stored in stems or roots and are utilized later for the development of new leaves or flowers. The nutrients relocated from senescing autumn leaves help in blooming of spring flowers.

Though leaf senescence is a deleterious process for the leaf organ but nevertheless critically contributes to the fitness of whole plants by ensuring optimal production of offspring and better survival of plants in their given temporal and spatial niches. Leaf senescence is therefore, an evolutionarily selected and genetically controlled developmental process that constitutes an important phase in the plant life cycle.

15.7.2 Regulation of Senescence

Senescence is a process which is a part of a developmental sequence of events and is a controlled process. It is controlled by both external and internal factors. Of the external factors, plant hormones, length of the day, temperature and nutrient supply play an important role. Of the internal factors, size and age of the plant, degree of flowering and time of ripeness of fruits determine the onset of senescence.

In one experiment where the normal time of senescence was 120 days, when mature fruits were removed from plants, the time of senescence was delayed. Senescence occurred after 140 days. And when young fruits and-flowers were removed from plants as soon as they were formed, the senescence was delayed to 160 to 180days. This means that the senescence is initiated as soon as the process of reproduction is set in. This is probably because a plant needs a lot of nutrition for the growth of flowers and fruits. So to increase the nutrient supply to fruits, the stored material from leaves and other parts is translocated to the growing fruits. The demand is so high that the fresh supply of nutrients and photosynthates cannot be replenished.

It is a simple case of more demand than supply. Naturally, the system collapses.

However, in the process the plant tries to ensure that the fruits mature and seeds are set so that it can continue with its progeny.

Internal and external factors get interconnected to form a complex network of regulatory pathways for senescence. Hormone signaling pathways mediate or

influence development responses in plants. Hormones play key role in the progression of senescence. Hormones induce activation of hydrolytic enzyme synthesis genes and trigger leaf senescence. The hormonal pathways regulate all the stages of leaf senescence including the initiation phase, progression, and the terminal phases. Cytokinins have been known to delay senescence. Cytokinins act as senescence-retarding hormones because the level of endogenous cytokinins falls during leaf senescence. Molecular analysis revealed that genes for enzymes viz. *cytokinin synthase* and *adenosine phosphate isopentenyl-transferase* (IPT) involved in cytokinins synthesis get downregulated and a gene for the enzyme, *cytokinin oxidase* (cytokinin degradation) gets up-regulated in senescing leaves. The gaseous hormone, ethylene is known to play a role in hastening leaf and flower senescence. Ethylene is supposed to act as an important positive regulator of leaf senescence. In many plant species, including *Arabidopsis* where the level of ethylene increases during leaf senescence. Exogenous application of ABA has also known to promote leaf abscission and senescence. The ABA level increases in senescing leaves and exogenously applied ABA induces expression of several senescence associated genes (SAGs), which is consistent with the effect on leaf senescence. High salicylic acid (SA) level in senescing leaves appears to be involved in up-regulation of several SAGs during leaf senescence.

Leaf senescence can be induced by the interaction of photoperiod and temperature. Studies have shown that photoperiod reduction increased leaf senescence by 61%, while low temperatures increased the process by about 39%. Photoperiod reduction triggers leaf senescence in several woody species. Thus, the photoperiod may be the abiotic factor which activates senescence. Temperature has been considered as the secondary factor that accelerates the deterioration process. Low temperatures activate leaf senescence in most deciduous species. The deciduous plants respond to these conditions by increasing electrolyte leakage, chlorophyll degradation, formation of reactive oxygen species and reducing enzyme activities.

Nitrogen deficiency accelerates leaf senescence. During this period, protein and amino acid degradation is high and this contributes to allocation of nutrients to developing seeds. The genes involved in the processes and transport of biosynthetic aromatic amino acids are regulated in early stages of leaf senescence. High nitrogen levels may delay leaf senescence in non-perennial species, in addition to maintaining high photosynthetic rates during reproduction and production. Wheat plants with higher leaf nitrogen content showed delayed senescence.

Water deficit also accelerates leaf senescence. Under water deficit, plants regulate dehydration by closing stomata in response to the production of the chemical messenger Abscisic Acid (ABA). Increased ABA levels increase ethylene production and consequently, the synthesis of the enzymes that act on the cell wall and middle lamella increases. Ethylene induces genes that encode specific hydrolytic enzymes of polysaccharides and cell wall proteins in the abscission zone. Thus, ABA accelerates leaf senescence, indirectly inducing abscission.

Besides environmental and endogenous factors, the biotic factors also play a role in inducing senescence. For example, attack of mites, insects or even parasitic fungi, hastens the process of senescence. Also, without realising

when you walk in a garden and pluck leaves or break branches and twigs, you are also contributing to initiating senescence in those plants (induced by injury). Fig. 15.27 gives a general view of the factors that affect senescence of various plants.

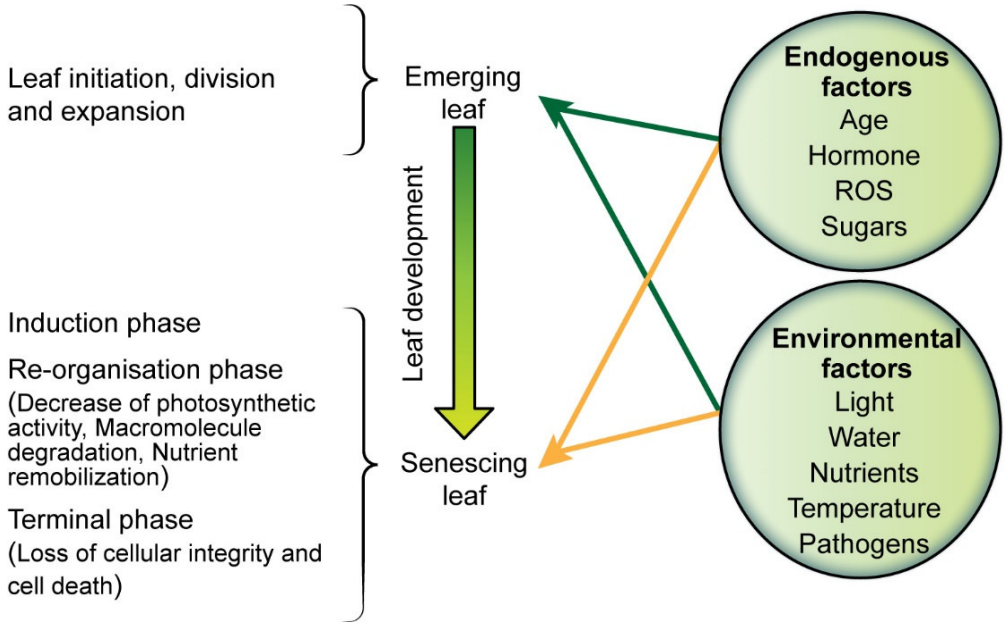

Fig. 15.27: The effect of various physical and chemical factors on senescence.

SAQ 5

a) Mention the major changes noted in leaves during senescence.

b) List various factors that induce senescence in plants.

c) Name some enzymes that get activated during abscission

15.8 CRYPTOCHROMES

These are blue-coloured photoreceptors present in almost all groups of plants. Earliest ideas regarding biological responses to the blue light were presented by Charles Darwin, who demonstrated that when light was passed through a solution of potassium dichromate, it failed to induce any heliotropic movements in plants. Plants sense and respond to blue light (400-500 nm) by production of anthocyanins and carotenoids. Interestingly, there exists a lot of common ground between the absorption spectrum of Flavin and many responses of blue light. For several years the nature of this photoreceptor remained elusive and debatable—a reason why it was called Cryptochrome. Cryptochrome is also known to regulate other aspects of growth and development like hypocotyl elongation, opening and closing of stomata, anthocyanin production, setting of flowering time and clocks. Cryptochromes function in coordination with phytochromes to bring about many responses.

Cryptochromes were first characterized in *Arabidopsis* and have helped us to explain the role of this photoreceptor in flowering by affecting the biological clock.

A gene *HY4* has been shown to encode for this blue photoreceptor. *CRY1* (which works under high blue light fluence rates). Another cryptochrome, viz. *CRY2* has been shown to be important under low blue light fluence rates.

The cryptochrome is believed to be a chromoprotein which is composed of a light harvesting chromophore- **pterin** or **deazaflavin** along with a catalytic part containing FMA and FAD, and an imino- terminal *photolyase* region. In bacteria, these flavoprotein photoreceptors repair the pyrimidine dimers produced by UV light, i.e., they act as *DNA photolyases*.

15.9 PLANT MOVEMENTS

Most of the plants, with the exception of Volvocales do not show any bodily movements, which is common in animals. Such movements of locomotion are however, absent in higher plants. Such plants respond to external environment by different type of movements like bending, twisting, elongation of certain parts, swelling or shrinkage and quite a few more.

Before studying the different movements in plants, let us first get familiar with some important terms used in plant movements.

- Any factor, external or internal which causes a reaction in a plant is called a ***stimulus*** due to its properties of irritability and sensitivity. The reaction in a plant due to this stimulus is the **response** which is expressed in various ways.
- Only certain specialized regions in a plant are sensitive to this stimulus. These are called **centres/sites of perception**.
- The manifestation of response occurs at the **site of response**. The two sites viz. site of perception and site of response may be same or different.
- The minimum time period for which a stimulus needs to be given so as to produce a response is called **presentation time**. Presentation time is inversely proportional to the intensity of stimulus.
- The time take by the plant between the given stimulus and the appearance of response is called **reaction time**.

Plant Movements are classified into two types (Fig. 15.28):

1. **Physical** - expressed by dead parts. These are **hygroscopic movements** or **mechanical movements/chasic movements**. Such movements are reversible. These are named accordingly to the nature of the stimulus and response.

   Hydrochasic = Swelling of wood.

   Xerochasic = Dehiscence of sporangia, elaters of bryophytes, peristome teeth in moss capsules, pseudo-elaters in *Equisetum* spores.

2. **Vital** - Expressed by living cells in response to internal or external stimuli. Movements taking place without any external stimulus are called

autonomicor spontaneous movements. Movements in response to external stimuli are termed **Paratonic** or **induced**. The paratonic movements are broadly classified as movements of locomotion and movements ofcurvature. Whereas the movements of curvature are generally found in the rooted terrestrial plants, locomotory movements are shown by some aquatic plants.

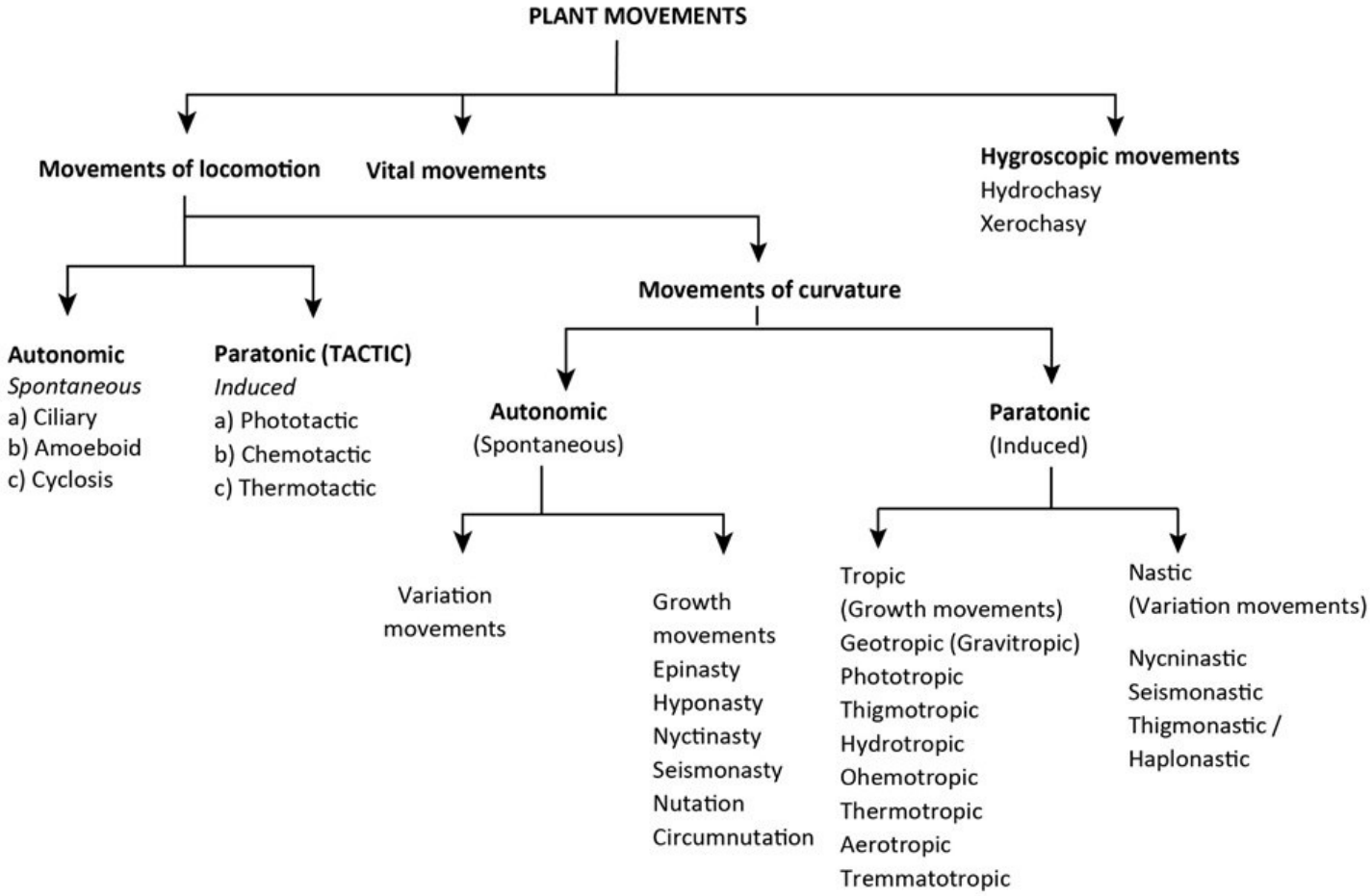

Fig. 15.28: Summary of Plant Movements

15.10 SUMMARY

- The development of the plant starts with the formation of seeds. A seed has stored food material and mRNA which it uses during the process of germination. By utilizing proteins that are already existing and newly synthesized on stored mRNA or whose transcription is induced, the stored food reserves like lipids, starch and seed storage proteins are degraded to yield compounds that can be utilized by the growing embryo for its growth and development.
- After a certain span of vegetative growth, plants undergo a reproductive development. During this process, flowers are produced. The flowering process is regulated by the duration of light/dark cycles. The dark period seems to be more critical to the flowering process. It is the leaf which perceives the photoperiodic stimulus. After perception the signal is sent to the vegetative meristems which then are converted into flowering meristems.

- The photomorphogenetic events in plants are regulated by a red light and far-red light absorbing pigment called phytochrome, which exists in two forms/states. The far-red light absorbing state—P_{fr} is biologically active and induces a large number of fast and slow responses in plants. The fast responses could be mediated by bringing changes in membrane properties. The slow responses may involve formation of new proteins which would control the transition from one developmental state to another.
- Each plant undergoes senescence after the completion of their life cycle. Senescence may be manifested in the form of seasonal leaf-fall or death of certain parts or the entire plant itself.
- Senescence involves a series of genetically controlled biochemical changes which also determine the various patterns of programmed death.
- The phenomenon of flowering itself is under the control of endogenous rythms and follows a strict biological clock- like precise pattern. Of course, these developmental processes are influenced by external factors.
- Many developmental responses are studied using *in vitro* culture technique. This technique allows the culture of any type of tissue to regenerate a complete plant. The tissue culture technique has many exciting possibilities and commercial application. The process of senescence shows variation in its pattern. This process also involves a series of biochemical changes which are genetically controlled.

15.11 TERMINAL QUESTIONS

1. What are the three major categories of plants bases on their day length requirement?
2. Give one example each of: i) Ambiphotoperiodic plant, ii) obligate short-day plant
3. Discuss the importance of dark period in flowering.
4. How are the two forms of phytochrome interconvertible?
5. Comment on the chemical nature of phytochrome.
6. Give examples of some phytochrome-mediated responses
7. What are the recent ideas regarding phytochrome action leading to flowering.
8. Discuss the concepts of florigens.
9. What is Vernalization? How is it useful in agriculture?
10. What are biological clocks? Give some examples.

15.12 ANSWERS

Self-Assessment Questions

1. a) i) Vegetative, floral ii) dark; iii) Day-neutral; iv) darkness

   b) i) False; ii) True; iii) False; iv) True; v) True

2. a) i) 660 nm, 730 nm

   ii) Far-red

   iii) Soluble protein, cytoplasm

   iv) Calcium

   v) *Pfr*, transcription

   b) Refer to Subsection 15.4.1.

3. a) Shoot apex; b) devernalization; c) Vernalin; d) GA

   e) Low temperatures, suitable photoperiod

4. i) Level of P_{fr}

   ii) *Phaseolus*

   iii) Floral clocks

   iv) Spore discharge in *Pilobolus*

   v) Absence of external factor

5. a) Refer to Section 15.7.

   b) Refer to Section 15.7.

   c) Refer to Section 15.7.

Terminal Questions

1. Long day, Short day and Day neutral plants;
2. i) *Setaria verticellata,* ii) *Chenopodium rubrum*
3. Refer to Subsection 15.2.2.
4. Refer to Subsection 15.3.1
5. Refer to Subsection 15.3.2.
6. Refer to Subsection 15.3.3.
7. Refer to Subsection 15.3.4.
8. Refer to Section 15.4.
9. Refer to Subsection 15.5.1.
10. Refer to Subsection 15.6.

UNIT 16

PLANT STRESS

Structure

16.1 INTRODUCTION

The previous units have provided you information about the various essential physiological processes in plants. These include photosynthesis, respiration, transpiration, translocation of solutes and assimilation of nutrients such as carbon, nitrogen, sulphur. Plants get exposed to environmental variations. These changes bring alterations in the growth and development of plants by affecting various biochemical and physiological processes. Exposure to abiotic and biotic stresses such as alteration in temperature, water availability, soil pH, salinity, alkalinity, heavy metals, pathogen infestation, infection and injury brings a change in the metabolic status of the plants. Exposure of plants to environmental conditions or stress triggers various metabolic responses in plants. The present unit introduces you to the concept of abiotic and biotic stress in plants and provides detailed information about plant response to stress.

Objectives

After studying this unit you would be able to:

- define stress and differentiate between abiotic and biotic stress to which plants gets exposed;
- describe changes in morphological, physiological and metabolic behaviour of plants in response to stress;
- explain plant response to water and osmotic stress, temperature stress;
- discuss plant response to pollution; and
- explain about plant response to infection and wounding.

16.2 What is stress?

Any change in the surrounding environment that affects the functioning of plants and alters the homeostasis is defined as **biological stress**. A sudden transition in the optimal environmental conditions which disturbs the homeostasis in plants and induces adverse effects on their physiology is referred as **plant stress**. Plant species get exposed to fluctuations in temperature (chilling, freezing and high temperatures), water availability, changes in the soil conditions such as salinity, heavy metals etc. Plants have an optimum requirement for various environmental variables and show susceptibility to any alteration. These changes impose stress in plants which include delay or alteration in growth and development, effects on productivity and in extreme cases, death.

Let us recall what happens within the natural communities occupying the same habitat. The relative location of two plants may place them under differing conditions with respect to a given environmental factor such as light. The top cover of a rainforest, for example, consists of relatively tall trees and receives maximal irradiance while the floor dwellers manage with sunflecks.

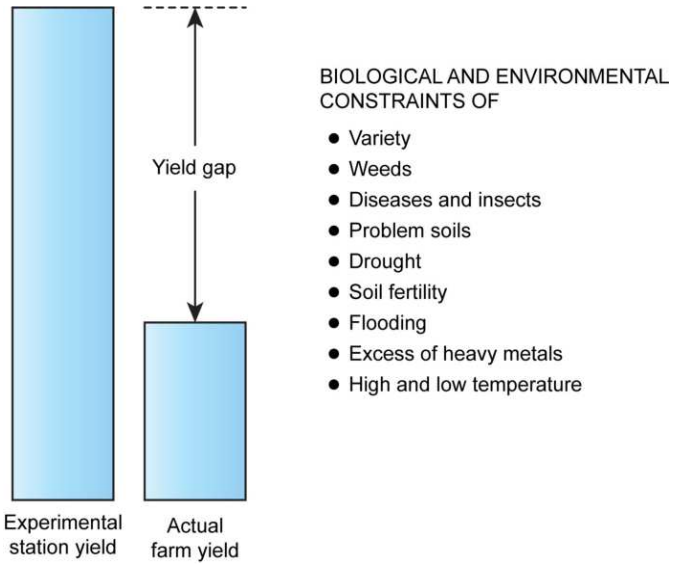

Fig. 16.1: Graphic representation of yield gap.

What will happen if we artificially shadow the outer cover plants and illuminate the forest floor? Being a deviation from the natural situation, this is likely to have adverse effects on growth of plants. Fig. 16.1 shows the yield gap due to biological and environmental constraints. Thus, any deviations from optimal environmental conditions usually with adverse effects are considered as stress. This in turn affects the yield of useful agricultural products

We must, however, realise that in no habitat, all conditions - temperature, light, availability of water, nutrient supply and soil characteristics can be controlled and fixed to optimal level for any species. But since the species must survive, it must adapt itself to the deviations in the environmental condition(s). This could be achieved either by breeding crop varieties tolerant to stress or by offering conditions that help the plants to withstand the stress. For example, if there is a deficit of some nutrient one could try to supplement the same. Plants capable of adapting themselves to changes in environmental conditions perform the best under stressful conditions.

16.3 NATURE OF STRESS

As mentioned in the preceding section, stress can be considered as any deviation in environmental condition from optimal for the overall performance' of the plant species under reference. If you recall the environmental factors, you can tell the various kinds of stress that plants may be subjected to. A broad outline on the nature of possible environmental stress is illustrated in Fig. 16.2.

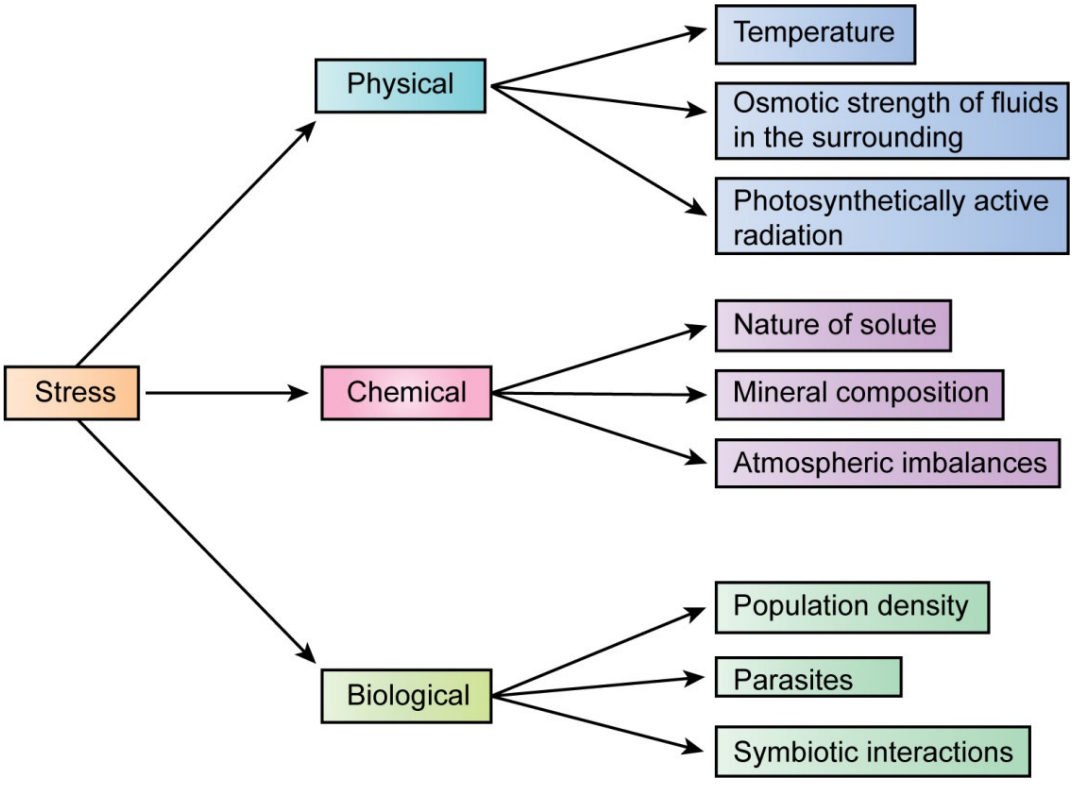

Fig.16.2: Nature of Environmental Stress Conditions.

16.3.1 Physical Stress

Physical stress originates from environmental conditions such as:

i) **Temperature :** We are familiar with the plants and other organisms that live at temperatures close to the temperature range in which we are

adapted to live i.e., 15° to 45° C. However, we know that there is life below 0° C in the arctic and above 90° C in the sulphur springs. In the subtropical zones, plants face stress -when they get exposed to freezing temperatures during the winters while in the deserts of the tropics the native plants withstand over 55° C during summer. High temperature can be inhibitory for photosynthesis (Fig. 16.3).

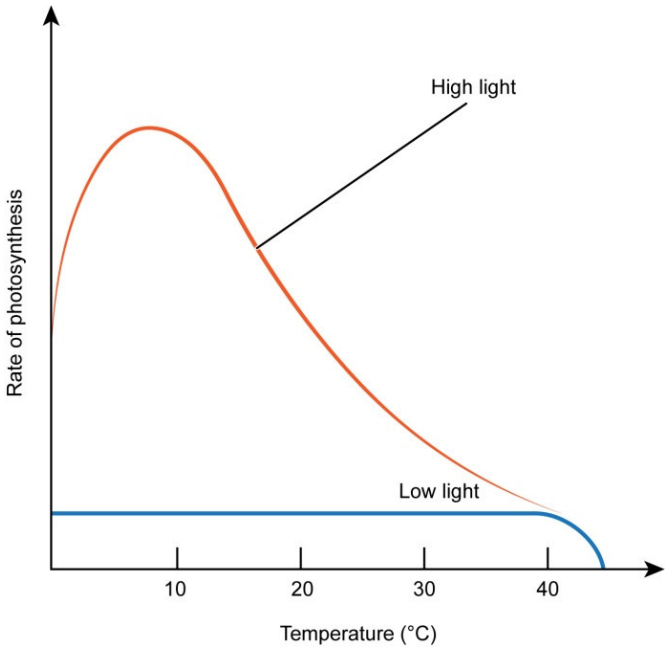

Fig. 16.3: Effect of light intensity and temperature on the rate of photosynthesis.

ii) **Osmotic strength of the fluids in immediate surrounding:** The availability of soluble mineral salts varies widely from habitat to habitat. In fact, a big chunk of land in our country has been classified as 'wasteland' because of high salinity of the soil cover or the water leachates. Common crop cultivars cannot be grown in such environments.

iii) **Photosynthetically active radiation**: This is usually in direct proportion with the incident solar radiation. You know that tropics are probably the best illuminated part of the globe. Also, variation in day length is minimal in this zone. No wonder, light-dependent life has attained maximal density in the tropics. Within a dense population of plants, different organisms may have different degrees of exposure. However, if a plant is exposed to increasing light intensity it shows a corresponding increase in the rate of photosynthesis upto a certain point, beyond which further increase in light causes either no change in the rate or causes an inhibition (Fig. 16.3). Thus, each plant species shows a characteristic photosaturation and/or photoinhibition of photosynthesis. In other words, each species has its own optimum with respect to light intensity. Plants get stressed if they get exposed to intensity above or below the optimum.

16.3.2 Chemical Stress

Survival of cells is dependent on carrying out of a set of chemical reactions (metabolic reactions) in a particular order. This results in a net gain in mass as well as energy in a growing cell. Even for dormant cells as in dormant buds

and seeds, where there may not be any net gain in mass, a certain amount of energy must be spent to maintain them in a viable state. Various catabolic and anabolic pathways operate according to the availability of chemical constituents present in the cytoplasm. This may in turn be influenced by the composition of the extracellular environment with respect to the following:

i) **Nature of the solutes**: The acidic or basic reaction of soil and water of a particular habitat reflects its geochemical history beginning with its formation and subsequent interactions with other constituents of the earth up to its current chemical activities. Mineral deposits in their oxide form ('Bhashma') are usually basic in their reaction. The reaction of chloride, sulphate and nitrate is acidic or neutral depending upon the nature of the conjugate ions. You can visualise the reaction of a salt through its acidic and basic radicals. On a natural course, one would expect the neutralisation reactions and consequently change in the character of the habitat towards neutral. However, there are soils which are very high and others very low in pH and certain plant species survive in such soils.

ii) **Mineral composition**: The living systems make use of several mineral ions that they might have encountered at the very origin or during evolution, particularly for transformation of matter involving proteins and nucleic acids. These elements continue to remain essential requirements for life. You have already learnt in Unit 12 that nitrogen, phosphorus, calcium, potassium and magnesium are familiar major requirements for plant growth besides carbon, hydrogen and oxygen. Apart from these, many other micronutrients such as manganese, iron, zinc, cobalt and molybdenum are required for healthy plant growth in much smaller quantities. Availability of these elements in the environment in quantities smaller than required cause's deficiency symptoms while a surplus may cause toxicity leading to stunted growth, necrosis, abnormal development of vegetative and reproductive parts.

iii) **Atmospheric imbalances:** At least two components of the environment, oxygen and carbon dioxide that plants require are predominantly in the gaseous form. As you have learnt, atmospheric nitrogen can be used by nitrogen-fixing bacteria, some prokaryotic algae and plant-bacterial symbiont. The biological cycling of these gases keeps their overall availability buffered within a reasonably stable range. Yet, intensive industrial activities that involve emission of one of these gases or high levels of the oxides of carbon, nitrogen and sulphur have visible effects on the performance of most plant species.

16.3.3 Biological Stress

Since in nature, the various organisms do not live in complete isolation from others, stress to a plant species might also be caused by what other organisms in the community require and consume. The following situations exemplify biological stress.

i) **Population density:** You are aware of what might follow an uncontrolled growth of human population. There will be competition for common

consumables and for space but this is not merely a human problem. Too thick population densities of plants can resist the competition for common nutrients, water and photosynthetically active radiation (PAR). The effect can be a self inhibition or inhibition of other plant species growing in the same environment. That is one of the reasons why weeds are removed from croplands.

ii) **Parasites:** Many insects and microorganisms can feed upon tissues and saps of living plants. Hence plants must be protected against such parasites either by expressing evasive devices such as inhibitors and toxins against the enzymes of parasites or by developing preventive morphological and biochemical alterations that keep the parasite away from aggregating near the plant.

iii) **Symbiotic interaction**: Presence of symbiotic microorganism can result in differential growth stimulation of those plants which can recognise the beneficial symbionts and utilise their presence in the environment. This interaction is a mutual one. For example in Rhizobium-legume symbiosis, the rhizobium causes a drain on the carbon fixed by the plant (refer Unit 13) while the plant gains access to the nitrogen fixed by the bacteria.

SAQ 1

What is the nature of stress faced by the following species if the change mentioned below is brought about in their environment?

a) A plant species growing in Manali is brought to Jaipur during summer.

b) A sun-loving plant gets shaded by other plants.

c) A wheat crop gets flooded due to heavy rains for several days.

d) Trees growing near the roadway are subjected to heavy traffic.

e) Plants acclimatised to grow near fresh water are grown in coastal areas of Goa.

16.4 WAYS TO ADAPT TO STRESS

Plant stress responses are dynamic and involve various regulatory pathways. These include physiological, morphological adaptations and adjustment of metabolism. Alterations in the physiology or morphology help the plants to survive the changing environmental conditions (Fig 16.4). Plants respond to stress by undergoing change in metabolic functions, morphological features and physiological features. These responses are referred as **stress responses**. In salt sensitive plants (**glycophytes**), a number of physiological responses get stimulated. These response help plants to cope salinity stress. This includes SOS pathway (known as salt overlay signaling) that leads to enhanced efflux of sodium (Na) ions and reduction in toxicity induced by salinity.

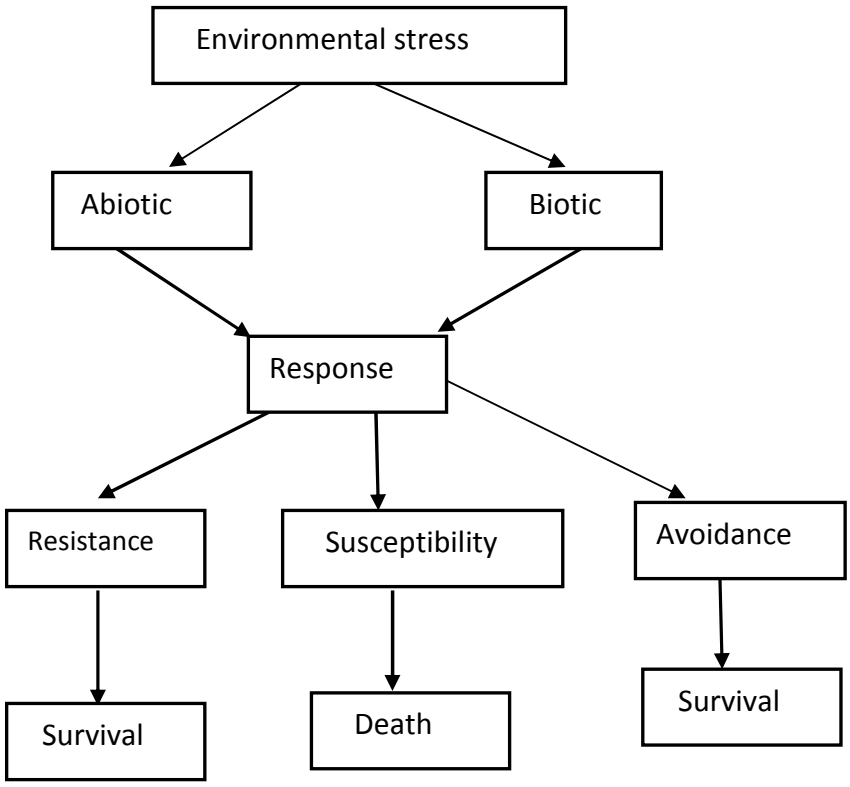

Fig. 16.4: Effect of stress on plants.

Stress responses require no genetic modification, hence are referred to as **phenotypic plasticity**. In phenotypic plasticity, the plants respond to changes in environmental conditions via changes in morphology and physiology. The changes are reversible. The ability of the plant to survive and establish in a given environment is related to balance between the genetic adaptation and phenotypic plasticity

Abiotic stress produces primary and secondary alterations in plants. Stress primarily causes reduction in hydrostatic pressure (turgor pressure) which leads to cellular dehydration. The change in cell hydration potential affects the physical and biochemical properties of cell. The secondary effects of stress include reduction in metabolic activities, production of reactive oxygen species (oxidative stress) and disruption of cellular integrity, ultimately death. Stress factors such as increase in temperature and exposure to ionizing radiation, UV light and environmental pollutants leads to generation of reactive oxygen species (ROS) which causes oxidative stress. The most common ROS formed include superoxide radical ($O_2.^-$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-).

Oxidative stress is caused when the ROS are produced and accumulated in high amounts in a cell or tissues than those that can tackled by the biological system. Oxidative stress leads to inhibition of major metabolic functions in plants. These include inhibition of photosynthesis and respiration. This results from down regulation or disruption of electron transport chains (ETC) in chloroplasts and mitochondria. Abiotic stresses such as water deficit, salinity and freezing disrupt cellular structures and impair physiological processes in plants. The damage to cellular structures leads to reduction in growth, fertility and cause premature senescence. The cell membranes become disorganized as the proteins get deactivated or denatured.

Box16.1: Tolerant plants

Two major strategies of combating stress have been recognized in plants. These are **avoidance** and **tolerance** strategies. Stress avoidance includes a various protective mechanisms that delay or prevent the negative effect of stress on a plant. Stress tolerance is the potential of a plant to acclimate to a stressful condition. Many plants have the capacity to tolerate stress. These are referred to as stress **resistant/tolerant plants** (Fig. 16.5). These plants exhibit the capacity to adjust or acclimatize to stress conditions. The plants ability to resist or tolerate stress depends on their genetic capacity to adjust/overcome stress and establish a new homeostatic state over a period of time. Some plants escape the stress. These include **ephemerals** or short lived plants. They germinate fast, grow and flower quickly. They complete their life cycle during a period of adequate moisture and form dormant seeds before the onset of dry conditions. Many arctic plants complete their life cycle during the short arctic summer and survive during winter in the form of seeds. Ephemerals never get exposed to stress because they survive the stress by **avoidance**. Plants of alfa alfa (*Medicago sativa)* survive dry habitats by sending their roots deep into the soil near the water table. In this way the plant ensures survival under conditions of drought. Other plants develop fleshy leaves that store water, thick cuticles and pubescence (leaf hairs) to reduce transpiration such as *Bryophyllum, Opuntia* etc.

Fig 16.5: *Medicago sativa* and *Opuntia*.

16.4.1 Changes at Cellular and Molecular Level

Responses to environmental stresses occur at all levels of organization. The changes can be at cellular or genetic level.

Cellular level - At this level, plants tackle stress by bringing modifications in the structure and architecture of the cell wall and membrane, cell cycle and cell division. Plants bring alteration in the metabolic events. These include production of compatible solutes such as proline, raffinose, glycine betaine. These solutes are the organic compounds that are osmotically active in the cell and play a role in stabilization of proteins, membranes and organelles. These compounds also contribute to maintenance of cell osmotic status by removing ROS and re-establishment of redox balance.

Molecular level - At this level, gene expression gets modified due to stress. Stress-inducible genes i.e. genes that are involved in protection of cell from stress via synthesis of osmoprotectants, detoxifying enzymes, transporters and those which encode regulatory proteins such as transcription factors, *protein kinases*, and *phosphatases* get induced.

SAQ 2

Define the following terms:

a) Stress

b) Tolerant plants

c) Ephemerals

d) Phenotypic plasticity

16.4.2 Biochemical Alterations

The plants exposed to stress depict alterations in their metabolic processes. Drought (water deficit), salinity and low temperature stress leads to change in osmotic/water potential resulting in loss of turgor in plant cells. The plants adjust to the condition of loss of turgor by accumulating solutes. The osmotic adjustment refers to the capacity of the plant cell to accumulate solutes which help in maintaining the osmotic potential during stress conditions.

High levels of sodium (Na) or chloride (Cl) ions exert detrimental effects in plants. Accumulation of ions (Na and Cl) in vacuoles (vacuolar compartmentalization) facilitates osmotic adjustment in halophytes growing in saline conditions.

Compatible solutes (osmolytes) help in tackling stress. These include a group of chemically diverse uncharged, polar, and soluble organic compounds and do not interfere with the cellular metabolism even at high concentrations. They mainly include proline, glycine betaine, sugar, and polyols. They act as source of carbon and nitrogen to the cell under normal conditions. Some plants show accumulation of amino acids on exposure to abiotic stress. This results from increase in amino acid synthesis or stress-induced protein breakdown.

Carbohydrates such as starch and fructans show accumulation under stress conditions. They act as storage substances and are used as energy source during stress. Fructans have high water solubility and possess resistance to crystallization at freezing temperatures. They stabilize membranes and indirectly contribute to osmotic adjustment by the release of hexose sugars. The roles played by these carbohydrates in stress mitigation are osmoprotection, carbon storage and scavenging of reactive oxygen species. Sugars function as osmolytes and help in maintaining turgor of cell and protect membranes and proteins from damage. Salt and drought stress generally leads to a depletion of starch content but accumulation of soluble sugars in leaves.

16.4.3 Changes in Plant Morphology and Behaviour

The plants adapt to extreme environmental conditions through modification in their life cycles. Desert plants have short life cycles and complete their life cycle when the water is available. The deciduous trees of the temperate zone shed their leaves before winter so that leaf tissue does not get damaged by low temperature. Besides these the growth habits of plants also contribute to

tolerance in plants. Studies have shown that plants which flower for long period of time are more tolerant to changing environmental conditions than those flowering for short periods.

The leaves of some plants develop certain characters that help them to escape extreme environmental changes. These include changes in leaf area, leaf orientation, thickness of leaf, presence of trichomes and cuticle. Large leaf area provides conditions for production of more photosynthates under stress conditions. Large surface area proves advantageous for leaf cooling as more water can be lost via evaporation, though excessive loss of water results in dehydration.

Plants growing under stress conditions generally reduce their leaf area by restricting cell division and expansion, altering leaf shapes and initiating senescence or abscission of leaves. The cell division and expansion of leaf gets restricted under conditions of water scarcity and salinity. Several signaling mechanisms slow down or stop cell cycle thereby limiting growth. Water deficit reduces turgor which affects cell expansion and reduces leaf expansion. The smaller leaf area reduces transpiration resulting in effective conservation of water. The thin film of air at the surface of the leaf (boundary layer) permits the transfer of heat from the leaf to the air. It helps the leaves to maintain surface temperature close to that of air so that transpiration is reduced and overheating can be prevented.

Alteration in leaf shape is another means by which plants overcome stress. Under conditions of water, heat, salinity stress, leaves become narrower or develop deeper lobes. This leads to reduced leaf area and prevents loss of water caused due to excessive heating. In some plants, leaf abscission occurs under conditions of water deficit and reduced leaf area prevents loss of water effectively. The plants adapted to drought show leaf abscission. Abscission results from the enhanced synthesis and responsiveness of the plant hormone ethylene. Some plants particularly crops such as corn, sorghum are able to maintain green leaf area during grain maturation stage under stress conditions. The retention of photosynthetically active leaves under stress is known as **stay green**. Some varieties maintain green stem and leaves even during drought conditions. The delay in senescence has been noted in these varieties.

The change in orientation also allows greater light absorption by leaves. Under conditions of high temperature and/or soil water deficits, plants can alter their leaves to avoid excessive heating. Leaves of some plants orient themselves away from sunlight to protect themselves from overheating. These leaves are called *paraheliotropic*. Some leaves gain energy by orienting themselves perpendicular to the sunlight is called as *diaheliotropic*. Wilting and leaf rolling also alters the absorption of light. Wilting changes the angle of the leaf while leaf rolling minimizes area exposed to sun.

The presence of trichomes on the surface of leaves help in keeping the leaf surface cool. The presence of densely packed hairs on the leaf surface reflects light. These hairs give leaf a silvery appearance. The cuticles (a layer of waxes and hydrocarbons present on the cell wall of epidermis) present on the leaf surface also reflect light thereby reducing heat. This layer also restricts diffusion of water, gases and entry of pathogens. Plants exposed to water stress develop thick cuticle to prevent water loss through transpiration.

Water stress affects the development of roots and shoots. It has been speculated that the shoot tends to grow until water uptake by the roots becomes limiting for further growth. In contrast the roots tend to grow until the demand for photosynthate from the shoot exceeds the supply. The functional balanced gets disturbed during water stress. When water supply becomes limiting, leaf expansion is reduced and a greater proportion of the plant assimilates can be allocated to the root system where they can support root growth. As the water deficit progresses the upper layers of the soil dry but the roots proliferate into deeper, moist soil. This change in root architecture is considered as defensive strategy against drought. Enhanced root growth into the deeper soil requires allocation of photosynthates to the growing root tips.

SAQ 3

a) Match the statements from the column A with the correct options from column B.

	Column A		Column B
i)	salt sensitive plants	a)	water deficit or salinity
ii)	dehydration	b)	osmolyte
iii)	results in loss of cell turgor	c)	trichomes
iv)	stabilizes proteins and membranes	d)	ephemerals
v)	present on the surface of leaves	e)	glycophytes
vi)	short lived plants	f)	Reduction in water content

b) Differentiate between:

i) abiotic and biotic stress

ii) paraheliotropic and diaheliotropic leaves

16.4.4 Alternate Metabolic Pathways

The metabolic pathways change in response to stress conditions. Water deficit decreases photosynthesis and consumption of assimilates in the expanding leaves. The decreased water potential inhibits movement of assimilates and less amount of photosynthates get exported from leaves. The ability of plants to continue translocating assimilates under drought conditions is considered as a key factor for plant resistance.

Some plants such as C4 and CAM plants change their mode of photosynthesis. In CAM plants (succulents such as cacti), stomata open at night and close during the day. This prevents loss of water through transpiration and increases water use efficiency. The change in CAM mode of photosynthesis has been noted in plants exposed to water deficit or salinity. This adaptation allows plant to acclimate to arid environments.

During flooding, oxygen levels are low (hypoxia) and roots, stem of the plants develop interconnected gas filled channels with in cells (aerenchyma) that provide resistance pathway for the movement of oxygen and other gases. The gases enter stomata or lenticels in woody stem and roots. They move by molecular diffusion or by convection driven by small pressure gradients. In many plants growing in wet habitats, the root cells develop into aerenchyma cells. When the oxygen supply is insufficient for respiration, roots begin to ferment pyruvate to lactate through the action of *lactate dehydrogenase*. The production of lactate lowers the intracellular pH inhibiting the *lactate dehydrogenase* and activating *pyruvate decarboxylase*. The change in the enzyme activity leads to ethanol production. Hence during fermentation, 2 moles of ATP are formed per mole of hexose sugar catabolized in comparison to 36 moles of ATP produced from each hexose catabolized in aerobic respiration. Thus injury to the root metabolism by oxygen deficiency leads to lack of ATP which affects essential metabolic processes.

16.5 PLANT REPONSES TO STRESS CONDITIONS

16.5.1 Water and Osmotic Stress

The condition that arises due to scarcity or excess of water is known as water stress. Water scarcity results in drought while excess of water leads to flooding. Water makes a large proportion of the cell volume and is used for expansion and metabolic processes. Flooding stress leads to anaerobic conditions i.e. oxygen stress. The decreased supply of oxygen limits respiration, nutrient uptake and other root functions. During drought conditions, the plant cells lose water; they shrink because of collapse of cell wall. The damage to root hairs affects the absorption capacity. The outer layers of root cortex get covered with suberin, a water impermeable lipid that increases resistance to water flow in the root. The resistance to water flow also results from breakage of water columns within xylem under tension.

Plant growth becomes limited under conditions of water deficit. This is because water deficit leads to cell dehydration. Water deficit causes reduction in cell turgor and dehydration which results in reduction in cell size and/or volume. The cell wall extensibility decreases due to increase in cell wall pH. In long term, water deficit leads to reduction in vegetative growth. Shoot growth and leaf production gets severely affected. There is reduction in leaf expansion and growth which is an adaptation in plants to reduce rate of transpiration. The loss in turgor of cells affects the cell enlargement. Reduction in low water potential enhances root growth gets thereby changing the root shoot ratio. Improvement in root shoot ratio improves water supply as the roots can extract more water by exploring larger volumes of soil.

Water deficit leads to closure of stomata. This has been considered as a strategy adapted by plants to conserve water. The closure of stomata leads to low internal CO_2 concentration in plants and this limits the photosynthetic capacity of the plant. Water stress affects the photosynthesis in two ways. First by blocking supply of carbon dioxide to the chloroplasts due to closure of stomata and second by changing the structural integrity of the photosynthetic

machinery due to low water potential. The change in the photosynthetic machinery affects electron transport and photophosphorylation.

The metabolic processes such as accumulation of solutes get triggered by stress. The solutes which take part in osmotic adjustment include inorganic ions (such as K^+), sugars and amino acids. The solutes maintain hydration of the protoplasm by decreasing osmotic potential under stress. They prevent loss of membrane integrity and maintain protein stability. The amino acid proline, sorbitol (sugar alcohol) and glycine betaine are the other solutes that help in the recovery of turgor under conditions of water stress.

Anatomical and physiological adaptive mechanisms limit transpiration in conditions of water deficit. The presence of thick cuticle, trichomes, sunken stomata limit the loss of water through transpiration.

16.5.2 Salinity

Excessive accumulation of salt in the soil is referred as **salinity**. High concentrations of Na^+, Cl^-, Ca^{2+}, Mg^{2+} and SO_4^{2-} are present in saline soils. High concentration of salts replaces the essential ions present in the soil resulting in toxicity. Salinity results in accumulation of Na and Cl ions in the cytosol. The soils having high sodium (Na) content are called sodic soils. They degrade soil structure by decreasing porosity and water permeability. High concentrations of salts cause denaturation of proteins and membrane destabilization by reducing hydration of molecules. The nutrient acquisition gets disturbed from accumulation of toxic ions in soil.

Some plants growing in saline soils are not or less adapted. They are called as *glycophytes*. These plants get exposed to stress referred as **salinity stress**. In contrast, some plants are well adapted to saline conditions. These are called as tolerant plants. For example-halophytes. These plants develop various physiological and biochemical mechanisms to survive in highly saline soils. The mechanisms involved in salt tolerance mainly include ion homeostasis, compartmentalization, biosynthesis of osmoprotectants/compatible solutes, activation of antioxidant systems (enzymes and compounds), synthesis of polyamines, generation of nitric oxide (NO) and hormone modulation.

Halophytes possess specialized salt glands on the surface of leaves that excrete salt. Some plants accumulate toxic ions in the older leavers which senesce and abscise to allow younger, more photosynthetically productive leaves to survive.

Compatible solutes function as a protector or stabilizer of enzymes or membrane structures that are sensitive to dehydration. Proline provides tolerance to stress and serves as a nitrogen reserve. It functions as a quencher of superoxide radical and hence exhibits antioxidant potential. It provides tolerance to salt stress by increasing the activity of enzymes involved in antioxidant defense system. Straight-chain polyols such as mannitol, sorbitol and cyclic polyols such as myo-inositol or its methylated derivatives increase in response to conditions of salinity. Trehalose accumulation caused due to salinity stress protects plants against several physical and chemical damages. Polyamines play an important role inducing tolerance in plants.

Although, abscisic acid (ABA) is a phytohormone which inhibits growth but its application ameliorates the effect of stress conditions in plants. This hormone gets upregulated in conditions of water deficit. Increased production of ABA has been observed in shoots and roots of plants exposed to salinity stress and water deficit. ABA acts as a vital cellular signal that modulates the expression of many salt and water deficit-responsive genes. The genes related to accumulation of K^+, Ca^{2+} and compatible solutes (such as proline and sugars) get upregulated by ABA. Hormones such as *salicylic acid (SA)* and *brassinosteroids (BR)* are also known to participate in plant responses to abiotic stresses.

A large number of genes and transcription factors are upregulated in response to salinity in different plant species. These are called *salt-responsive genes*. These genes are mainly involved in ion transport or homeostasis (e.g., *SOS* genes, *AtNHX1*, and *H^+-ATPase*), senescence [*senescence associated gene* (*SAG*)], molecular chaperones (such as heat shock proteins), and dehydration-related transcription factors (DREB). Upregulation of *metallothionein* and water channel proteins has also been noted in plants exposed to stress.

16.5.3 Pollutant Stress

Plants have adapted various ways to tackle stress caused by exposure of various gaseous pollutants and toxic trace elements present in the environment.

Exclusion - The process/means by which the plants maintain level of contaminants below a toxic threshold. It mainly involves removing the element out of the cell/body.

Tolerance - The process/means by which plants develop various biochemical adaptations which allow the plant to survive stress conditions. These mainly include compartmentalization and chelation.

Plants get exposed to high levels of heavy metals present in the soil. Some plants show high accumulation of heavy metals such as copper (Cu), nickel (Ni), zinc (Zn), chromium (Cr), lead (Pb), cadmium (Cd), cobalt (Co), iron (Fe) in their tissues. These are called hyperaccumulators. High concentration of heavy metals induces oxidative stress in plants. Antioxidant defensive mechanisms play an important role in tackling oxidative stress caused by heavy metal accumulation.

The plants exposed to air pollutants show alterations in the physiological and biochemical features in plants. These features help in determining susceptibility or tolerance to stress caused by pollutants. Changes in stomatal behavior, carbon and nitrogen assimilation have been noted in plants exposed to air pollutant stress. Air pollution stress leads to stomatal closure which reduces availability of carbon dioxide to leaves and inhibits carbon fixation. As a result the net phostosynthetic rate decreases thereby limiting the plant productivity. Absorption of air pollutants such as sulphur dioxide (SO_2), oxides

of nitrogen (NO_X) and carbon dioxide (CO_2), suspended particulate matter (SPM) by leaves causes reduction in the concentration of photosynthetic pigments viz. chlorophyll and carotenoids. This affects the plant productivity. The plants respond to pollutant stress by closing their stomata. This depicts the avoidance mechanism adapted by plants. In contrast, synthesis of antioxidant compounds reflects strategy adapted by tolerant plants.

SAQ 4

Answer in one word:

a) The plant hormone that accumulates in the water stressed leaves.

b) Soils having high concentrations of Na^+, Cl^-, Ca^{2+}, Mg^{2+} and SO_4^{2-}.

c) Solutes that protect or stabilize enzymes or membrane structures during stress.

d) The process by which plant maintains the level of elements below a toxic threshold.

e) Some plants show high accumulation of heavy metals in their tissues.

f) Some plants possess specialized salt glands on the surface of leaves that excrete salt.

g) The condition in which roots and stem of the plants develop interconnected gas filled channels that provide low resistance pathway for the movement of oxygen and other gases.

16.5.4 Temperature Stress

Plants exhibit sensitivity to variations in temperature. Each plant requires an optimum temperature for growth and development. Exposure to extremely high and low temperatures adversely affects growth in plants.

Low temperature stress

An exposure to moderate chilling temperature may kill or cause injury to plants. Maize, tomato, soybean, cotton are susceptible to injury when exposed to temperatures between 10 to 15^o C. The seedlings show wilting and chlorosis in conditions of chilling stress. The low temperature causes reversible changes in the physical state of the membranes. This occurs due to change in the proportion of saturated and unsaturated fatty acids present in the membrane lipids. The temperature at which the transition from liquid to solid state occurs is called as **transition temperature.** At temperature above the transition temperature, the membrane remains fluid but becomes solid or gel like at temperatures below the transition temperature. With the drop in temperature, the membranes show a transition from a flexible liquid crystalline structure to a solid gel state. The rate of transition varies with the species and depends upon the lipid composition of the membrane. Sensitive plants have

high proportion of saturated fatty acids while chilling resistant species possess low proportion of saturated fatty acids but more of unsaturated fatty acids. The membranes with this composition tend to solidify into a semicrystalline state at temperature above 0º C. For functioning and stability, membrane needs to be maintained in a liquid gel like state but under stress they are changed to solid state which affects the membrane structure and activity. Freezing leads to intra and intercellular crystal formation. Intercellular crystal formation damages membranes and organelles. Extracellular ice crystal formation cause cellular dehydration. This is because ice formation lowers the water potential in the apoplast. It forms a gradient from high water potential gradient in the symplast to low water potential gradient in the apoplast. Water moves from the symplast to apoplast resulting in cellular dehydration which affects the membranes. The formation of a stable ice crystal involves participation of several hundred water molecules. This is called **ice nucleation**.

The damage to the plant cell membranes i.e. structure and function affects the metabolism. Enzymes and enzymatic reactions are sensitive to temperature. The rate of reaction doubles for each 10^{o}C rise in temperature until an optimum is reached beyond which the rate of reaction declines. The decline in enzymatic activity results from unfolding of protein and is referred as thermal denaturation. The Q_{10} increases linearly with short term rise in temperature. Q_{10} measures change in rate of respiration. An increase in ambient temperature causes change in the rate of respiration. .

The herbaceous species grown at low temperature exhibit a short, compact growth habit, thicker leaves due to an increase in leaf mesophyll cell size and/or an increase in the number of palisade layers.

Deciduous trees, conifers and shrubs such as birch, willow, survive cold stress because they are able to adapt to low temperature conditions. The adaptation in woody tissues begins in autumn when the growth and photosynthesis ceases and plant enters dormancy. Hence the plants enter the dormant phase prior to the onset of frost to prevent damage caused due to freezing. During frost the respiratory activities sufficiently provide the energy required for the numerous metabolic changes to attain maximum cold acclimated stage. During this period the conversion of starch to sugars and level of organic phosphate increases. The glycoproteins accumulate and the protoplasm becomes more resistant to dehydration.

During adaptation to cold conditions, the temperate woody trees withdraw water from the xylem vessels thereby preventing the stem from splitting in response to the expansion of water during freezing. The physical properties of lipids get altered at low temperatures resulting in increased membrane rigidity.

High temperature stress

High temperature (HT) stress is injurious to growth and development of plants. High temperature stress causes loss of water content from the cell as a result cell size gets reduced and ultimately the plant growth gets affected. Reduction in seed germination potential, seedling growth, reduced radicle and plumule

growth has been noted as the major impact of heat stress in plants (Fig.16.6). Reduction in relative growth rate (RGR) due to decrease in net assimilation rate (NAR) has been noted in conditions of HT stress. The negative effect of heat on leaf includes reduction in water potential, leaf area and pre-mature leaf senescence.

Exposure to high temperature alters the total phenological duration of the plant life. Increases in temperatures affect the grain filling periods. A short period of heat stress can cause significant decrease in production of floral buds. The increase in sterility is caused due to impairment of meiosis in both male and female organs, negative effects on pollen germination, reduced pollen tube growth, reduced ovule viability, abnormality in stigmatic and style positions, disturbance in fertilization process, hindrance in growth of endosperm and proembryo. High temperature treatment reduces anther dehiscence and pollen fertility. The reduced fertilization results from decrease in number of pollens on the stigma.

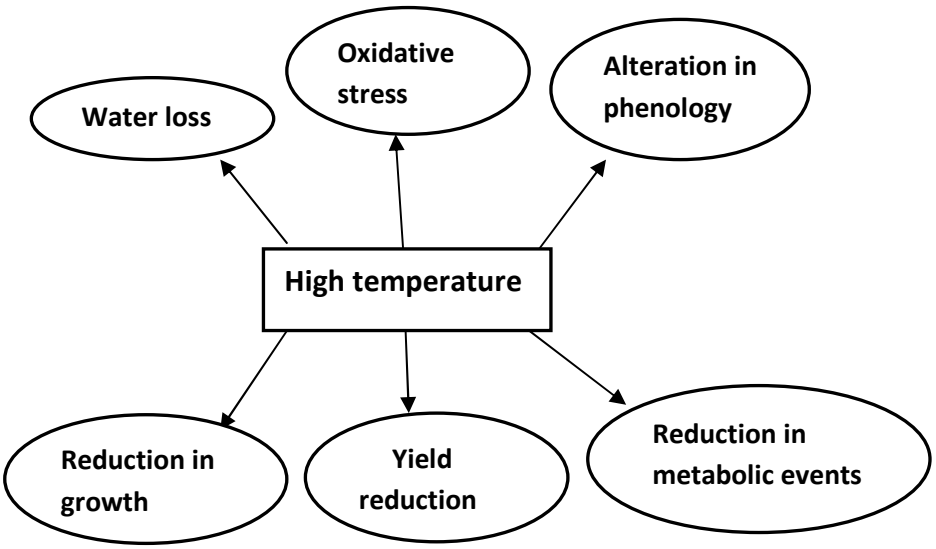

Fig. 16.6: Major effects of high temperature stress on plants.

The plants that can adjust to high temperature conditions are called **thermotolerant plants**. Plants exposed to cold temperature exhibit a lower optimum temperature for photosynthesis while those growing at high temperatures exhibit high temperature optima for photosynthesis. The tissues of the plants are not able to survive temperatures above 45^{o}C but some of the tolerant species show survival at these temperatures because of their ability to show of evaporative cooling. Leaf temperatures can raise to 4 to 5^{o}C above ambient air temperature in bright sunlight when soil water deficit causes stomatal closure to high relative humidity reduces gradient driving evaporative cooling.

Plants exhibit various mechanisms to ensure survival under high temperature conditions. These include phenological changes, morphological adaptations and short-term avoidance or acclimation (adaptation) mechanisms such as changing leaf orientation, transpirational cooling, or alteration of membrane lipid compositions. Closure of stomata and reduced water loss, increased

stomatal and trichome densities and larger xylem vessels are common heat induced features in plant.

Early maturation noted in many crop plants has been considered as a mechanism to escape heat stress. Plants growing in a hot climate avoid heat stress by reducing the absorption of solar radiation. This ability is supported by the presence of small hairs (tomentose) that form a thick coat on the surface of the leaf as well as cuticles, protective waxy covering. In such plants, leaf blades often turn away from light and orient themselves parallel to sun rays (*paraheliotropism*). Solar radiation may also be reduced by rolling leaf blades.

Plants with small leaves are also more likely to avoid heat stress. They evacuate heat more quickly due to smaller resistance of the air boundary layer in comparison with large leaves. In well-hydrated plants, intensive transpiration prevents leaves from heat stress, and leaf temperature may be 6°C or even 10-15° C lower than ambient temperature. High temperature can affect the degree of leaf rolling in many plants

When plantlets or tissues of plants are shifted to 42° C and above, the synthesis of normal proteins rapidly declines, instead synthesis of new proteins is induced. These proteins are known as **heat-shock proteins (HSP)**. These proteins are self-regulatory i.e. their synthesis is switched off after 6 to 8 h of exposure to elevated temperature while synthesis of the normal proteins resumes. The molecular weight of heat-shock proteins ranges from 15 to 102 kDa. HSPs are known to be induced also by heavy metals and arsenites. The HSPs occur in representatives of all the major groups of organisms. A pre-treatment at elevated temperature (at 45°C for 2 h) eliminates the heat-shock response. It is believed that heat shock protein 2 protects essential enzymes and nucleic acids from denaturation.

Induction of synthesis of heat-shock proteins has also been observed under field conditions. In dry fields during summer when the leaf temperature reaches or exceeds the ambient temperature (>40° C), synthesis of HSPs is induced as under experimental conditions. The synthesis of HSP shows a transcriptional as well as translational control.

How do HSPs help in heat-shock avoidance? They probably help important cellular proteins to acquire conformations that would be safe and functional under high temperature and the protein will remain in soluble state in the cytoplasm.

Molecular approaches are also being followed to improve plant tolerance. Plants tolerate HT stresses by modulating multiple genes involved in various pathways. Heat stress up-regulates several heat inducible genes referred as "**heat shock genes" (*HSGs*)** which encode HSPs. These proteins protect intracellular proteins from being denaturation and preserve their stability and function through protein folding. HSPs act as chaperones. High temperature also induces synthesis of low molecular mass proteins known as **heat shock proteins (HSPs).** The synthesis of these proteins in cells improves thermal tolerance. HSPs are also induced by water deficit, ABA treatment, wounding, low temperature and salinity. Three distinct classes of these proteins have

been recognized on the basis of their molecular mass in plants. These include HSP90, HSP70 and heterogenous group of proteins in the range of 17 to 28 kDa.

16.5.5 Stress by Infection and Wounding

Plants respond to various pathogens through an intricate and dynamic defense system. The mechanism of defense has been classified as innate and systemic plant response. An innate defense is exhibited by plant in two ways, viz., specific (cultivar/pathogen race specific) and non-specific (non-host or general resistance). A large array of proteins and other organic molecules are produced prior to infection or during pathogen attack. Constitutive defenses include morphological and structural barriers (cell walls, epidermis layer, trichomes, thorns, etc.), chemical compounds (metabolites, phenolics, nitrogen compounds, saponins, terpenoids, steroids and glucosinolates), proteins and enzymes. These compounds confer tolerance or resistance to biotic stresses by not only protecting the plant from invasion, but also giving the plant strength and rigidity. The production of toxic chemicals, pathogen-degrading enzymes (*chitinases* and *glucanases*) is used by plants. These compounds may be present in their biologically active forms or stored as inactive precursors that are converted to their active forms by host enzymes in response to pathogen attack or tissue damage. Plant defense strategies involving these compounds can fall in the category of either innate or systemic acquired resistance (SAR). Innate immunity is of greater efficiency and is the most common form of plant resistance to microbes.

Imbalances in biotic factors reduce cell proliferation, photosynthesis, membrane integrity, protein stability and induce production of ROS, oxidative damage and death. The plant exposed to insects or pathogens responds with changes in the composition and properties of the cell walls and synthesis of secondary metabolites that limit the infection.

Secondary metabolites associated with the hypersensitive reaction constitute signal transduction pathways that prepare cells and tissues to resist secondary infection. Because of this effect the plant reacts to infection by slowly developing a general immune capacity. The phenomenon is called as systemic acquired resistance (SAR). One important component of this signaling pathway is salicyclic acid (SA). It is a naturally occurring secondary metabolite with analgesic properties.

Methyl jasmonate is the principle constituent of the essential oil of *Jasminium* and high concentrations of Jasmonic acid (JA) has been isolated from fungal culture filtrates. Both JA and methyl ester of jasmonate mediate insect resistance. Besides the role in insect and pathogen resistance, jasmonic acid is supposed to be involved in many physiological processes such as seed and pollen germination, protein storage, root development and coiling of tendrils.

Plants show specific responses to abiotic or biotic stress. These responses mainly include production/synthesis of specific hormones, proteins and accumulation of solutes that helps in maintenance of the physiological and metabolic processes (Fig. 16.7).

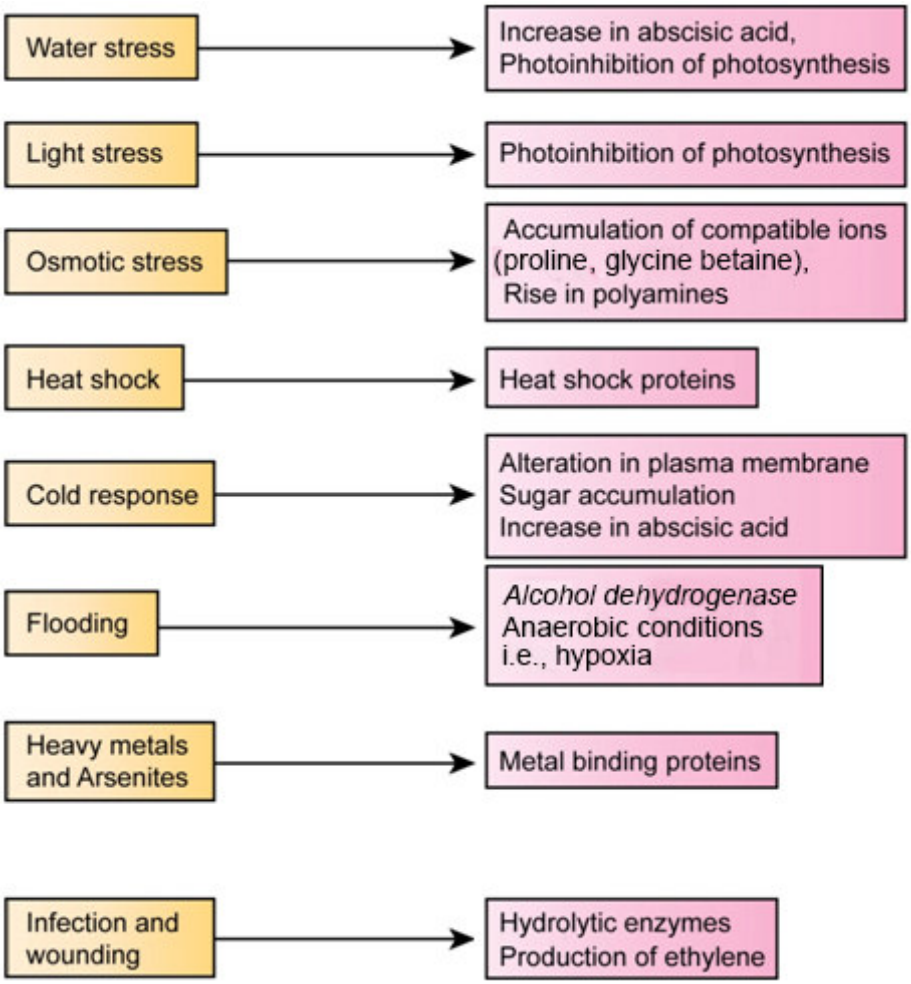

Fig.16.7: Plants responses in various kinds of stress.

SAQ 5

Complete the statements given below:

a) The low temperature causes reversible changes in the …… .

b) The chilling injury can result in many …… .

c) The measure of Q_{10} depicts change in …….. .

d) The negative effect of heat on leaf mainly include …… .

e) High temperature has a greater influence on the photosynthetic capacity of …….. .

f) The plants that can adjust to high temperature conditions are …….. .

g) Synthesis of low molecular mass proteins in cells that improve thermal tolerance are …….. .

h) …….. acts as important component of the signaling pathway in systemic acquired resistance.

i) Hormone that mediates insect resistance in plants is …….. .

16.6 FUTURE PROSPECTS

Plants have evolved a variety of mechanisms to withstand stress conditions. In many cases we can precisely define the way plants enable themselves to survive and perform well under stress conditions. Based on such knowledge, it should be possible to construct and breed plants tolerant to many environmental extremes found in our country and elsewhere on earth while trying to cultivate plants. There are so many salt affected areas in our country. Salt tolerant varieties can be planted in large areas affected by salinity.

One of the ways to manipulate stress tolerant varieties is through genetic engineering technique. With this technique it is possible to isolate a gene and introduce it in a desired organism. This results in the expression of transferred gene in the new organism which then starts behaving like the organism from which this gene was originally isolated. The gene products related to a particular phenomenon also get synthesised in the organism**.** The synthesis of one or more proteins can be achieved easily through genetic engineering technique. Thus, for instance, if genes for betaine synthesis can be transferred to a plant sensitive to osmotic stress, it might confer tolerance against such stress or an elicitor like phytoalexin can be produced by plants by artificial gene transfer, it would be possible to have disease resistant plant.

These programmes can be directed towards incorporating the following traits among common crop plants.

1) **Non-photoinhibition** of photosynthesis particularly in the tropics would mean several fold increase in biomass produced over the same period of time as water and carbon dioxide supply are non-limiting.

2) **Resistance to high temperature** beyond mesophilic ranges can allow agriculture in several areas that are left uncultivated because of prohibitive temperatures. To a large extent any strategy to achieve this would depend on the water status of the environment.

3) **Cold-hardiness** in cultivars can help curb the losses often incurred because of extremely low temperatures reached during winters in some parts of the world.

4) **Drought resistance** in crop plants will be particularly helpful to our primarily rain-fed agriculture.

5) **Salt tolerance** in plants will bring a lot more of territory under green cover.

6) **Disease resistance** has always been a trait sought after in plants adopted for cultivation. This can improve the present yield by 20 to 50% (depending on the plant in question and the climatic zone).

7) **Pest tolerance** in the crop plants can be achieved by producing *proteinase* inhibitors or by producing bacterial gene coding for pesticidal protein. This limits their nutritional value but in cases like cotton where our prime interest is fibre instead of food, this can still be a way to achieve upto 50% improvement in yield.

16.7 SUMMARY

- Transition in the optimal environmental conditions which disturbs the homeostasis and induces adverse effects on their physiology in plants is referred as stress. Stress is of two types: abiotic and biotic. Plant species get exposed to various stresses such as fluctuations in temperature (chilling, freezing, high temperatures), water scarcity, salinity, infections and pathogen infestations etc.
- Plants respond to stress in various ways. These mainly include physiological, morphological and metabolic adaptations. These changes help the plants to survive the changing environmental conditions. These responses require no genetic modification and hence are referred as phenotypic plasticity.
- Plants combat stress by two major ways i.e. avoidance and tolerance. Stress avoidance includes a variety of protective mechanisms that delay or prevent the negative effect of stress on plant. Stress tolerance is the potential of a plant to acclimate to a stressful condition.
- Abiotic stress produces primary and secondary effects in plants. Primary effects include reduction in water potential, and cellular dehydration. These effects alter the physical and biochemical properties of cell. The secondary effects include reduced metabolic activity, production of reactive oxygen species (oxidative stress) and disruption of cellular integrity.
- Plants respond to these stresses at various levels of organization. The changes can be at cellular or genetic level. At cellular level, plants tackle stress by alteration in the metabolic events such as production of compatible solutes.
- Studies on plant responses to stress provide useful information. In future, it would be possible to take measures by genetic engineering and other means to prevent losses in yields of crops, fruits, vegetables and other useful products due to stressful environmental conditions. It would also be possible to bring into use chunks of land that at present cannot be used for cultivation.

16.8 TERMINAL QUESTIONS

1. What are the two different strategies adapted by plants to overcome stress?
2. What is oxidative stress and how does it affects plants?
3. What compatible solutes are synthesized by plants under conditions of abiotic stress?
4. What are the main components of the antioxidant defense system in plants?
5. Explain the reason for closure of stomata under conditions of water stress.
6. What are heat shock proteins? What role do they play in plants exposed to stress?

16.9 ANSWERS

Self-Assessment Questions

1. a) physical stress i.e., heat/temperature stress

   b) physical stress i.e., photosynthetically active radiation

   c) physical stress i.e., flooding

   d) chemical stress i.e., Pollution

   e) physical stress

2. a) Stress-Any change in the surrounding environment that affects the functioning of plants and disturbs homeostasis in plants is referred as stress**.**

   b) Tolerant plants – The plants that have the capacity to tolerate stress by adjusting or acclimatizing to stress conditions are referred as resistant/tolerant plants. The plants tolerance capacity depends on their genetic capacity to adjust/overcome stress and establish a new homeostatic state over a period of time.

   c) Ephemerals- Some plants escape the stress. These are called ephemerals or short lived plants. They germinate fast, grow and flower quickly. They complete their life cycle during a period of adequate moisture and form dormant seeds before the onset of dry conditions. They survive the stress by avoidance. Many arctic plants complete their life cycle during the short arctic summer and survive during winter in the form of seeds.

   d) phenotypic plasticity- Stress response in which plants respond to changes in environmental conditions via changes in morphology and physiology without any genetic modification. The changes are reversible.

3. a) i) glycophytes

      ii) water deficit or salinity

      iii) reduction in water content

      iv) osmolytes

      v) trichomes

      vi) ephemerals

   b) i) Stress imposed on plants due to change in physical or chemical environment is called as abiotic stress while the stress caused by living organisms, specially viruses, bacteria, fungi, nematodes, insects, arachnids and weeds is called as biotic stress.

      ii) Leaves of some plants orient themselves away from sunlight to protect themselves from overheating. These leaves are called paraheliotropic. Some leaves gain energy by orienting themselves perpendicular to the sunlight is called as diaheliotropic.

4. a) Abscisic acid (ABA)

   b) Saline soils

   c) Compatible

   d) Exclusion

   e) Hyper accumulators

   f) Halophytes

   h) Hypoxia

5. a) physical state of the membranes

   b) metabolic dysfunctions in plants

   c) rate of respiration

   d) reduction in water potential, leaf area and pre-mature leaf senescence.

   e) C_3 plants

   f) thermotolerant plants

   g) heat shock proteins (HSPs)

   h) Salicyclic acid (SA)

   i) Jasmonic acid (JA)

Terminal Questions

1. Two major strategies of combating stress recognized in plants are avoidance and tolerance. Stress avoidance includes a variety of protective mechanisms that delay, avoid or prevent the negative effect of stress on plant. The plants which are able to tolerate stress are called as stress resistant/tolerant plants. The plants ability to resist or tolerate stress depends on their genetic capacity to adjust/overcome stress and establish a new homeostatic state over time.

2. Oxidative stress occurs due to generation of reactive oxygen species (ROS). During oxidative stress, the ROS are produced and accumulated in high amounts in a cell or tissue than those that can tackled by the biological system. The most common ROS include superoxide radical ($O_2.^-$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-). ROS possess strongly oxidizing capacity and potentially affect metabolic activities such as photosynthesis, damage cellular structures leading to reduction in growth, reduction in fertility and premature senescence.

3. The plants respond to abiotic stress such as drought, salinity by undergoing metabolic alterations. One of the strategies adapted by plants to overcome stress is synthesis of compatible solutes. Compatible solutes are the organic compounds which help in curtailing stress. They

mainly include amino acids such as proline and sugars. Proline acts as a ROS scavenger, molecular chaperone and helps in stabilization of protein structure thereby protecting cells from damage caused due to stress. The non-protein amino acid, γ-aminobutyric acid (GABA) helps in maintaining carbon–nitrogen balance and ROS scavenging. Carbohydrates such as starch and fructans act as storage substances and are used as energy source during stress conditions. They stabilize membranes and indirectly contribute to osmotic adjustment upon freezing and dehydration by the release of hexose sugars. Sugars function as osmolytes to maintain cell turgor and protect membranes and proteins from damage caused due to stress. Trehalose (non-reducing disaccharide) functions as an osmolyte and stabilizes proteins and membranes in desiccation-tolerant plants. The level of trehalose increases under stress. Polyols play a role in stabilizing macromolecules and scavenging hydroxyl radicals. Accumulation of polyols such as mannitol and sorbitol has been noted in several plants species exposed to stress.

Glycine betaine (GB) is a quaternary ammonium compound produced in plants exposed to abiotic stress such as cold, drought, and salinity. The compound protects photosystem II, stabilizes membranes, and mitigates oxidative damage. Various stresses such as drought, salinity and cold induce synthesis of polyamines (PA). High PA levels have been positively correlated with stress tolerance. PAs have been implicated in protecting membranes and alleviating oxidative stress.

4. Antioxidant system includes antioxidant enzymes and non-enzymatic compounds. These play critical role in detoxifying ROS. The activity of antioxidant enzymes, such as *superoxide dismutase* (SOD), *catalase* (CAT), *glutathione peroxidase* (GPX), *ascorbate peroxidase* (APX), and *glutathione reductase* (GR) and accumulation of nonenzymatic antioxidant compounds increases under stress conditions. Antioxidant compounds such as ascorbate, glutathione also help in mitigation of stress. They react with superoxide radical, hydroxyl radical, and hydrogen peroxide thereby functioning as a free radical scavenger.

5. Abscisic acid (ABA) accumulates in the water stressed leaves and mediates the loss of solute from guard cells. The plant regulates ABA metabolism by modulating ABA synthesis in response to dehydration. ABA accumulation in the chloroplast lowers the pH. The pH increases in the region surrounding the mesophyll cells stimulates release of ABA from the mesophyll to the apoplast. This supports efflux of K ions from the guard cells. The guard cells lose turgor resulting in closure of stomata.

6. Heat stress leads to up-regulation of genes referred as “heat shock genes” (HSGs). These genes encode proteins called heat shock proteins (HSPs). These proteins protect denaturation of intracellular proteins from being and preserve their stability, function through protein folding. HSPs act as chaperones. The synthesis of these proteins improves thermal tolerance in cells.

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GLOSSARY

Antioxidant : A substance that reduces damage caused by free radicals.

Actinorhizal : Pertaining to several woody plant species, such as alder trees, in which symbiosis occurs with soil bacteria of the nitrogen fixing genus *Frankia.*

Apoplast : Space in between the cells creating a pathway through which materials may diffuse freely.

Arbuscles : Branched structures of mycorrhizal fungi that form within penetrated cells; the sites of nutrient transfer between the fungus and the host plant.

Arbuscular Mycorrhizal fungi : Symbiotic fungi that form hyphae growing in a loose arrangement, both within the root itself and extending outward from the root into the surrounding soil. The hyphae form unique structures, such as the arbuscules that enhance the exchange of nutrients between the fungus and its host.

Bacteroids : Nitrogen-fixing organelles that develop from endosymbiotic bacteria upon a signal from the host plant.

Cessation : The ending of a process.

Chelates : Substances such as EDTA that from a complex with divalent cations eliminating their biological activity.

Chelator : A carbon compound that can form a non-covalent complex with certain cations facilitating their uptake (e.g., malic acid, citric acid).

Coleoptile : A sheath protecting a young shoot tip in a grass or cereal.

Denaturation : Breaking of weak linkages or bonds within a molecule.

Eccentrically : Deviation from conventional or usual pattern or style.

Endogenous : Anything that originates internally.

Heterogenous : Consisting of different, distinguishable parts or elements.

Homeostatic : A stage by which an organism tends to maintain stability or adjusting to conditions required for its survival.

Hydroactive : Activated by water

Indeterminate : Not exactly known or defined.

Leghemoglobin : An oxygen-binding heme protein found in the cytoplasm of infected nodule cells that facilitates the diffusion of oxygen to the respiring symbiotic bacteria.

Mesophilic : growing or thriving best in an intermediate environment (moderate temperature)

Nitrate reductase : Enzyme that reduces nitrate NO_3^- to nitrite NO_2^-. Catalyzes the first step by which nitrate absorbed by roots is assimilated into organic form.

Nitrite reductase : The enzyme that reduces nitrite NO_2^- to ammonium (NO_4^+.).

***Nitrogenase* enzyme complex** : The two-component protein complex that conducts biological nitrogen fixation in which ammonia is produced from molecular nitrogen.

***Nod* factors** : Lipochitin oligosaccharide signal molecules active in gene expression during nitrogen fixing in which ammonia is produced from molecular nitrogen.

Nodules : Specialized organs of a plant host containing symbiotic nitrogen-fixing prokaryotes.

Nodulation genes (*nod)* : Rhizobial genes, the products of which participate in nodule formation.

Nodulin (*Nod*) genes : Plant genes specific to nodule formation.

Photomorphogenesis : Light mediated development of plant.

Phytoalexin : substances produced by plants that inhibit the growth of pathogen (such as a fungus)

Rhizobia : Collective term for the genera of soil bacteria that form symbiotic (mutualistic) relationships with members of the plant family Leguminosae.

Skotomorphogenesis : The development of a seedling in the dark.

Stimulus : Thing that triggers a specific functional reaction in an organ or tissue.

Susceptibility : The state of being influenced or harmed by something.

Symplast : Inner side of the plasma membrane in which water and low-molecular-weight solutes can freely diffuse.

Synchronization : Two activities/events happening at a same time.